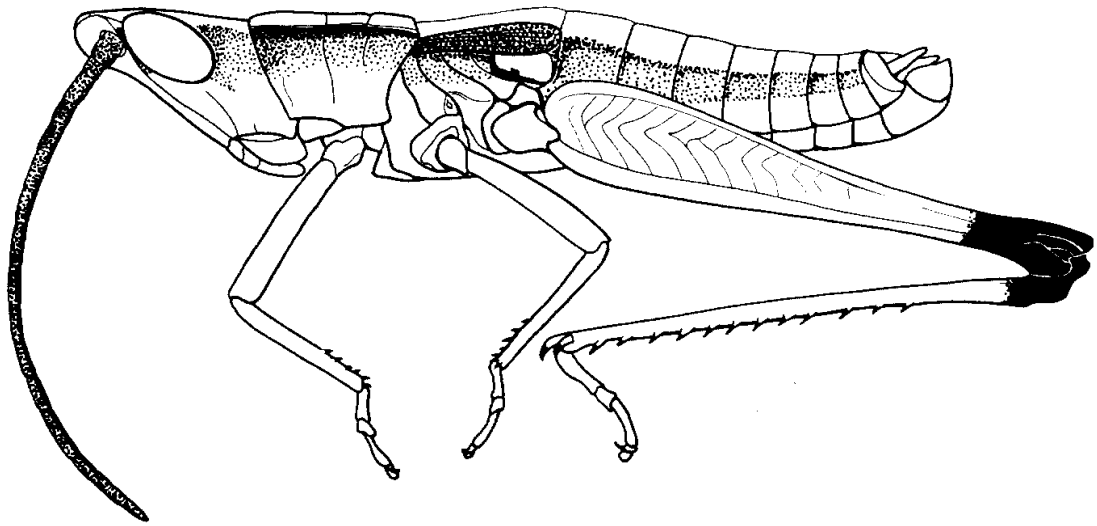
A Phylogenetic Analysis of the East African Grasshopper Genus *Afrophlaeoba* JAGO, 1983 (Orthoptera: Acridoidea: Acridinae)



Dissertation zur Erlangung des Doktorgrades

Dr. rer. nat.

vorgelegt von

Axel Hochkirch

Universität Bremen
Fachbereich Biologie / Chemie

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“If we can walk on the moon or search for life in outer space, we can fully explore life on Earth. We know that life occurs here, but we have only an inkling of its diversity, grandeur, and wonderment”

Miller & Rossmann (1996)

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1 Introduction

Tropical evergreen forests are of major biological interest due to their high biological diversity. Recent estimates of worldwide species diversity have risen to 30 million species following canopy fogging studies in tropical evergreen forests (Erwin 1982). Other estimates range from five million to 100 million species (Stork 1996), and although the variation of these estimates is striking, there is little doubt that tropical evergreen forests make a huge contribution to worldwide biodiversity (Myers et al. 2000), offering home to more than 50% of the world's species (Linsenmair 1990). However, diversity is generally not high in all taxa (Scharff 1992), nor is it equally high in all tropical evergreen forests. There are so-called "hot spots" among them, which are usually defined as regions with high within-habitat-diversities and a high percentage of small-range endemics (Myers et al. 2000). Nowadays there is great interest to locate these regions for subsequent conservation (Burgess et al. 1998a) because of the rapid decline of tropical moist forests (Sayer & Whitmore 1991). Since the distribution patterns of species are not only determined by the present environment but also by processes which have operated in the past (Moreau 1966), the genesis and maintenance of those patterns of biodiversity pose major questions for research. Or, as Erwin (1996) points out, "attention must focus on the underlying evolutionary processes that have resulted in such diversity and evaluate these in terms of present human activities."

In Africa four main hotspots of biodiversity have been identified: The Central African forests in Cameroon, the Cape Floristic Province, the Lake Tanganyika / Lake Malawi area and the Eastern Arc Mountains / Coastal Forests (Barthlott et al. 1996). The Eastern Arc Mountains / Coastal Forests have been ranked as the second most important "endemism hot spot" of mainland Africa, based on a global analysis of plant and animal endemism (Myers et al. 2000). Approximately 1,500 endemic plant species and 121 endemic vertebrates occur in 2,000 km^2 of remaining primary vegetation, topping a world wide list of hotspots in concentration of endemics, but also in terms of degree of threat (Myers et al. 2000). Conservation priority-setting assessments have ranked the Eastern Arc as second most important area in Africa for the conservation of restricted-range bird species (Dinesen et al. 1993). The presence of relicts with close affinities to SE Asia (Dinesen et al. 1994), Madagascar (Emberton et al. 1997, Pócs 1998), and the Guinea-Congolian forest block (Lovett 1988, Howell 1993) as well as recently diversified groups (Fjeldså & Lovett 1997, Roy et al. 1997), makes this area extremely interesting for studies of evolution and biogeography (Burgess et al. 1998a).

The factors causing such a high degree of endemism have been a subject of controversy, but several interacting factors are discussed. Factors influencing the generation of centres of endemism can be divided into factors related to time (e. g. evolutionary times, times of separation) and factors related to space (e. g. refuge areas, geographic separation). Persistence in time and space is probably a main factor for the survival of endemics (Anderson 1994). According to the refuge concept (Haffer 1974),

tropical forest species have evolved by isolation in areas which remained stable. This can also be concluded from the fact that most hotspots are situated in areas which were climatically more or less stable or at least buffered during the Pleistocene climatic fluctuations (Fjeldså & Lovett 1997). They are often located close to the sea or in mountainous regions, which received high rainfall even during dry periods. The accumulation of species in mountain regions is probably also influenced by the topography. The altitudinal climatic variation allows an altitudinal shift of species with special climatic requirements. Moreover, mountainous streams may provide small humid areas, in which at least some endemic insect species may survive dry periods (Fjeldså & Lovett 1997). The separation of the single mountain blocks of the Eastern Arc by adjacent savannah or dry miombo woodland caused the isolation of congeneric species (Lovett & Wasser 1993). Thus each of the forests contains a number of small-range endemics, resulting in a high landscape diversity (Hochkirch 1998). However, there is still some controversy on the past effects of climatic fluctuations on the vegetation of East Africa, since the relative effects of different climatic factors are difficult to disentangle.

Several authors have urged for more phylogenetic and historical biogeographic research linking speciation with forest refuges. According to Howell (1993) there is a "lack of knowledge about exact timing and nature of the lost connections among various forests and climatic variations." He proposes to apply biochemical techniques to study the evolutionary relationships of the faunal assemblages. Scharff (1993) suggests that an analysis of the phylogenetic relationships of a monophyletic group endemic to the Eastern Arc may provide further insight into the natural history of those faunas. A group of allopatric taxa with small ranges and low vagility, such as flightless insect species may well serve as study object for such a purpose, since those species depend on habitat connections rather than on long-distance dispersal (Brühl 1997). Since nearly all forest grasshoppers of the Eastern Arc are flightless, this group is probably suitable for such a study. The grasshopper faunas of the Eastern Arc are characterized by numerous endemic taxa, each of which consist of closely related species belonging to the same genus, and replacing each other on different mountains. While in the agricultural cultivations similarities can be found on species level, endemic genera or even subfamilies occur inside forests of single mountain blocks (Hochkirch 1998). The ranges of grasshopper species and genera are much smaller than in plant taxa and therefore, grasshoppers provide a suitable study group for phylogeographical studies (Hochkirch unpubl.).

The main motivation for this study was the lack of information about the evolutionary history of endemic Orthoptera of the Eastern Arc. The research objectives of this study can be summarized as follows:

1. In which way have separated populations of a typical Eastern Arc taxon been connected?
2. When and where did those connections exist?
3. Under what ecological conditions could gene flow occur between the populations?
4. How strong is the ecological, morphological, genetic and ethological differentiation between the separated taxa?

In this study the genus *Afrophlaeoba* JAGO, 1983 was chosen for the analysis. Four species from four separated mountain blocks are known (Jago 1983). The closely related genus *Parodontomelus* RAMME, 1929 was also included in the study, as an outgroup. A combined approach was chosen to gain more information on the degree of molecular, morphological, ethological and ecological differentiation of the species:

1. Molecular makers (three DNA sequences of parts of mitochondrial genes) were analysed to gain phylogenetic information. This method may also allow rough estimates on the time of separation (Li 1997).
2. Discrete morphological characters of the male external anatomy and genitalia were examined, to study the differentiation. In addition to these, morphometric measurements of 27 external characters were made to provide some information on inter- and intraspecific morphometric discontinuity (Blackith & Reyment 1971).
3. Behavioural records of male displays were included to provide information on interspecific differences in this regard, since communication differences are known to be important for specific mate recognition in Orthoptera (Ragge & Reynolds 1998).
4. Habitat requirements were studied to gain some information about niche differentiation between the species and the possible ancestral biology. Information about the ecological origins of the species can be important for the reconstruction of possible paths of gene flow (Knox & Palmer 1998).

2 Study Area: The Eastern Arc

A series of ancient crystalline mountain blocks situated east of the arid corridor and running from southeast Kenya to southwest Tanzania (figure 1) is referred to as Eastern Arc (Lovett 1985). The more prominent rocks of the Arc consist of Taita Hills (Kenya), North and South Pare Mts., East and West Usambara Mts., Nguu Mts., Nguru Mts., Ukaguru Mts., Rubeho Mts., Uluguru Mts., Malundwe Hill, Udzungwa Mts. and Mahenge Mts. (all in Tanzania). On the whole the Eastern Arc mountain forests cover less than 2% of Tanzania's total land area (Kingdon & Howell 1993). They are under the direct influence of the Indian Ocean monsoon (Lovett 1993b) and separated by dry woodland and savannah. The exact coordinates of the mountains relevant for this study are given in table 1.

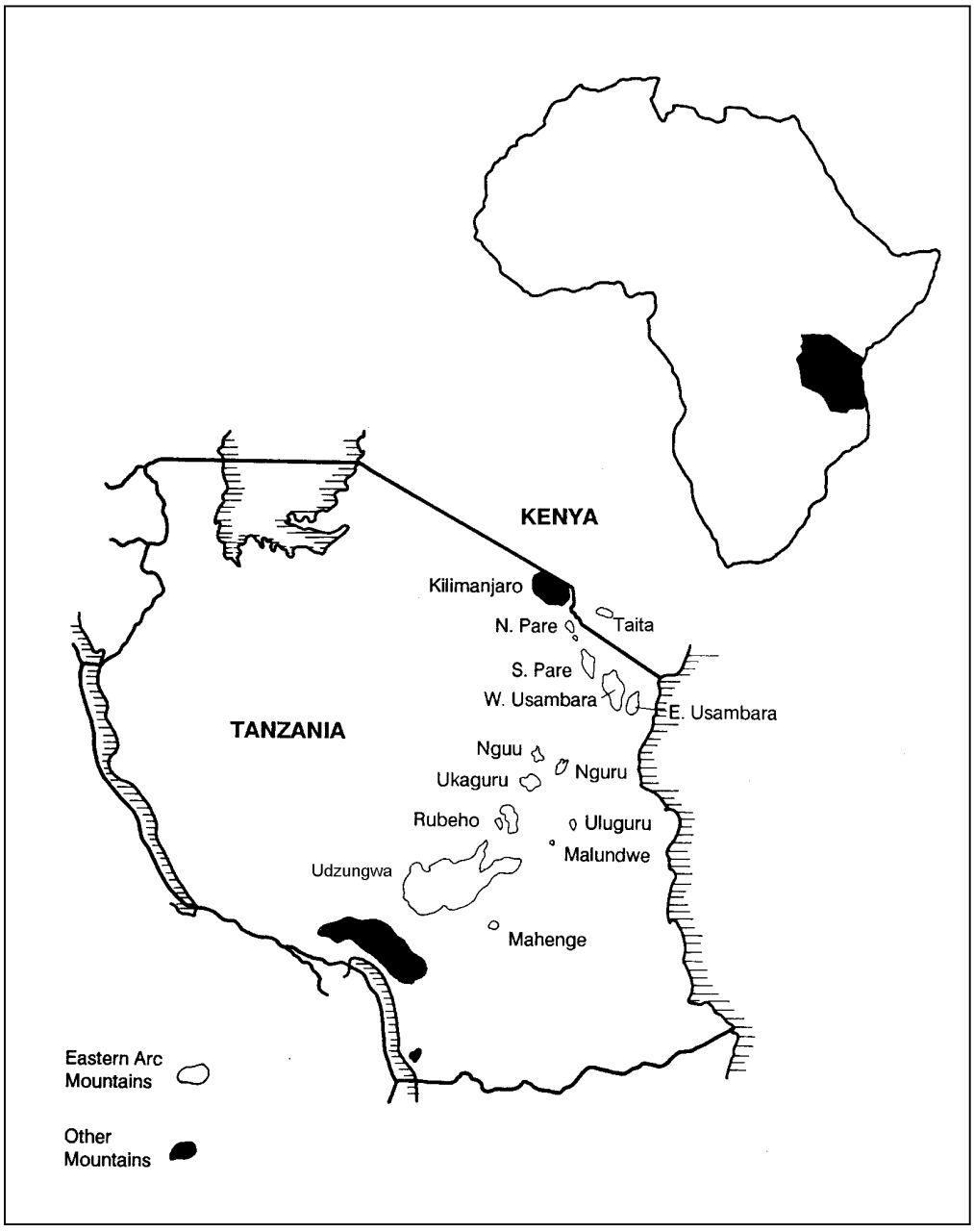


Fig. 1: Location of Tanzania in Africa and the Eastern Arc Mountains in Tanzania (according to Hamilton & Bensted-Smith 1989).

Tab. 1: Location, distance to coast, elevation and altitudinal range of the forests of the Eastern Arc (data from Burgess et al. 1998a and Cordeiro 1998), from which the genus under study was recorded.

Sites	Coordinates	Distance to coast	Altitude	Altitudinal range of forest
West Usambara Mts.	04°20'-05°07' S. 38°06'-38°41' E.	100 km	2250 m	1200-2200 m
East Usambara Mts.	04°45'-05°20' S. 38°26'-38°48' E.	40 km	1500 m	130-1500 m
Nguru Mts.	05°27'-06°13' S. 37°26'-37°37' E.	150 km	2400 m	400-2000 m
Ukaguru Mts.	06°19'-06°35' S. 36°53'-37°03' E.	220 km	2250 m	1500-2200 m
Rubeho Mts.	06°48'-07°22' S. 36°34'-36°58' E.	300 km	2050 m	520-2050 m
Uluguru Mts.	06°51'-07°12' S. 37°36'-37°45' E.	180 km	2650 m	300-2400 m

2.1 Natural History of the Eastern Arc

The first uplift of Precambrian basement rocks took place between Ethiopia and Mozambique during the Mozambiquian orogeny, 650 to 450 my BP (Griffiths 1993). These Precambrian geological formations are usually referred to as Mozambique belt (Saggerson 1962) and a particular uniform series within this belt is called Usagaran system (Quennel et al. 1956). Nowadays only the deeper root zones of those mountains remain and later uplifts are usually defined as the origin of the Eastern Arc. During the Karoo period (290 to 180 my BP) vertical movements led to early rift faulting (Griffiths 1993), including the genesis of the lake troughs such as Nyasa (Rodgers 1998) and the progenitors of the Eastern Arc (Lovett 1993a). The Gondwana break-up separated Africa from the other continents 165 to 120 my BP (Griffiths 1993). Currents from the surrounding oceans brought moisture to large areas of Africa, with moist forests even in northern Africa 60 to 40 my BP (Lovett 1993a). Based on the distribution patterns of birds, Lönnberg (1926) and Braestrup (1935) already assumed a former forest connection to Western Africa. These huge forested areas in Africa are usually referred to as pan-African forests. When the African continent reached Eurasia during the Miocene (c. 20 my BP), the closed Tethys Sea changed the rainfall supply from the north and aridity was spreading in large parts of Africa (Lovett 1993a). This changing rainfall pattern and the uplift of the Central African Plateau separated the Guinea-Congolian forests from the East African rainforests by the "arid corridor", which simultaneously connected the arid regions of northeastern Africa with the southwestern parts (Axelrod & Raven 1978, Werger 1978, Lovett 1993a). During the same period C_4 grass-dominated ecosystems spread in Africa (Jacobs et al. 1999). The development of the East African rift started 30 to 20 my BP in the northern parts, continuing 15 to 12 my BP in Southern Kenya and 7 my BP in Tanzania. This latest period was accompanied by volcanism and block faulting again (Griffiths 1993), including the uplift of mountain blocks further east (Rodgers 1998). These topographic changes altered the drainage patterns dramatically (Lovett 1993a)

with successive desiccation of large parts of eastern Africa. The Kenyan Rift Valley was still occupied by woodland 14 my BP (Cerling et al. 1991).

There is still some controversy about the date of the latest faulting and uplifting of the Eastern Arc. Teale (1936) considered the late Mesozoic (100 my BP) as the time for the uplifting of the basement complex, but although these tectonic events probably were significant for the genesis of the Eastern Arc, later uplifts are likely to have occurred (Iversen 1991b). According to Quennel et al (1956) the Pare Mountains, Uluguru Mountains and Usambara Mountains are of Neogene age (38 to 7 my). Saggerson (1962) and Hamilton (1982) dated the latest uplifts to the Miocene (25 to 20 my BP). Other theories (e.g. Lundgren 1978, Ezaza 1988) date the latest Eastern Arc faulting to the Pliocene-Pleistocene (5 to 2 my BP). Iversen (1991b) points out that these theories of a later origin are incompatible with vicariant distribution patterns of some plants. However, there is little doubt that the Eastern Arc is much older than the volcanic mountains of the Kilimanjaro / Meru region or the Southern Highlands of Tanzania, which were formed by volcanic activity during the Pleistocene, 2 to 1 my BP (Coe 1989). Many elements of the Eastern Arc fauna and flora are believed to be relicts from the Miocene (Burgess et al. 1998a). Consequently most authors regard the Miocene as the time of the latest uplift. It should be mentioned that some present-day deep-sea areas were land during the Neogene (26 to 2.5 my BP), while some land areas were deep sedimentary basins during the same time (Kajoto 1982). This is probably of importance, as there are many faunistic affinities between the islands and the Coastal Forests respectively the Eastern Arc (e. g. *Parodontomelus arachniformis*). The offshore island of Zanzibar is separated from the mainland by a distance of only 35 km and a depth of 70 m, while Pemba island is separated by a distance of 60 km and a depth of 1,000 m. Both Pemba and Zanzibar are thought to be fault blocks like the Eastern Arc (Kent et al. 1971). Pemba is thought to have been separated from the mainland for 6 my, while Zanzibar was connected to the mainland during the Pleistocene (Burgess et al. 1998b).

The location of the Eastern Arc Mountains close to the Indian Ocean is widely accepted as the source of higher rainfall at the eastern flanks of those blocks (Rodgers 1998), supporting the growth of evergreen forests and aridity in the areas west to them. The glacial fluctuations started in Equatorial Africa 2.5 my BP with a first cold and dry period. However, climatic fluctuations probably already existed before. Three major climatic periods during the Pliocene have been identified (Lovett 1993a):

1. 9 to 6.4 my BP the climate was humid and warm – moist forests existed even in north-western Ethiopia
2. 6.4 to 4.6 my BP aridity spread – the Kalahari desert reached the river Congo
3. 4.6 to 2.5 my BP the climate became warm and humid again – with rich riverine forests near Lake Turkana

Jacobs et al. (1999) emphasize that widespread grass dominated environments were found not earlier than in the Pliocene in East Africa. During the Pleistocene at least 21 glacials or near glacial periods occurred since 2.3 my BP (van Donk 1976), but the influence on the vegetation in Africa has not been

completely understood yet. Marine drill-core data suggest that the coastal waters of Tanzania were less influenced by Pleistocene climatic fluctuations (1-2°C) than other oceans (Prell et al. 1980). Such stable temperatures of the Indian Ocean may have supported the high rainfall pattern of the Eastern Arc or in parts of it and the persistence of evergreen forests. This climatic stability is a frequently used argument for the high biodiversity and endemism in the Eastern Arc and the Coastal Forests. Although the southerly Indian Ocean monsoon was still weaker during glacials than during interglacials, the rainfall pattern was possibly still high enough for the existence of forests on the Eastern Arc (Hamilton 1982). In other parts of Africa the glacial fluctuations had a more severe influence on the climate and vegetation. There are presumptions that during wetter periods (interglacials) the forests of the usually separated mountains have been connected through the lowlands, allowing species to disperse. During drier periods (glacials) the populations were separated again and evolved to new species (Rodgers 1998). A striking argument against such a hypothesis is the absence of sympatric congeneric species in most of the mountain blocks (Hochkirch 1998). Interglacial periods with warm and wet climates are thought to have occupied only a relatively small proportion of at least the last 1 million years (Hamilton 1982). According to Cordeiro (1998) global cooling depressed the vegetation belts during the early part of the Pleistocene, which may have served as forest corridors for dispersal between the single mountain blocks. A high rainfall period was documented for the mid-Pleistocene (c. 525,000 y BP, Rossignol-Strick et al. 1998).

Hamilton (1982) presented detailed evidence that the climate was cool and moist before 22,000 y BP with high lake levels. During the last glacial maximum (22,000 to 12,500 y BP) the temperature depression and dry climate led to aridification. At 18,000 BP, the vegetation zones of high mountains were lowered by about 1,000 m and the temperatures depressed by 3-6°C (Hamilton 1982, 1988). However, it must be kept in mind that a changing climatic situation probably does not lead to a simple downward movement of complete plant associations. Each species reacts differently to a changing environment and it is likely that during humid periods lowland forests had a different species composition than montane rainforests or lowland forests of today. Lake Victoria was nearly dry 14,370 y BP and the water levels of Lake Tanganyika and Lake Malawi were 250 to 500 m lower than today (Lovett 1993a). The deserts of Kalahari and Sahara were spreading during this period (Hamilton 1982). On the other hand the high number of endemic species within the lowland forests and the constant water temperatures at the East African coast suggest that the rainfall pattern stayed more stable close to the coast, allowing forest taxa to survive in small refuges (Lovett 1993a). 12,500 to 10,000 y BP the East African lake levels rose again and the wettest postglacial period was dated to 9,000-8,000 y BP, with rainfall 125 to 135% higher than today (Gasse et al. 1990). After minor drier periods with a recession of some lake levels from 8,000 to 6,000 y BP, an obvious switch to a drier climate occurred 4,000 y BP, with a spreading Sahara and lower lake levels (Hamilton 1982, Ambrose & Sikes 1991). Those climatic forces have been accelerated by the influence of man. The use of fire started in the rift valley 1.5 my BP (Clarke & Karoma 2000), and the spread of modern man occurred

40,000 BP, but according to Hamilton (1982) the human influence in montane areas did not start before 2,000 BP, during the time when the Bantu spread and replaced the older Bushman and Hottentot stocks. In the lowlands, however, human beings might have caused bushfires since 150,000 y BP (Clarke & Karoma 2000). During recent times some smaller climatic fluctuations occurred. Based upon the lake level and salinity fluctuations of Lake Naivasha (Kenya) some evidence for a drier climate during the "Medieval Warm Period" (c. AD 1,000-1,270) and a wet period during the "Little Ice Age" (c. AD 1,270-1,850) was presented (Verschuren et al. 2000). The latter period was characterized by glaciations on the high mountains of East Africa (Hamilton 1982). Despite these extensive records of climatic fluctuations, the length of time the Eastern Arc forest blocks have been separated from each other remains virtually unknown (Scharff 1990).

2.2 Climate and Soils of the Eastern Arc

The climate of the tropics is mainly influenced by the seasonal movements of the intertropical convergence zone (ITCZ), which is following the movements of the zenith. Solar heating leads to the convergence of hot air and a high convectional rainfall pattern in the ITCZ (Hamilton 1982). Close to the equator the ITCZ crosses twice a year and consequently two rainy seasons can be found here (e. g. in the East Usambara Mts.). Further south the rainy seasons fuse to just one longer-lasting rainy season (e. g. in the Udzungwa Mts.). Therefore, forest growth is dependent on the relative position of the solar equator (Lovett 1993a). The higher rainfall pattern in the Sahara during the Cretaceous was mainly due to a position further south of Africa, with the equator running through the Sahara (Clarke 2000b). In addition to the influence of the ITCZ, the rainfall pattern is modified by oceanic currents and the positions of mountains and lakes. The currents of the Indian Ocean (South Equatorial current) are quite important for the Eastern Arc, as they bring warm and humid waters to the East African coast, increasing the rainfall close to the coast and at the eastern slopes of mountains (Wasser & Lovett 1993). The Eastern Arc Mountains are known to receive the highest rainfall in East Africa (2,000-3,000 mm per year), but some regions are markedly drier. The high rainfall is the main condition for the existence of evergreen forests. The temperatures are exceptionally cool, with frosts occurring frequently above an altitude of 2,000 m during July and August (Lovett 1998) and exceptionally occurring as low as 1,500 m in the West Usambara Mts. (Moreau 1935). The depression of mean and mean maximum temperatures in those high rainfall areas is due to the cloudiness, which decreases the quantities of incoming radiation (Kenworthy 1966). Apparently some small areas in the Eastern Arc possess exceptionally stable climatic regimes (Fjeldså et al. 1997). Parts of the Usambaras and Ulugurus have an almost per-humid climate (Lovett 1993b).

In the East Usambaras the average yearly rainfall is 2,235 mm at Kwamkoro and 1,919 mm at Amani (Iversen 1991a), but much variation occurs (Phipps 1959). The long rainy season lasts from March to May and a short one from November to December. The long rains provide about 50% of the annual

precipitation (Iversen 1991a). Amani has an annual mean of 162 rainy days (>0,25 mm). The climate is humid or perhumid throughout the year with no month having less than a rainfall of 75 mm (Rodgers & Homewood 1982a). The rainfall decreases towards the lowlands and to the North (Iversen 1991a). The mean humidity is 87% in the morning and 75% at midday. Fog or mists have been recorded on 130 days annually. Within the forest the humidity is rarely lower than 70%, caused by the dense canopy (Rodgers & Homewood 1982a). The East Usambaras have anomalous low average temperatures, with the 20°C isotherm passing Amani at 900 m altitude (in the highlands of Kenya at 1,400 m). The lapse rate reaches 1.7°C / 100 m in the East Usambaras, while the general lapse rate for East Africa has been estimated to be 0.5°C / 100 m (Iversen 1991a). The mean annual temperature at Amani is 20.6°C. The mean daily maximum is 24.9°C, the mean daily minimum 16.3°C (Iversen 1991a).

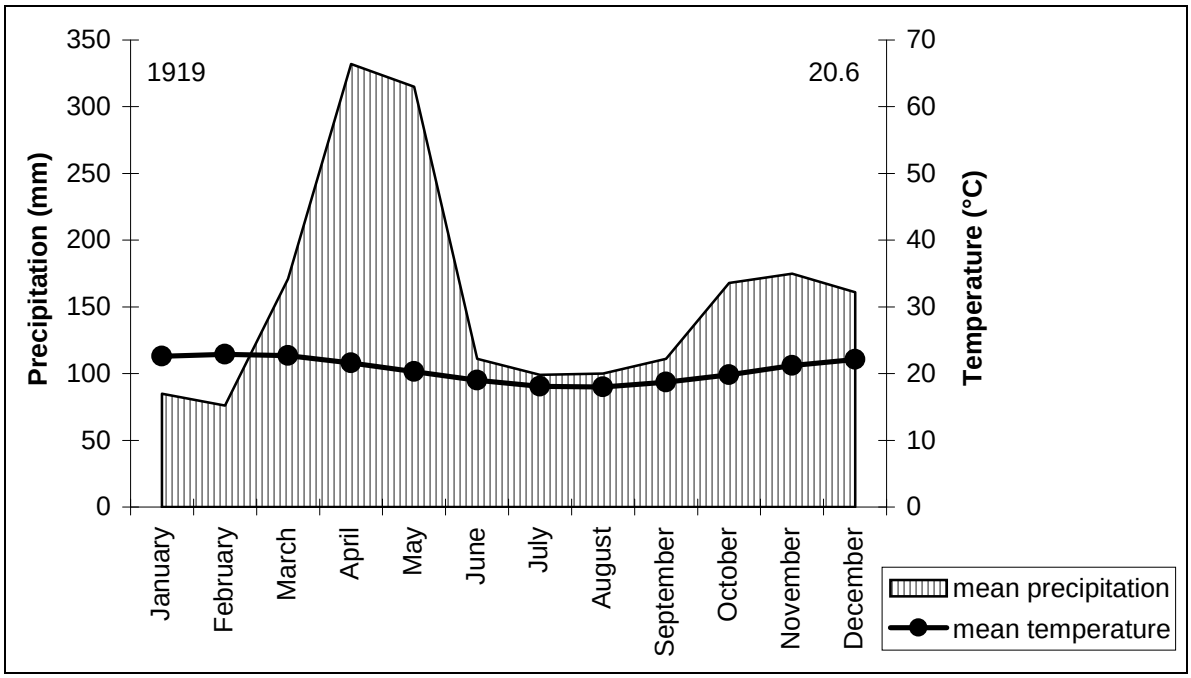


Fig. 2: Climatic diagram for Amani, East Usambara Mts. (1902-1982, Data: meteorological station Amani)

The exact climatic data for the other regions are not available. The southern parts of the visited mountains (Ulugurus, Rubehos) lie in the transition zone of unimodal and bimodal rainfall pattern. The Uluguru Mountains receive two annual peaks of rainfall with the rainy seasons lasting from March to May and from October to November (Nummelin & Nshubemuki 1998). The annual rainfall of the eastern slopes of the Ulugurus exceeds 3,000 mm, while the arid land in the rain shadow receives less than 600 mm rain per year (Wasser & Lovett 1993). The estimated annual rainfall for the western slopes of Uluguru North Catchment Forest Reserve is 1,200-3,100 mm, for the eastern slopes 2,900-4,000 mm. The dry season is not marked. Average temperatures vary from 17°C in July to 22°C in December (Lovett & Pócs 1993). Morogoro, situated at the northern edge of the Ulugurus receives a rainfall of about 800 mm a year, falling mainly between March and May, and sometimes there is a substantial amount in November.

The total annual rainfall at the foot of the Nguru Mountains, as reflected by the Mtibwa Sugar Company rainfall data, is 1,206 mm with a considerable variation from one year to the next (Lovett 1996). The bimodal pattern peaks in April and December. The Nguru South Catchment Forest Reserve near Turiani probably receives higher rainfall due to its higher elevation (the Mtibwa Sugar Company is located in the plains east of the Nguru Mts.). The estimated annual rainfall varies from 2,100 mm to 4,000 mm at an altitude of 2,000 m. The dry season is not marked on the eastern side. Mean annual temperatures vary with the altitude from 12-24°C (Lovett & Pócs 1993).

Kilosa is situated between the Rubeho and the Ukaguru Mountains on the Mkondoa River. The parts south of the Mkondoa River belong to the Rubeho Mountains. Kihiliri Catchment Forest Reserve, 3 km south of Kilosa town, is estimated to receive an annual rainfall of 1,050 mm. The dry season lasts from June to October and the mean temperatures vary from 21°C in July to 26°C in December (Lovett & Pócs 1993).

In the Udzungwa Mountains the rainfall is unimodal in pattern, starting in November with a peak in April and ending in June (Wasser 1993). The climate is influenced by the November-May Southeast monsoon winds. High altitudes above 1,600 m receive more rain than the lower slopes (Shangali et al. 1998). The rainfall in that area ranges from 1,800 to 2,000 mm per year (Rodgers & Homewood 1982b) and sometimes reaches over 3,000 mm per year in wetter areas (Shangali et al. 1998). The northern mountain blocks Taita and Pare are much drier and resemble the Kenyan Highlands in ecoclimatic parameters (Fjeldså et al. 1997). The Taita Hills receive an annual rainfall of 1,329 mm (Brooks et al. 1998).

The well-drained soils of the Eastern Arc are derived from crystalline gneissic rocks. Within the mountain chain one type is rather similar to the other. Thus the description focuses on the East Usambara Mountains. The soils of the East Usambaras are described as red laterite (Phipps 1959) and belong to the humic ferrisols according to the FAO soil classification scheme (Rodgers & Homewood 1982a). In the valleys, grey-black sandy clay soils occur, which are quite deep and fertile. On the escarpments the soils are younger, less leached and more fertile than the higher plateau soils (Iversen 1991a), which are very acidic and highly leached (Hamilton 1989a). According to Hamilton (1998) a remarkable drop in acidity from about pH 6.5 at 850 m to below pH 5.0 at 900 m and pH 4 at 1,050 m occurs, while the pH declines only gradually from pH 7 at 300 m to pH 6.5 at 850 m. This seems to be caused by the typical position of the cloud cover at 850 m, which also might be the reason for the high temperature lapse rate. A thick (10 cm) upper layer of mor-type humus can be found underneath the litter at higher altitudes, which cannot be found in the lowland forests (Hamilton 1998).

2.3 Vegetation of the Eastern Arc

The vegetation zones of the Eastern Arc largely follow the altitudinal and moisture gradients (Lovett 1993c). The floristic variation with the altitude is continuous, without any abrupt changes (Hamilton 1998) and the forests change in a similar way with the altitude on the mountains of eastern Tanzania (Lovett 1996). The highest mountain elevation within the Eastern Arc is the Kimhandu peak in the Uluguru Mts. (2,635 m altitude). From this height down to 2400 m elfin woodlands, montane grasslands, heaths and bogs can be found (Burgess et al. 1998a, Lovett 1998). Forests naturally extend from 2400 m down to the lowlands, but many have been cleared at lower slopes. These forest formations are divided into three zones, including the upper montane (2,400-1,800 m), the montane (1,200-1,800 m) and the sub-montane (800-1,200 m) forests (Pócs 1976). At lower altitudes the forest vegetation grades into that of the lowland Coastal Forests, which occur fragmented along the eastern seaboard of Africa (Burgess et al. 1998b). In addition to the altitudinal changes of vegetation, the different rainfall patterns influence the forest structure. Lovett (1993b) therefore also distinguishes dry and wet forest types with dry montane forests above 1,500 m (with an annual rainfall of 1,000 to 1,200 mm), upper montane forests (altitude: >1,800 m, rainfall: >1,200 mm), montane forests (1,200-1,800 m, >1,200 mm), submontane forests (800-1,400 m, >1,500 mm), lowland forests (<800 m, >1,500 mm) and dry lowland forests (<800 m, 1,000-1,500 mm). The lowlands between the southern parts of the mountains are mainly covered by miombo woodland (composed of the tree genera *Brachystegia*, *Isoberlinia* and *Julbernardia*), receiving an annual rainfall of 750-1,000 mm with a long dry season in between (Hamilton 1982). The driest montane forest type is the *Shume-Juniperus* forest in the West Usambaras (Lovett 1993c). The cloud forests at high elevations on wetter ridge-tops are characterized by low canopy and large quantities of epiphytes, herbs, mosses and ferns. The wettest upper montane forest type is the northern Uluguru cloud forest (Lovett 1998). The upper montane forests of the Udzungwa and Rubeho mountains seem to be secondary, having been cleared for cultivation within the last few hundred years (Lovett 1993b). Since the general vegetation zones are quite similar within the Eastern Arc (Shangali et al. 1998), a more detailed description is given only for the East Usambaras.

Early descriptions of the vegetation of the East Usambaras give the impression that large parts were forested (Engler 1903), including even the higher populated Mt. Mlinga. At steep slopes and higher altitudes heathlands, grasslands, bushes and open rocks were more common. In the northwestern part of the East Usambara plateau grassy areas with patches of forest have been reported. Particularly near the villages grassland occurred, possibly caused by burning (Iversen 1991a). Due to the lower elevation of the East Usambara Mts., the submontane forest is the main forest type of the plateau – an exception within the Eastern Arc. Iversen (1991b) distinguishes five forest types for the East Usambaras: Lowland evergreen forest up to 750 m, dry lowland evergreen forest, occupying smaller patches south and north of the mountain range, submontane evergreen forest between 750 and 1400 m

altitude, ericaceous shrub forest on exposed and xerophytic summits and ridges and riverine forests at low altitudes along the Zigi river. Four types of grassland are distinguished (Iversen 1991a): grassland and wooded grassland, *Pteridium* heaths, swamps and rocky outcrops at the steepest parts of the mountains. It is still a matter of controversy, whether grasslands naturally occur on the mountains or whether they are anthropic in origin. At least there is palynological evidence for the dominance of montane grasslands in the southern African mountains since the last glacial maximum (Meadows & Linder 1993). Nowadays induced vegetation can be found in large parts of the Eastern Arc, including plantation forests (e. g. *Pinus spp., Eucalyptus grandis, Grevillea robusta, Maesopsis eminii, Tectona grandis, Terminalia ivorensis* and *T. superba*) and cultivated areas (tea, sugarcane, cardamom, banana, maize, coffee, cassava, sweet potato, pineapple, cocoyam).

2.4 Endemism within the Eastern Arc

The high degree of endemism of the Eastern Arc is well documented (Verdcourt 1968, Myers et al. 2000). White (1978) mentions, that specific endemism of individual mountains of tropical Africa is comparatively low, but exceptionally high in the Usambaras, Ulugurus and Ngurus. About 25% of the c. 2,000 plant species occurring in the Eastern Arc are endemic (Lovett 1988). A total of 66 trees (> 20 cm dbh) are strictly endemic to the Eastern Arc (Lovett 1998). According to Pócs (1998) 700 bryophyte species occur in the Eastern Arc, including 32 endemics (4.6%). Burgess et al. (1998a) list 74 vertebrates, which are strictly endemic to the Eastern Arc, including ten birds, eleven mammals, 23 reptiles and 30 amphibians. While rodents are broadly distributed across the Eastern Arc, five shrew species may be endemic (Stanley et al. 1998). Howell (1993) lists 82 amphibian and reptile species for the Eastern Arc, including 54 (65.8%) endemic species. 82% of the linyphiid spiders are endemic to the Eastern Arc and the single-site endemism varies from 58% to 86% (Scharff 1993), while Mt. Elgon and Mt. Kenya have only 25% respectively 33% endemics,. Burgess et al. (1998a) mention an endemism of 95% for the carabid beetles of the Uluguru Mts., but this is based on older taxonomic studies. Of the 89 butterfly species recorded from the Eastern Arc, 56 species (62.9%) are endemic (De Jong & Congdon 1993). The mollusc faunas of the East Usambaras (94 species) and the Ulugurus (77 species) belong to the richest land snail faunas worldwide with 57 species in Mbomole forest (Tattersfield et al. 1998). According to Myers et al. (2000) the Eastern Arc / Coastal Forest hotspot contains the highest density of endemics worldwide (75 plant species / 100 km^2).

Nearly all endemics are closed-forest specialists, but some prefer montane grasslands (Burgess et al. 1998a). According to Lovett et al. (in press) restricted forest tree taxa occur in all altitudes of the Eastern Arc, but mostly in narrow elevational bands. Wetter forest types contain more endemic plant species and the submontane forests are the richest in endemic species of large trees (Lovett 1998). The degree of endemism also varies between the single mountain blocks. The wettest mountains with the most endemics are the East and West Usambaras, the Udzungwas and the Ulugurus, while the

drier areas, like the Rubehos, Ukagurus, Ngurus and Pares are poor in endemics (Burgess et al. 1998a). It is believed that the wettest mountains had a more stable climate during the Pleistocene (Fjeldså & Lovett 1997) and served as staging areas for dispersal (Cordeiro 1998).

The endemics of the Eastern Arc are divided into palaeoendemics (relicts, which survived in a limited portion of the former range) and neoendemics (rapidly evolving species complexes with closely related taxa in the same or nearby region; Lovett 1993b). Among the palaeoendemics, some genetically ancient birds may be from lineages stretching back to the Miocene c. 30 my BP (Fjeldså & Lovett 1997). The endemic *Xenoperdix udzungwensis* has Indo-Malayan affinities (Dinesen et al. 1994). However, recent radiations seem to dominate (Fjeldså & Lovett 1997), indicated by DNA data on the bird genus *Andropadus* (greenbuls) and the plant genus *Saintpaulia* (African violets) (Roy 1997, Lindqvist & Albert 1999).

According to Iversen (1991b) the degree of real endemism in the Usambara Mts. is lower than usually thought (6% for the whole Usambaras, 3% for the East Usambaras). He assumes that isolation has been relatively short or not fully effective. A number of plants also occur on other mountains or in the Coastal Forests. The affinities to the Coastal Forests are generally high within the Eastern Arc, particularly in the East Usambaras and the Ulugurus, which are situated close to the coast. Some authors, therefore, regard the lowland forests of the Eastern Arc as Coastal Forests (Lovett 1998).

Tab. 2: Species diversity and endemism in the Usambara Mountains and in the Uluguru Mountains for different groups; E = East Usambaras, EW = East and West Usambaras, U = Ulugurus, - = Data not available, * = Figure includes subspecies, + = Figure includes "near endemics."

Group	Area	Species	Endemics	Forest species	Forest endemics
Trees (>5 m)* (Iversen 1991b)	EW	684	42 (6.1%)	-	41
Vascular plants* (Iversen 1991b)	EW	2855	169 (5.9%)	1627	157 (9.7%)
Bryphyta (Pócs 1998)	EW	464	11 (2.4%)	-	-
Mammals (Rodgers & Homewood 1982)	EW	55	2 (3.6%)	-	-
Reptiles (Howell 1993)	EW	-	-	29	6 (20.6%)
Amphibians (Howell 1993)	EW	-	-	23	4 (17.4%)
Gastropoda* (Rodgers & Homewood 1982)	EW	122	55 (45.1%)	-	-
Linyphiidae (Scharff 1993)	EW	12	6 (50.0%)	7	6 (85.7%)
Butterflies* (De Jong & Congdon 1993)	EW	37	15 (40.5%)	-	-
Vascular plants* (Iversen 1991b)	E	2083	64 (3.1%)	-	64
Birds*+ (Rodgers & Homewood 1982)	E	219	5 (2.2%)	100	5 (5.0%)
Land snails+ (Tattersfield et al. 1998)	E	94	12 (12.8%)	-	-
Diplopoda (Hoffman 1993)	E	41	5 (12.2%)	37	5 (13.5%)
Sphecidae (Rodgers & Homewood 1982)	E	131	27 (20.6%)	74	27 (36.5%)
Grasshoppers (own data)	E	63	13 (20.6%)	36	10 (27.8%)
Birds (Stuart et al. 1993)	U	-	-	41	2 (4.9%)
Reptiles (Howell 1993)	U	-	-	24	6 (25.0%)
Amphibians (Bhatia & Buckley 1998)	U	-	-	26	6 (23.1%)
Diplopoda (Sørensen 1995)	U	28	23 (82.1%)	-	-
Linyphiidae (Scharff 1993)	U	17	12 (70.6%)	14	12 (85.7%)
Butterflies (De Jong & Congdon 1993)	U	37	10 (27.0%)	-	-
Grasshoppers (own data)	U	48	13 (27.1%)	29	13 (44.8%)

Discrepancies in the assessments of endemism are mainly due to different definitions of the term "endemic." As Anderson (1994) points out, the term "endemic" is used in rather different ways. Unless an area of endemism is not exactly defined, it is useless to argue with the term. These problems become obvious, if the summary of biodiversity and endemism of the Usambara Mountains presented by Rodgers & Homewood (1982a) is analysed. The comparison of different taxa includes so-called "near endemics" without any clear definition. For some taxa subspecies are included, for others the West and East Usambara fauna is combined or even only forest species are regarded. Consequently endemics should be defined as restricted to a single mountain block. An analysis of the East Usambara grasshopper fauna showed that affinities to the Coastal Forests near Tanga are higher (16 species; 25.4%) than the number of endemics (13 species; 20.6%, Hochkirch unpubl.). Hawthorne (1993) mentions that the foothills of the East Usambara Mountains share many species with coastal forests, including a number of endemics. This is probably caused by the small distance between the mountains and the coast (40 km). In contrast to the East Usambaras the species of the West Usambara Mountains and of most other Eastern Arc mountains are more closely restricted to the mountain area, since they are surrounded by dry grassland or miombo woodland. Table 2 shows the number of endemic taxa for the Usambara Mts. according to literature and own data.

In comparison to vascular plant genera of the Usambaras (Iversen 1991b), grasshoppers of the East Usambaras have no cosmopolitan or pantropical genera, which make up 34% of the plant genera. The number of genera endemic to East Africa is 26% in grasshoppers and most of them are endemic to the Coastal Forests and the Eastern Arc, while this proportion is only 4% in vascular plants (Hochkirch unpubl.). At species level the degree of endemism in the East Usambaras also is higher (21%) than in plants (3.1%), birds (2.2%) and mammals (0%), but lower than in Diplopoda (76%). The vicariant distribution of many grasshopper genera and the high degree of endemism is mainly due to the small ranges of flightless forest species (Hochkirch 1998). If only the flightless species of the East Usambaras are regarded, the percentage of grasshopper species occurring in no other of the Eastern Arc Mts. is 71.4%.

A number of authors stress the significance of geographical separation of the mountain blocks and their climatic stability within periods of drought for the biodiversity and endemism of the Eastern Arc (e. g. Rodgers 1998). However, the degree of endemism is influenced by many different interacting effects (Anderson 1994). The geographical level includes the age of an area, its dimensions, the distance to source areas (i. e. other forests), the distance to the ocean (climatic stability), latitude, altitude, barriers and geographical separation. The climatic level includes seasonality and stability. The ecological level includes the age of the habitat type, the habitat persistence and its diversity. The taxon level includes factors depending on the evolutionary history of the taxon such as life history, vagility, ecological tolerance, abundance, minimal range, minimum viable population size and also body size as it influences the minimum viable population size and vagility. These interacting factors either influence the spatial level (degree of separation) or the temporal level (time of separation).

2.5 Conservation of the Eastern Arc

The human influence on the forests of the Eastern Arc started probably with the use of fire. Charcoal layers in the soil of the East Usambaras illustrate that fire influenced the forests quite early. The earliest stone-bowl cultures (Azanian culture) were recorded from the early Iron Age, 2,000 y BP in the Usambaras, Pares and Taita Hills (Newmark 1998). Forest clearance began at approximately the same time in Rwanda and Burundi, some 2,300 y BP (Jolly et al. 1997). A later wave of village settlements in the East Usambaras was reported for the time from 900-1,000 AD, but these vanished later (Rodgers 1993). Most tribes in Tanzania have some traditionally protected forests, which are often referred to as "sacred forests." In the North Pare Mountains the traditionally protected forests are almost the only remaining natural forests (Mwihomeke et al. 1998). Recently settled tribes, like the Waluguru, who moved into the hill forests of the Uluguru Mts. less than 200 y BP, are characterized by only little traditional anti-erosion works in comparison to earlier settled tribes like the Wasambaa in the Usambara Mts. (Rodgers 1993).

At the end of the 19th century the first Europeans found large forested areas in the East Usambaras, but some amounts of deforestation were already noted (Engler 1903). Under German rule (1885-1916) the pressure on the forest increased due to colonial settlements at climatically preferable higher altitudes (Rodgers 1993). Deforestation was accelerated in order to plant tea and coffee and the developing infrastructure and agriculture attracted people from the lowlands (Rodgers & Homewood 1982a). However, this also led to a rapid reservation of forests. Some 80% of the present forest reserves within the Eastern Arc were gazetted during this period (Rodgers 1998), but these reserves also included plantations of induced vegetation. The biological and agricultural research station at Amani and the Amani Botanical Garden were founded in 1902 by German scientists. One of the trees planted on larger scale was *Maesopsis eminii*, which soon spread into deforested areas and is now causing problems for conservation (Binggeli 1989). Until 1914, a total of 231 forest reserves had been established, covering 7,500 km^2 of mainly montane and costal forests. The British administration (1916-1961) enlarged the number of forest reserves in the 1930s and 1950s, so that in 1961 some 9,500 km^2 of natural closed forest was protected (Rodgers 1993). The "Uluguru Land Usage Scheme" (ULUS) was established in the mid 1940s to stop erosion, and in the 1950s bench terracing was introduced in the Ulugurus (Bhatia & Buckley 1998). In the 1950s the attention to forest production increased in forest policy leading to plantations of fast growing exotic softwoods. Since the independence, Tanzania has increased the forest reserves to 13,700 km^2 (1985), but 605 km^2 have been degazetted, including 120 km^2 of valuable natural forest in the West Usambara Mountains (Iversen 1991a, Rodgers 1993). Due to the high annual population growth in Tanzania ranging from 2.8% to 3.2%, or even 6.5% in the Ulugurus (Lulandala 1998), the increasing demand for land led to high deforestation pressure (Rodgers 1998). Polhill (1968) already noted the high population in the East Usambaras and the need for conservation. While during the 1950s and 1960s the forest was

cleared mainly for agriculture, timber exploitation was the main cause for the loss of forest during the 1970s and 1980s, supported by the Finnish developmental organization FINNIDA (Chachage 1998). In the Usambaras c. 50% of the public land forest disappeared between 1954 and 1978 (Rodgers & Homewood 1982a).

A new recent wave of conservation actions started with the "4th East African Wildlife Symposium" in 1978, in which a section of forest conservation was included. The "Tanzania Forest Conservation Group" was established in 1983 (Rodgers 1998). In the mid 1980s the Tanzanian government realized the necessity of a natural forest cover for maintaining water, soil and climatic resources (Rodgers 1993). The "Tanzania Forest Action Plan" (TFAP) in 1986 included the Eastern Arc as key ecosystem conservation project (Lovett 1998). In 1987 the IUCN (International Union for Conservation of Nature) started the "East Usambara Conservation and agricultural Development Project" (EUCODEP) in the East Usambara Mts. and the FINNIDA started to focus on conservation of reserves in the "East Usambara Catchment Forest Project" (EUCFP) (Ningu pers. comm.). In the Uluguru Mountains the "Uluguru Mountains Agricultural Development Project" (UMADEP) and the "Uluguru Slopes Planning Project" started in the 1990s (Bhatia & Buckley 1998). The "Udzungwa National Park" was gazetted in 1992, covering an area of 1,990 km^2 and an altitude ranging from 250 m to 2,500 m (Iddi 1998). In 1997 the "Amani Nature Reserve" was gazetted, which covers some formerly smaller forest reserves and the Amani Botanical Garden (Iddi 1998).

Tab. 3: Forest cover in the some blocks of the Eastern Arc Mountains (according to Newmark 1998)

Mountain block	Natural forest	Nr. of main forest patches	Closed forest	Loss of forest cover
East Usambara	413 km^2	8	221 km^2	57%
Nguru	647 km^2	8	120 km^2	82%
Ukaguru	184 km^2	1	100 km^2	90%
Rubeho	499 km^2	6	100 km^2	37%
Uluguru	528 km^2	5	120 km^2	65%

At present, there is still a need for conservation measures. According to recent estimates by Newmark (1998) the Eastern Arc total forest area covers approximately 5,327 km^2 and is highly fragmented and disturbed (table 3). Over the last 2,000 years 77% of the original forest cover has been lost – most of it during the last 100 years. The estimate for the closed forest cover is 1,497 km^2, which is 27% of the remaining forest (Newmark 1998). According to Lovett (1998) the submontane forests in the Usambaras have been largely replaced by plantations or disturbed by logging, in the Ulugurus they have been mostly lost to cultivation, but some are left at steep slopes of the Ngurus. The lowland forests of the East Usambaras have been partly replaced by teak plantations (Rodgers 1993). In the Ulugurus, shifting cultivation has converted most of the mountain slopes below the gazetted forest reserves into treeless grassland, and erosion is still a severe problem (Fjeldså et al. 1995). In 1993 hundreds of tonnes of mud flooded the Morogoro Municipality. Moreover, each year fires destroy

extensive areas (Lulandala 1998). At present c. 150 forest reserves or forest patches in nature reserves and national parks are left (Lovett 1998). In the East Usambaras c. 32,000 ha (76% of the forest left) are protected in 13 forest reserves and the Amani Nature Reserve, but poles and timber are still extracted from the forest (Johansson et al. 1998). Most parts of the Eastern Arc forests still suffer from logging, pit-sawing, fuelwood collecting and hunting due to inadequate legal protection, field protection and management (Rodgers 1993). This is probably also caused by the high level of negative or neutral attitudes to protected areas by local people in Tanzania (Newmark et al. 1993). In the Udzungwa Scarp Forest Reserve the main human activities include tree felling, animal hunting and trespassing (Zilihona et al. 1998).

3 The Study Object

The choice of the right taxon for a phylogenetic analysis concerning the Eastern Arc is influenced by several aspects, such as (a) the vagility of the group, (b) the degree of endemism, (c) the dependence on forests, (d) the taxonomic knowledge, (e) the number of species or separated populations within a species and (f) the abundance of the species in the area of research.

a) The vagility is of importance to avoid the influence of long-distance dispersal (Brühl 1997). The vagility of grasshoppers is mainly affected by the presence of wings. Most tropical forest species are flightless and thus have an extremely low vagility (Hochkirch 1998).

b) Flightlessness is also the main reason for the small ranges of forest grasshoppers (Hochkirch 1998). Most of the forest species are restricted to single mountain blocks, but some East Usambara species also occur in the Coastal Forests (see above).

c) Nearly all endemic grasshopper species of the Eastern Arc are forest specialists, although some Eastern Arc endemic birds and butterflies are confined to montane grasslands and heathlands (Burgess et al. 1998a). However, many of the grasshoppers are not restricted to the forest interior, but inhabit clearings, paths, forest edges, or disturbed forest types (Hochkirch 1995, 1996a).

d) The taxonomic knowledge of East African Acridoidea has strongly increased during the last century (Ritchie 1987, Green 1998) and a handbook on East African grasshoppers is in preparation (Jago pers. comm.). Although new species can still be found even at such well-collected places as the East Usambaras (Hochkirch 1996b), and some huge genera are in need of revision, the taxonomic knowledge is far greater than in many other insect groups.

e) Since the intention of this study was to present a broad overview of a typical group, including mtDNA data, morphometric data, behaviour records and ecological records, the taxon under study should not contain too many species. A higher number of species would also enlarge the number of study sites and thus cause logistic problems, since not all places in Tanzania are easy to reach with public transport.

f) Many species of tropical rainforests occur only in small abundance, which might influence the number of records. Grasshoppers of forest edges usually reach a higher abundance than species within the forest (Hochkirch 1996a).

3.1 Eastern Arc Grasshopper Faunas

The choice of the right grasshopper taxon for a phylogenetic analysis requires some information on typical Eastern Arc grasshopper faunas. A list of typical genera is given in table 4.

Table 4: Typical grasshopper genera of the Eastern Arc with habitat, number of species (+: number of additional subspecies; ?: number uncertain, awaiting revision), distribution type (C: Coastal Forests, EA: Eastern Arc, TN: Tanganyika-Nyasa Mountain Forest Group (incl. Eastern Arc), GC: Guinean-Congolian Forest block, K: Kilimanjaro area: young volcanic mountains, V: Lake Victoria basin), availability (based on habitat or abundance) and last taxonomic revision (in need: in need of revision, De: Descamps, Di: Dirsh, Gre: Green, Gru: Grunshaw, H: Hochkirch, Ja: Jago, Jo: Johnsen, K: Kevan, U: Uvarov)

Genus	Habitat	Spec.	Distribution	Avail.	Revision
Euschmidtia KARSCH, 1889	arboricolous	13	TN – C – GC	bad	De 1973
Chromomastax DESCAMPS, 1964	arboricolous	5	C – EA	bad	De 1973
Stenoschmidtia DESCAMPS, 1973	arboricolous	5	C – EA	bad	De 1973
Mastarammea DESCAMPS, 1977	arboricolous	1	TN	bad	De 1977
Pieltainidia RAMME, 1925	arboricolous	1	East Usambaras	bad	De 1977
Plagiotriptus KARSCH, 1899	arboricolous	7	TN – C – K – V	bad	Jo 1986
Acanthothericles DESCAMPS, 1973	arbusticolous	2	Ulugurus	bad	De 1977
Dimorphothericles DESCAMPS, 1977	?arbusticolous	2	Ulugurus	bad	De 1977
Loveridgacris REHN, 1954	terricolous	1 + 1?	C – EA	bad	K 1977
Ixalidium GERSTÄCKER, 1869	terricolous	? 8	C – EA – GC	good	in need
Burttia DIRSH, 1951	herbicolous	1	Ulugurus	good	Di 1951
Aresceutica KARSCH, 1896	herbicolous	? 3	EA – K	good	in need
Rhainopomma JAGO, 1981	herbicolous	5	EA – C	good	Ja 1981
Physocrobylus DIRSH, 1951	herbicolous	2	EA	bad	H 1996b
Parodontomelus RAMME, 1929	graminicolous	6	EA – C	bad	H 1999b
Parepistaurus KARSCH, 1896	herbicolous	20 + 4	TN – C – K – V	good	Gre 1998
Kassongia I. BOLÍVAR, 1908	herbicolous	6 + 1	C – TN – V	bad	Gru 1986
Afrophlaeoba JAGO, 1983	graminicolous	4	EA	good	Ja 1983
Oxyaeida I. BOLÍVAR, 1914	herbicolous	3	C – EA – V	good	Ja 1994b
Gymnobothroides KARNY, 1915	graminicolous	6 + 2	C – EA – K – V	good	Ja 1968
Paraspathosternum RAMME, 1929	graminicolous	1	C – EA – V	good	Gru 1988
Phaeocatantops DIRSH & UVAROV, 1953	herbicolous	16	Ethiopian	good	Ja 1982
Eupropacris WALKER, 1870	arbusticolous	? 19	Ethiopian	bad	in need
Heteracris WALKER, 1870 (*pulchripes*-group)	arbusticolous	9	Ethiopian	good	Gru 1991
Phyteumas I. BOLÍVAR, 1904	arbusticolous	3	TN – C – K – V	med.	K 1977
Cyphocerastis KARSCH, 1891	?	10	GC – EA	bad	Jo 1987
Anischnansis DIRSH, 1959	?	1	East Usambaras	bad	Di 1959

Twenty-seven grasshopper genera can be regarded as typical Eastern Arc elements, although the transition to Coastal Forest elements is fluent. Their suitability for a phylogenetic analysis, however, is not similar. Genera in need of revision are not suited, since a taxonomic revision of a genus was not the intention of this thesis. Eumastacoids (the first eight genera) are taxonomically badly known. Some species have been described on the basis of single males, others on the basis of single females. Fully winged genera (the last six genera) are too vagile and do not serve for the purpose as well, since they might be long-distance dispersers. Some genera (*Loveridgacris*, *Burttia*, *Gymnobothroides*, *Paraspathosternum, Anischnansis*) contain only one or two species. Others (*Parepistaurus*) consist of a very high number of species with several subspecies or separated populations, which would be beyond the scope of this thesis. Some genera are difficult to collect, since the populations of the

species are usually small or difficult to locate (*Kassongia, Parodontomelus, Physocrobylus*). Other genera are arboricolous (Eumastacoidea). In the end only two genera seem to be suitable for this study: *Rhainopomma* and *Afrophlaeoba*. Both have four or five allopatric species and both have been revised recently (Jago 1981, 1983). They usually occur in high abundances and have exceptionally small ranges (with the exception of *Rhainopomma usambaricum*, which is also present in the Coastal Forests and in the Shimba Hills, and *Afrophlaeoba longicornis* which has been recorded from the Rubeho Mts. and the West Usambara Mts.).

3.2 Why *Afrophlaeoba*?

A direct comparison of *Afrophlaeoba* and *Rhainopomma* leads to the conclusion that the former genus has several advantages. First of all, the whole *Afrophlaeoba* genus group (sensu Popov in press) seems to be restricted to evergreen forest regions, such as the Coastal Forests, the Eastern Arc, the Tanganyika-Nyasa Forest block, the Lake Victoria basin and Madagascar (Jago 1983, Popov in press). A close relative genus, *Parodontomelus*, has several endemic species in the Eastern Arc and in the Coastal Forests of Tanzania. The sympatric occurrence of a potential outgroup (*P. arachniformis*) with *A. usambarica* in the East Usambaras is of high value for direct ecological comparison. All members of the *Afrophlaeoba* genus group are flightless, which is actually a good argument for future extension of this study on the whole group. In comparison to *Afrophlaeoba* some relatives of *Rhainopomma* are known to occur in the drier parts of Tanzania. The comparatively wide distribution of *Rhainopomma usambaricum* suggests a broader ecological niche for this species.

The choice of *Afrophlaeoba* also offers the opportunity to test Jago's (1983, 1994a) hypothesis that the morphologically very similar genus *Odontomelus* belongs to a completely different group (Pargaini sensu Popov in press). This genus would offer another suitable outgroup for the study, since it is rather widespread in Eastern Africa. *Odontomelus* also contains several flightless species with limited ranges. Some of them are endemic to the drier parts of the Eastern Arc (Pare Mts., Nguru Mts.), to the Coastal Forests, Kilimanjaro or the Lake Victoria basin. Jago (1983, 1994a) assumed that the *Afrophlaeoba* genus group consists of ancient relicts, while *Odontomelus* is a recent radiation. This hypothesis might be proved with the help of mtDNA markers.

The combined approach does include studies on the habitat requirements of the species and on the courtship behaviour. While *Rhainopomma usambaricum* has been subject of ecological and ethological studies (Hochkirch 1995, 1999a), the knowledge on *Afrophlaeoba* is low. Hence, the study of *Afrophlaeoba* would add something new to the ecological information of the Eastern Arc grasshopper fauna, which is of importance for a better understanding of its biodiversity and for conservation measures (Rodgers and Homewood 1982a). *Rhainopomma* belongs to the family Lentulidae, a primitive Caeliferan group, without tympana, wings and wing muscles. Its courtship behaviour is less pronounced than that of *Afrophlaeoba*, which belongs to the rather modern group of

Acridinae. Jago (1983) already made some remarks on the courtship behaviour of *Afrophlaeoba* and *Parodontomelus*: "*P. arachniformis* uses elaborate semaphoring of the hind femora following careful cleaning of the antennae, while *A. usambarica* uses rapid vibration of the femora and body without antennal cleaning, probably transmitting signals through the substrate." This statement suggests that the species is interesting for studies on communicative behaviour. Finally, the decision was also influenced by the practical experience. During a first trip to Tanzania all *Afrophlaeoba* species were found without difficulty and in adequate abundance for a comprehensive sample. These preliminary results encouraged the continuation of the study on *Afrophlaeoba*.

3.3 History of the genus *Afrophlaeoba*

The systematic relationships within the subfamily Acridinae are poorly understood (Jago 1983). A recent phylogenetic analysis, based on mtDNA data, suggests that African Acridinae are monophyletic (Rowell pers. comm.). Based on the anatomy of the epiphallic plate, Jago (1983) distinguished two major groups, which he called "*Phlaeoba* genus group", including the Phlaeobinae (sensu Dirsh 1975) and the "*Parga* genus group", including the Gymnobothrinae and Pargainae (sensu Dirsh 1975). While in the *Parga* genus group the epiphallic lophi form slender hook-liked structures, they form broad lobate structures in the *Phlaeoba* genus group. Jago (1983) stated that the *Phlaeoba* genus group consists of small (often monotypic) genera with small ranges in forested areas, while in the *Parga* genus group the genera are large and widespread in grasslands or dry woodlands. The latter is particularly true for the genus *Odontomelus*, of which Jago (1994a) suggested that it consists of recently evolved species. Popov (in press) established the tribes Phlaeobini, Pargaini and Gymnobothrini within the Acridinae. He distinguished five genus groups within the Phlaeobini, including an "*Afrophlaeoba* genus group" containing the following genera:

1. *Afrophlaeoba* JAGO, 1983 (Eastern Arc Mts., Tanzania);
2. *Brachyphlaeobella* JAGO, 1983 (W Uganda);
3. *Chlorophlaeobella* JAGO, 1983 (Madagascar);
4. *Chokwea* UVAROV, 1953 (Tanganyika-Nyasa Mountain Group);
5. *Chromochokwea* JAGO, 1983 (Ufipa Plateau, Tanzania);
6. *Parodontomelus* RAMME, 1929 (Coastal Forests and Eastern Arc Mts. of Tanzania and Kenya);
7. *Paralobopoma* REHN, 1914 (Lake Victoria basin);
8. *Platyverticula* JAGO, 1983 (Zambia, SW-Tanzania, Somalia);

The genus *Afrophlaeoba* was erected by Jago (1983) for *Odontomelus usambaricus* RAMME, 1929 which was described from some specimens collected in 1905-1907 at Amani in the East Usambara Mts. by J. Voßeler, the former Zoologist of the Amani Biological Research Station. Jago (1983) transferred the species to his *Phlaeoba* genus group (now: Phlaeobini sensu Popov in press) based on the epiphallic morphology and described three more species: *A. nguru*, *A. euthynota* and *A. longicornis*. Dirsh (1965) missed the epiphallic differences between *Odontomelus* and *Afrophlaeoba*, since he studied *Afrophlaeoba usambarica* as a typical *Odontomelus*, instead of the type species *Odontomelus brachypterus* (GERSTÄCKER, 1869) (Jago 1983).

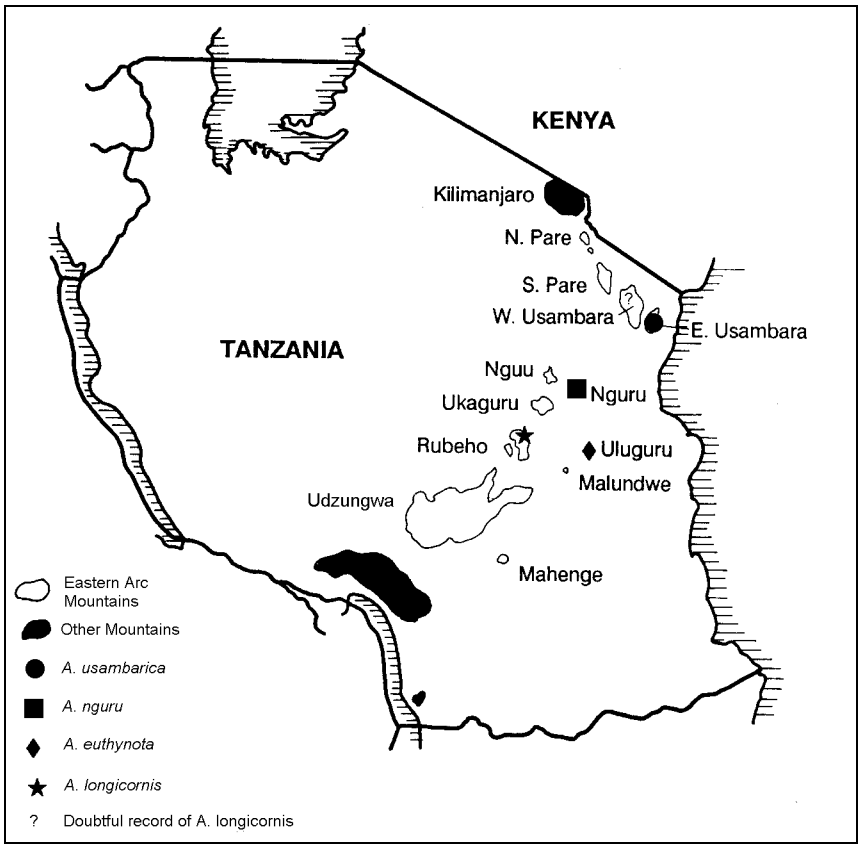


Fig. 3: Present knowledge of the distribution of the *Afrophlaeoba* species.

A species list with localities is given below (Jago 1983 and own data). The collection of the Natural Resources Institute (NRI) (the former Centre for Overseas Pest Research, COPR) has now been transferred to the Natural History Museum, London (NHM). Further abbreviations are: MNHU: Museum für Naturkunde der Humboldt-Universität zu Berlin; UMB: Überseemuseum Bremen.

1. ***euthynota*** Jago, 1983: Locus typicus: **Tanzania**: Uluguru Mts., W side of Bunduki For. Res. fishing camp, 20.-23.10.1964 (Jago) (NHM) (Jago 1983); further records: **Tanzania**: Uluguru Mts., W side of Bunduki For. Res. fishing camp, 20.-23.10.1964 (Jago) (NHM); Morogoro, Morningside, 11/1953 (Phipps) (NHM); Morogoro, Morningside, 02.02.1970 (Brown) (NHM); Uluguru Mts., Mkata, 11/1953 (Phipps) (NHM) (Jago 1983)
2. ***longicornis*** Jago, 1983: Locus typicus: **Tanzania**: Ilonga, 16.06.1967 (Jago) (NHM) (Jago 1983); further records: **Tanzania**: Ilonga, 16.06.1967 (Jago) (NHM); West Usambara Mts., Mkussu For. Res., 11.11.1964 (Jago) [possibly mislabelled] (NHM) (Jago 1983)
3. ***nguru*** Jago, 1983: Locus typicus: **Tanzania**: E foot Nguru Mts., montane forest above Turiani, 05.-07.11.1964 (Jago) (NHM) (Jago 1983); further records: **Tanzania**: E foot Nguru Mts., montane forest above Turiani, 05.-07.11.1964 (Jago) (NHM); E foot Nguru Mts., Mtibwa For. Res. nr Turiani, dry woodland, 05.11.1964 (Jago) (NHM) (Jago 1983)
4. ***usambarica*** (Ramme, 1929): Locus typicus: **Tanzania**: Amani, 09.01.1905 (Vosseler) (MNHU) (Jago 1983); further records: **Tanzania**: E Usambara Mts.: Amani, 06.12.1907 (Vosseler) (MNHU); Sigi, nr Amani, 18.-31.12.1965 (Jago) (NHM); Kizugu, nr Amani, 18.-30.12.1965 (Jago) (NHM); nr. Amani, 09.11.1964 (Jago) (NHM); Mbomole summit, nr Amani, 03.04.1966 (Jago) (NHM); Longuza For. Res., 15.04.1966 (Jago) (NHM); nr. E Usambara Mts., Ngomeni, Makuyuni, 27.06.1954 (Phipps) (NHM); Korogwe-Msata rd, 103 km N of Msata, summit of kopje, 27.09.1982 (Jago) (NHM); 10 km N of Mbwewe, bush fire, 30.09.1982 (Jago) (NHM) (Jago 1983); E Usambara Mts., Amani East Forest Reserve, 06-11/1994 (Hochkirch) (UMB); E Usambara Mts., Mbomole Hill, 06-11/1994 (Hochkirch) (UMB); E Usambara Mts., Road from Amani to Muheza, near Zigi, 06-11/1994 (Hochkirch) (UMB); E Usambara Mts., Road from Amani to Mbomole, 06-11/1994 (Hochkirch) (UMB); E Usambara Mts., Centre of Amani, 06-11/1994 (Hochkirch) (UMB); E Usambara Mts., Road from Amani to Monga, 06-11/1994 (Hochkirch) (UMB); Mt. Mlinga, nr. Magrotto, 07-08/1994 (Hochkirch) (UMB) (Hochkirch 1996a)

3.4 Ecological Knowledge on the Genus *Afrophlaeoba*

Although the East Usambara Mts. belong to the best-studied areas in East Africa, the ecological knowledge of its fauna is very limited. During the German colonial times, the zoologist of the biological research station at Amani, J. Voßeler, was probably the first to note some observations on Eastern Arc grasshoppers (1905, 1906, 1907a, b, c). However, due to the agricultural focus of his studies, he exclusively published data on pest species, like the locust *Schistocerca gregaria* or *Zonocerus elegans*. Nevertheless, he collected the holotype of *Afrophlaeoba usambarica* at Amani (09.01.1905), which was later described by Ramme (1929) at the Berlin Museum. Since Ramme's (1929) description, nothing but the name *Odontomelus usambaricus* was known for a long time. This is partly due to the relatively recent taxonomical assignments. Older literature usually refers to *Afrophlaeoba* species as *Odontomelus* species.

At the end of the 1950s a wave of ecological studies started in Tanzania with works of Phipps (1959, 1966, 1968), Kevan & Knipper (1961), Robertson & Chapman (1962), Anderson (1963), Vesey-Fitzgerald (1964), Robertson (1967) and Jago & Masinde (1968). Of these authors Phipps (1959, 1966, 1968), Kevan & Knipper (1967) and Jago & Masinde (1968) studied in the range of *Afrophlaeoba*. Phipps (1959) described life cycles and habitats of several grasshopper species, which he collected at Muheza, Kibaranga and Mlingano located at the foothills of the East Usambara Mts. and at Morogoro located at the foothills of the Uluguru Mts. In a table of dissected specimens he lists *Odontomelus brachypterus* for the Usambaras and for Morogoro. Since *Odontomelus brachypterus* is endemic to the region of Mt. Meru and Kilimanjaro and other species of the genus *Odontomelus* are not known to occur at the locations, these records probably refer to *A. usambarica* respectively *A. euthynota*. This can also be followed from the body lengths given by Phipps (1959). The only data, which are presented for those specimens, are average numbers of ovarioles of 16.2 for *A. euthynota* and 16.5 for *A. usambarica*. These are quite low values, which are suggested to be economisation strategies influenced by high predation pressure (Hochkirch 1998). In the second paper, Phipps (1966) deals with the habitat and seasonal distribution of grasshoppers from the same regions. Again he lists *Odontomelus brachypterus* instead of the *Afrophlaeoba* species and includes the information "thicket edge" and the dates of collection (Magrotto, East Usambaras: December; Morogoro: February, March, May, June). In the third paper Phipps (1968) summarizes data on the ecology and life cycles of several African species, but he does not include *Afrophlaeoba* any more. Kevan & Knipper (1961) list several species from Tanzania, including new species from the Uluguru Mts. (Morningside), but any data on *Afrophlaeoba* are missing. Jago & Masinde (1968) present some general information on the faunistic differences of northern and southern slopes within the East Usambara Mts. near Amani, but they did not work at species level.

After the 1960s the number of ecological studies on grasshoppers in Tanzania decreased abruptly. However, taxonomists of the Natural Resources Institute (NRI), formerly the "Anti-Locust Research

Centre" or "Centre of Overseas Pest Research" (COPR), published many revisions and descriptions of species collected during the colonial times, including the huge collections of specimens collected by N. D. Jago in Tanzania from 1964 to 1967. It was Jago, who enforced the revisions for a handbook of the grasshoppers of East Africa. As noted above, this work also included the description of the genus *Afrophlaeoba* and fortunately also some information on the ecology and behaviour of these species (Jago 1983), which were described above. For *A. euthynota* he gives some more accurate information on the habitat ("edge of lush relic montane forest, especially sunny well watered gullies with *Dracaena* and ferns") and food (grasses). In the discussion of his description of *A. nguru*, he mentions that the "Nguru and Uluguru sites are isolated from each other by savannah and dry woodland unsuitable to the genus." In 1994 the habitat preferences of the grasshoppers were studied at the East Usambara Mts. (Hochkirch 1996a), including also *A. usambarica* and *P. arachniformis*. Nymphs and adults of *A. usambarica* were common at grassy forest edges and at large clearings from June to November. The graminivorous diet was confirmed and a continuous life cycle assumed. *P. arachniformis* occurred dispersed at grassy patches inside the forest and small clearings from July to September. This species also seems to be graminivorous and has similar habitat requirements as *P. luci* (Hochkirch 1999b).

4 Molecular Systematics

4.1 Introduction

The analysis of DNA data has become a frequently used method for inferring relationships of different taxa or genes. This is partly due to the development of the PCR technique, which allows rapid amplification of gene sequences. Amazingly, "the PCR only works because of an enzyme discovered in a bacterium [*Thermus aquaticus*] found in the hot springs of Yellowstone […], an area [which] was set aside as the world's first National Park in 1872" (Lovejoy 1996).

DNA sequences are believed to evolve in a more regular manner, compared with morphological or physiological characters (Li 1997), although Omland (1997) points out that in most cases there is a correlation between morphological and genetic evolutionary rates. DNA data are more amenable to quantitative treatments, they are more abundant and easily accessible. The high number of available characters in genes is also an advantage, especially in cases where discrete morphological characters are missing (see chapter 5). Mitochondrial DNA (mtDNA) is well suited for phylogenetic studies, as it is maternally transmitted and non-recombining. All parts of the molecule share the same historical pattern of common descent (Wilson et al. 1985). Genes of the mtDNA demonstrate a wide range of evolutionary rates, and therefore can provide resolutions across a large time scale (Hillis et al. 1996a). Full sequences of the mtDNA of several taxa are already available and so-called "universal primers" have been developed (Kocher et al. 1989). These primers are highly conserved and allow the access to gene sequences of taxa without genetic background information (Palumbi 1996). Since mtDNA is present in multiple copies in many cells, the use of mtDNA provides a large number of starting copies for the polymerase chain reaction. However, sometimes gene trees may fail to reflect the relationship of the organisms from which they were sampled (Swofford et al. 1996). This may happen if introgression events occur (Ballard 2000) or if the time of a species split is short (Li 1997). To avoid these errors, it is necessary to use different genes or different methods for phylogenetic reconstructions.

The use of mtDNA markers in the field of phylogeography is executed by genotyping different taxa to maternal lineages, and relating the resulting phylogeny to patterns of geographic distribution (Weir 1996). In this study a gene tree is inferred for the mitochondrial DNA of the genus *Afrophlaeoba* using three different gene fragments. Although the occurrence of introgressed haplotypes cannot be excluded, such events would also contribute to the research objectives, as they indicate gene flow between the taxa, for which a direct connection of the populations (and the habitats) is necessary.

4.2 Methods

4.2.1 Collection of Specimens

During the first field trip to Tanzania in March and April 1997 all four *Afrophlaeoba* species, *Parodontomelus arachniformis* and *Odontomelus phloiodes binervis* were collected by hand. Additional specimens were obtained during the second field trip from 30 November 1997 to 27 February 1998, including *Odontomelus brachypterus* and *Parodontomelus luci*. The exact data on the specimens are listed in table 5. Despite intense searches during both field trips, *Afrophlaeoba longicornis* was not found in the West Usambara Mountains, from where Jago (1983) lists one specimen. The species is quite common in the region of Ilonga (the type locality) and Kilosa (8 km south of Ilonga). All other species were found rather frequently at the type localities. Thus it is likely, that the record from the West Usambara Mountains is the result of mislabelling. The type localities were chosen to avoid misidentifications. All specimens were stored in 80% ethanol. The pronotum was cut at one side to allow a better penetration with ethanol. One day later the ethanol was replaced by fresh 80% ethanol. The comparatively small number of individuals sampled allows the collection of sizeable DNA sequences, which frequently yield highly, resolved, well-supported mitochondrial phylogenies (Funk 1999).

Table 5: Specimens used for the genetic analysis

Species	abbreviation	location	date
Afrophlaeoba usambarica (RAMME 1929)	A_usam1	East Usambara Mts.: Top of Mbomole Hill near Amani (05°05.9' S., 38°37.3' E.)	27.03.1997
Afrophlaeoba usambarica JAGO 1983	A_usam2	East Usambara Mts.: Road to Kwamkoro near Amani (05°06.2' S., 38°37.6' E.)	04.01.1998
Afrophlaeoba nguru JAGO 1983	A_nguru	Nguru Mts.: Grasses under mango tree near Mvaji village (06°07.3' S., 37°33.9' E.)	15.03.1997
Afrophlaeoba euthynota JAGO 1983	A_euthy	Uluguru Mts.: Morningside Hotel near Morogoro (06°53.4' S., 37°40.3' E.)	09.03.1997
Afrophlaeoba longicornis JAGO 1983	A_longi1	Rubeho Mts.: Grasses under mango tree near Ilonga (06°47.7' S., 37°01.8' E.)	08.04.1997
Afrophlaeoba longicornis JAGO 1983	A_longi2	Rubeho Mts.: Grasses under mango tree near Kilosa (06°50.0' S., 36°58.7' E.)	08.02.1998
Parodontomelus arachniformis JAGO 1983	P_arach	East Usambara Mts.: Amani Nature Reserve (East slope) (05°05.5' S., 38°38.4' E.)	27.03.1997
Parodontomelus luci HOCHKIRCH 1999	P_luci1	Udzungwa Mts.: Sanje Falls (07°50.7' S., 36°53.0' E.)	05.12.1997
Parodontomelus luci HOCHKIRCH 1999	P_luci2	Udzungwa Mts.: Sanje Falls (07°50.7' S., 36°53.0' E.)	05.12.1997
Odontomelus phloiodes binervis JAGO 1994	O_phloiod	Amboni Caves near Tanga (05°04.4' S., 39°07.0' E.)	24.03.1997
Odontomelus brachypterus (GERSTÄCKER, 1869)	O_brach	Kilimanjaro: near Kidia (03°18.1' S., 37°24.9' E.)	26.12.1997

4.2.2 DNA Extraction

All laboratory work was executed with sterile tubes and disposable pipette tips (sterilized with an autoclave) to avoid contamination. Sterile and double distilled water was used for all solutions. Forceps, scissors, scalpels and other dissecting material were flamed in ethanol to avoid contaminations. All specimens were dissected under a stereomicroscope to gain some muscle tissue from the thorax. Thoracic muscle tissue is suitable for mtDNA extraction as the muscle cells contain mitochondria in high concentration. The spin column procedure (QIAamp Tissue Kit, QIAgen) was used to extract DNA, following the tissue protocol of QIAgen handbook. The muscle tissue was transferred to a 1.5-ml microfuge tube with 180 µl buffer ATL and afterwards crushed and squashed with a mortar to decrease the lysis time. 20 µl proteinase K stock solution was added and mixed by vortexing. The cup was incubated for 1-3 hours in a water bath at 55°C until the tissue was completely lysed. This procedure was necessary to destroy the membranes and DNA-degrading enzymes. Afterwards 200 µl buffer AL were added to the sample and vortexed again. The tube was now incubated in a water bath at 70°C for ten minutes. Thereafter it was centrifuged for three minutes at 10,000 rpm (Hettich mikro-rapid) to eliminate insoluble fragments of the cells. The supernatant was transferred to a fresh tube. 210 µl 95% ethanol was added and vortexed. This mixture was applied to a QIAamp spin column in a 2 ml collection tube. The spin column with the cup was centrifuged at 6,000 rpm and afterwards placed in a clean 2-ml collection tube. The filtrate was decanted. The whole procedure was executed to bind the DNA to the spin column. 500 µl buffer AW were added and centrifuged for one minute at 6,000 rpm to wash the sample. This washing procedure was repeated. Afterwards the cup was centrifuged for 2 minutes at 10,000 rpm. The spin column was transferred to a clean 2-ml microfuge tube and 200 µl buffer AE (preheated to 70°C) were added to elute the DNA. This cup was transferred to a water bath at 70°C for 5 minutes. Afterwards 200 µl buffer AE (70°C) were added again and for another five minutes the cup was placed in the water bath of 70°C. Thereafter the mixture was incubated at room temperature for 1 minute and then centrifuged for 1 minute at 10,000 rpm. The supernatant was transferred to a clean tube and frozen. The template for the PCR was diluted 1:50 (1 µl DNA fluid with 50 µl dd H_2O).

4.2.3 DNA Amplification

The Polymerase Chain Reaction (PCR) is an enzymatic in vitro procedure to amplify specific nucleotide sequences. It was introduced by Mullis et al. (1986), although the principles were already described by Kleppe et al. (1971). The four DNA-specific nucleotide triphosphates (NTP), buffer, cofactors (such as Mg^{2+}) and a heat-stable DNA-polymerase (= *Taq* polymerase, isolated from the hot springs bacterium *Thermus aquaticus*) are given to the template DNA. The specificity of the reaction is given by two short oligonucleotides (primers) complementary to a short specific region of the template DNA and flanking the region of interest. Due to increasing temperatures the DNA is

denatured into both single strands. If the temperature is lowered again, the oligonucleotide primers bind to the appropriate sites of the template DNA (annealing). The primers anneal faster than the complementary strand, since they are shorter and a vast overdose of them is available. The double-stranded part of the DNA functions as a starting signal for the polymerase, which extends the primer sequences in the 3'-direction (Palumbi 1996). Thus the original sequence between the primers is copied. This procedure is repeated in successive cycles and in each cycle the product is doubled, leading to an exponential increase of the copies required. To ensure the success of the PCR, the knowledge of short conserved sequences is needed for the construction of the primers. Since the complete mitochondrial genome of several insect species has been sequenced (including also *Locusta migratoria*), primers can easily be found (Clary & Wolstenholme 1985, Crozier & Crozier 1993, Mitchell et al. 1993, Flook et al. 1995). At the beginning, universal primers were used and those, which had been used in the workgroup for DNA studies on carabid beetles. These primers usually work well, if the number of mismatches is low and not located at the 3'-end (Palumbi 1996). Two additional primers have been designed later. Parts of three mitochondrial genes have been analysed: Those genes were the NADH-dehydrogenase subunit 1 (ND1), the NADH-dehydrogenase subunit 5 (ND5) and the 12S rRNA. The locations of the amplified sequences are given in figure 4, the primer sequences in table 6.

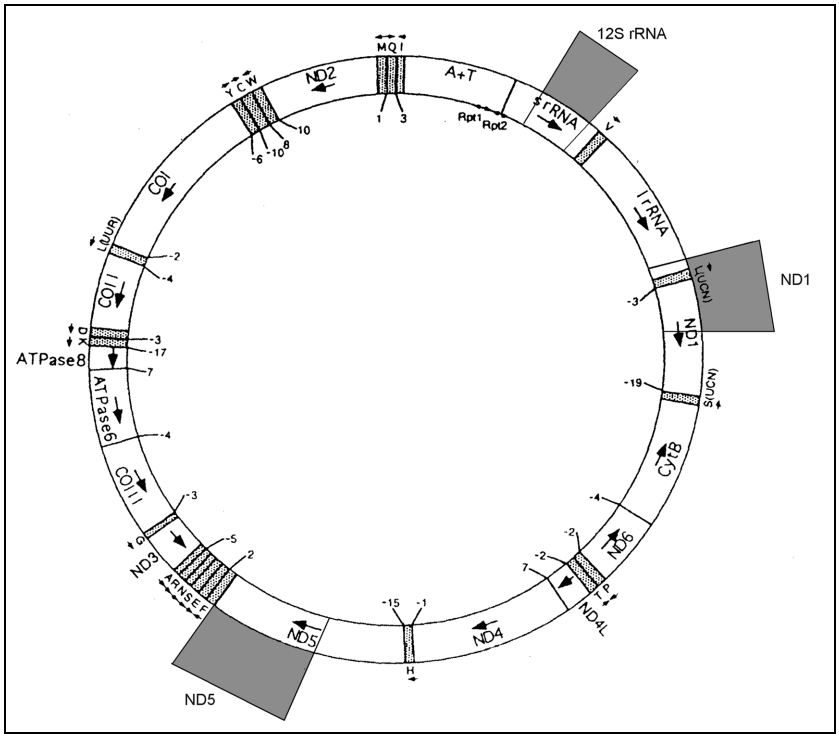


Fig. 4: Genetic map of the complete mitochondrial genome of *Locusta migratoria* (according to Flook et al. 1995)- the grey shaded areas represent the location of the amplified sequences 12S rRNA, ND1 and ND5

Tab. 6: Names, location, sequences and length of primers used for amplification

Name	location	sequence	length	Reference
12S ai	12S rRNA	5′ AAA CTA GGA TTA GAT ACC CTA TTA T 3′	25 bp	Kocher et al. 1989
12S bi	12S rRNA	5′ AAG AGC GAC GGG CGA TGT GT 3′	20 bp	Kocher et al. 1989
ND S	ND 1	5′ TAG AAT TAG AAG ATC AAC CAG C 3′	22 bp	Pashley & Ke 1992
ND II	16S rRNA	5′ ACA TGA TCT GAG TTC AAA CCG G 3′	22 bp	Vogler & DeSalle 1993
ND V-His	ND 5	5′ CCT GTT TCT GCT TTA GTT CA 3′	20 bp	Su et al. 1998
ND V-Phe	ND 5	5′ GTC ATA CTC TAA ATA TAA GCT A 3′	22 bp	Su et al. 1998
ND V-400	ND 5	5′ AGC TGG TTT TTA TTC AAA GG 3′	20 bp	self-designed
ND V-400r	ND 5	5′ ATC CTT TGA ATA AAA ACC AG 3′	20 bp	self-designed
ND V-850	ND 5	5′ GAT TTA TAC CTA ATA TTT CTA C 3′	22 bp	Düring & Brückner 2000
ND V-850r	ND 5	5′ GTA GAA ATA TTA GGT ATA AAT C 3′	22 bp	Düring & Brückner 2000

The PCR war performed according to the *Taq* PCR handbook of QIAgen using the following protocol. A total of 100 µl were pipetted into a 0.5 ml Eppendorf tube, including the following substances according to the *Taq* PCR handbook of QIAgen:

50 µl vortexed PCR Master Mix of QIAgen (incl. Taq polymerase, buffer, NTP, $MgCl_2$)
2,5 µl Primer A
2,5 µl Primer B
3,0 µl DNA template (1:50)
42 µl sterile dd H_2O

This mixture was overlaid with approximately 100 µl mineral oil to avoid evaporation. The tube was transferred to the thermal cycler (Perkin Elmer DNA-Thermal Cycler). The cycle programmes are given in table 7. The first annealing temperature (Nr. 2, Step B) varied according to the optimal temperature of each primer from 37-46°C. The lower annealing temperature of the first ten cycles is mainly caused by the fact that universal primers are often not matching perfectly. After the start of the synthesis, the ends of the PCR fragments are identical to the reaction primers and a higher annealing temperature can be chosen (Palumbi 1996). If the primers bind at more than one site, it is necessary to perform the first cycles at a higher annealing temperature to assure that only the correct products are obtained.

Tab. 7: Programme of the thermal cycler for the PCR

Nr.	temperature	time	number of cycles	function
1	A: 96°C	00:05:00	1	Denaturation
2	A: 96°C	00:01:30	5	Denaturation
	B: 37-46°C	00:01:30		Annealing
	C: 68°C	00:01:30		Extension
3	A: 94°C	00:01:30	28	Denaturation
	B: 50°C	00:01:30		Annealing
	C: 68°C	00:01:30		Extension
4	A: 68°C	00:03:00	1	Extension
5	A: 4°C	endless	1	Cooling

To check the success of the PCR, 2 µl loading solution was added to 10 µl of the product and transferred to a 1.5% agarose gel (submerged in 1x TBE buffer: 40mM Tris-HCl, 0.114% glacial acetic acid, 1 mM EDTA). 4 µl Hind III/Eco-R1-digested lambda bacteriophage DNA were included in the same gel as a size standard. The electrophoresis lasted 1-1.5 hours at 100 V. Afterwards the gel was stained in an ethidium bromid solution (200 ml H_2O + 200 µl ethidium bromid solution 1 mg/ml) for at least 0.5 hours. The result was examined on an ultraviolet lamp desk (Vetter Chroma 43) at 302 nm.

4.2.4 DNA Purification

The efficiency of sequencing reactions can be improved by purifying the PCR-generated templates prior to sequencing (Hillis et al. 1996a). For this reason the PCR product (90 µl product) was precipitated with 10 µl ammonium acetat (10 M) and 300 µl ice-cold absolute ethanol at -20°C overnight. The tube was centrifuged 20 minutes at 10,000 rpm and the supernatant decanted. The pellet was dried in a vacuum centrifuge (DNA Speed Vac DNA 100, Savant) for 8 minutes. Afterwards the pellet was dissolved in 20 µl TE buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA). 4 µl loading solution was added to this solution and transferred to a 1.5% agarose gel (submerged in 1x TBE buffer: 40mM Tris-HCl, 0.114% glacial acetic acid, 1 mM EDTA). The electorphoretic separation of the sample was performed with an agarose gel apparatus (Isco Little Blue Tank, Biorad Wide Mini sub^{TM} Cell). To estimate the fragment lengths, 4 µl Hind III/Eco-R1-digested lambda bacteriophage DNA was included in the same gel as size standard. The electrophoresis lasted 1-1.5 hours at 100 V. Afterwards the gel was stained in an ethidium bromid solution (200 ml H_2O + 200 µl ethidium bromid solution 1 mg/ml) for at least 0.5 hours. The result was examined on an ultraviolet lamp desk (Vetter Chroma 43) at 302 nm. The length of the DNA fragments was checked, and the DNA band was excised from the agarose gel with a clean, sharp scalpel and transferred to a tube. The next steps of the purification followed the Qiaex II Handbook of QIAgen. The gel slice was weighed and three volumes of buffer QX1 were added to one volume of excised gel. A silica-binding matrix (the glassmilk QUIAEX II) was vortexed for 30 seconds and 10 µl were added to the sample. The tube was incubated at 50°C in a water bath for 10 minutes to solubilize the agarose and bind the DNA to the glassmilk. Every 2 minutes the sample was mixed by vortexing. Afterwards the tube was centrifuged at 10,000 rpm for 30 seconds. The supernatant was carefully removed with a pipette and the pellet washed with 500 µl of buffer QX1. This washing procedure was repeated twice. Afterwards the pellet was air-dried for 15 minutes until the pellet became white. 20 µl of 10 mM Tris-Cl (pH 8.5) were added and the pellet was resuspended by vortexing to elute the DNA. The sample was incubated at 50°C in a water bath for 10 minutes and centrifuged at 10,000 rpm for 30 seconds. The supernatant containing the purified DNA, was carefully pipetted into a clean tube and frozen.

4.2.5 DNA Sequencing

Before the sequencing procedure is started, it is necessary to quantify the purified DNA product. For this purpose 4 µl purified DNA are mixed with 1 µl loading solution (to mark it and to enlarge the weight) and transferred to a 1.5% agarose gel (submerged in 1x TBE buffer: 40mM Tris-HCl, 0.114% glacial acetic acid, 1 mM EDTA). To estimate the DNA content 2.5 µl KB-marker and 5 µl KB-marker were separated in the same gel. The electrophoresis lasted 1-1.5 hours at 100 V. The result was examined on an ultraviolet lamp desk (Vetter Chroma 43) at 302 nm and photographed. The brightness of the DNA bands was used to estimate the DNA content.

Cycle Sequencing is based on the dideoxynucleotide chain-termination method of Sanger et al. (1977). The primer is annealed to a complementary single-stranded DNA fragment in the presence of a polymerase, deoxynucleotide triphosphates (dNTPs) and fluorescently labeled dideoxynucleotide triphosphates (ddNTPs). The extension of the DNA is terminated by incorporation of a ddNTP, since it lacks the 3' OH group. Successive cycles of denaturation, annealing, and synthesis result in a linear amplification of the labelled product (Hillis et al. 1996a). The different labels (in this case dRhodamine acceptor dyes) for each base allow to separate all bases simultaneously in one gel slot later. The main purpose of cycle sequencing is to ensure an adequate signal for the automated sequencing. The automated sequencing was performed by the workgroup biotechnology of Prof. Blohm, University of Bremen with an AB 373A Stretch DNA-Sequencer. The fluorescently labelled DNA fragments are detected during electrophoresis by a tunable laser as they pass a single point. The sequence is recorded directly into a computer, printed as a chromograph and interpreted by computer software (Hillis et al. 1996a). These chromographs were visually controlled for uncertainties.

Cycle Sequencing was performed according to the protocol for a kit (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, Biosystems, Warrington, England). The template volume was chosen according to the fragment length and to the concentration estimated above and transferred to a 0.5-ml tube. 4 µl pre-Mix (incl. dye terminators, dNTPs, Ampli*Taq* DNA Polymerase FS, r*Tth* pyrophosphatase, $MgCl_2$ and Tris-HCl buffer, pH 9.0) and 0.5 µl primer A were added and the volume filled up to 10 µl with H_2O. This mixture was overlaid with a drop of mineral oil to avoid evaporation. The same mixture was transferred to a second tube, but using primer B. The sample was transferred to the thermal cycler and a program run, which is presented in table 8. The product of cycle sequencing was precipitated with 1.0 µl 3M $NaCO_3$ and 25 µl 95% ethanol. To purify the product, it was centrifuged at 13,000 rpm (Heraeus Pepatch Biofuge A) for 30 minutes. The supernatant was pipetted and 125 µl 70% ethanol added to the pellet. The fluid was pipetted again and the tube was centrifuged in a vacuum centrifuge for eight minutes.

Tab. 8: Programme of the thermal cycler for sequencing

Nr.	temperature	time	number of cycles	function
1	A: 96°C	00:02:00	1	Denaturation
2	A: 96°C	00:00:30	25	Denaturation
	B: 44-50°C	00:00:15		Annealing
	C: 60°C	00:04:00		Extension
3	A: 4°C	endless	1	Cooling

4.2.6 Data Analysis

The DNA sequences are analysed by means of several computer programmes. In a first step the sequences are aligned, so that exclusively homologous sequences can be compared. In a second step the homoplastic content is estimated. If the homoplasy is low, the data are added to the complete data set and analysed with distance methods and parsimony programmes. The topologies rendered by these programmes are rooted with outgroup(s) and transposed to phenograms (distance methods) or cladograms (parsimony methods). The cladograms are tested by bootstrapping and the character distribution is presented.

4.2.6.1 Alignment

Sequence alignment is one of the most important and least understood component in the phylogenetic analysis of DNA sequences. Only if the characters under study are homologous, a comparison for a phylogenetic purpose is possible. Homology suggests that the character under study must be defined in a way that all character states observed among the taxa must have been derived from a corresponding state in a common ancestor of those taxa (Swofford et al. 1996). In DNA sequences, the characters are represented by the corresponding positions in the sequences, and the character states are the nucleotide residues observed at these positions. The alignment is a method to interpret the homology of DNA sequences based on similarities or distances. The similarity approach tries to maximize the number of matched base pairs, while the distance approach tries to minimize the number of mismatched pairs and gaps. If the compared sequences are highly similar the visual inspection can be feasible as well (Li 1997). Alignments may be simple for closely related protein genes, but may be rather difficult if the taxa under study are distantly related (Hillis et al. 1996a). This is particularly true, if deletions or insertions (indels) occur in longer sequences of a single nucleotide (e. g. microsatellites). In DNA sequences of protein coding genes single indels hardly ever occur, as they would cause a shift of the reading frame (in the protein coding data presented here no indels have been found at all).

The specificity of the primer region is of high importance for finding homologous (orthologous) sequences. This criterium might cause problems, if a gene or a part of it that includes the primer region is copied to another part of the genome, as it is known for pseudogenes (Bensasson et al.

2000). Such a copy would also be homologous (paralogous) to the required sequence, as it is derived from a common ancestor, but as it has lost its function and has become selectively neutral, it is not possible to infer phylogenies from comparing pseudogenes with the original, despite genomic evolution. To avoid the comparison with such paralogous non-functional DNA sequences, the transcription to the amino acid sequence is helpful. In the case of functional genes most substitutions should be silent substitutions. These synonymous substitutions do not change the amino acid sequence due to the degenerate code (70% of the substitutions at the third codon position). If a nonsynonymous substitution takes place, amino acids with similar physicochemical properties should be substituted (e. g. Leu–Ile) to guarantee the function of the protein (Li 1997). In the case of pseudogenes, which lost their function and which are believed to be selectively neutral, the substitution may occur at all positions. In RNA specifying genes, a comparison with the highly conserved regions is necessary, as they were identified by Hickson et al. (1996).

A first step of pairwise alignment is given by matrix plots, which allow quick determination of similarities and their portrayal (Lawrence 1990). The two sequences under comparison are portrayed along the x and y axes of a graph, and the similarities are marked. This procedure allows the identification of indels (Hillis et al. 1996a). The pairwise alignment of two sequences usually follows the algorithm of Needleman & Wunsch (1970). This algorithm tries to find a path through the matrix plot of two sequences, causing as few substitutions and indels as possible (Hillis et al. 1996a). Substitutions are usually penalized less severely than indels, since they should occur more frequently. It is also possible to assign different penalties for the number or size of gaps or for different kinds of substitutions (transitions, transversions). The aim of the algorithm is to find the least costly interpretation of the data (Hillis et al. 1996a). If multiple sequences have to be compared, a multiple alignment is necessary. A multidimensional matrix would exponentially increase the number of calculated cells and exceed the capacity of many computers. Therefore, the sequences are compared serially by pairwise alignment of each sequence and combination of the most similar ones. The alignment was performed with the computer programme Clustal V (Higgins et al. 1992), using the default parameters. In this programme the order of the pairwise alignments is obtained from clusters in an initial tree produced from a matrix of distances across all pairwise alignments (Hillis et al. 1996). The alignments were visually inspected to ensure that the most reasonable one has been generated.

4.2.6.2 Homoplastic Content of the Data Set

Skewness

If a data set was randomly constructed, some random covariation would lead phylogenetic reconstruction methods to prefer some trees to others, although any true hierarchical structure was absent. A suitable method for estimating the non-randomness of a hierarchical structure is the examination of the tree length distribution of a high number of random trees from the data set (Swofford et al. 1996). While little or no hierarchical structure produces relatively symmetric distributions, a high amount of hierarchical structure produces left-skewed distribution. The degree of skewness is quantified with the g1 statistics.

$$g_1 = \frac{\sum_{i=1}^{n}\left(T_i - \overline{T}\right)^3}{n \cdot s^3}$$

g1 = skewness
T = tree length
n = number of trees
s = standard deviation of the tree length distribution

A g1 = 0 is given by a normal distribution, since the data set contains as many longer trees as shorter trees and there is only little covariance. A skewed left (g1 < 0) indicates that the data set contains covariances, which support similar branches. The lower amount of necessary evolutionary steps is mainly based on the lower amount of homoplastic information. However, strong skewness can be misleading, since highly asymmetric tree-length distribution can also be produced by very localized structures (Swofford et al. 1996). In this study one million random topologies were computed for each data set. The calculation was executed with the computer programme PAUP 3.1.1 (Swofford 1993) on a Power MacIntosh 6100/60, using the default parameters.

Proportion of Transitions

Substitution is a long lasting process, whereby the frequencies of mutant alleles increase or decrease randomly, until the alleles are fixed or lost by chance. A mutation becomes significant only if its frequency increases with time (Li 1997). Due to chemical constraints, transitions (substitutions within purines or pyrimidines) occur more often than transversions (substitutions between purines and pyrimidines, Brown et al. 1982). Since multiple substitutions may occur at the same site (back substitutions, parallel substitutions, convergent substitutions), the number of transitions between two evolutionary lineages increases first, but reaches a plateau after some time. The number of transversions increases at a lower rate and reaches a plateau later. Therefore, the proportion of transitions among the number of substitutions gives a good estimate for the homoplastic content of the data. A high proportion of transitions indicates that the plateau is not reached and the number of back

substitutions is low. If the proportion of transition decreases, the number of back substitutions is higher and the homoplastic content of transitions rises. The number of transversions, transitions and total substitutions between the taxa was calculated by the computer program MEGA 1.02 (Kumar et al. 1993).

Consistency and Retention Indices

For the evaluation of the performance of a method, consistency, efficiency, robustness, computational speed, discriminating ability, and versatility are important criteria (Hillis et al. 1996b). The ability of an estimation method to converge to a true value as more data become available is known as consistency. Methods become inconsistent, if their assumptions are violated. Efficiency describes the speed in which a method converges to a correct solution as more data are accumulated. A method is called robust if it is more or less insensitive to violations of its assumptions. The computational speed of distance methods is generally higher than that of parsimony methods, but the discriminating ability is much lower, which means that they do not necessarily find an optimal solution. Versatility is the possibility to include more information about the data, such as the weighting of different codon positions (Hillis et al. 1996b). The computer programme PAUP 3.1.1 calculates the following indices for estimating the accuracy of the method. The CI (Consistency index) gives the proportion of homoplastic events (multiple substitutions) among all variable sites. Noninformative sites can be excluded from the analysis. The RI (Retention index) gives the relation of the highest possible homoplasy to the existing homoplasy. Both values can vary from 0 to 1. A high CI or RI indicates a low homoplastic content. A third index given by PAUP is the RC (Rescaled consistency index), which is the product of RI and CI.

4.2.6.3 Phylogenetic Inference

Generally spoken two approaches to infer a phylogeny with molecular data can be distinguished, algorithms and optimality criteria (Swofford et al. 1996). Since no information about the past is available, the methods try to find the best estimate of an evolutionary history. Algorithms try to define a specific sequence of steps that lead to determination of a tree, optimality criteria try to find a criterion for comparing alternative phylogenies and choosing the best (Swofford et al. 1996). Algorithmic methods include distance methods (UPGMA, Neighbor joining), which are computationally fast. Optimality criteria methods (parsimony methods, maximum likelihood) are computationally much slower, since they first define an objective function (optimality criterion) for evaluating a tree and then calculate the values of the trees to find the one with the best values according to the criterion. The advantage of criterion-based methods is the possibility to rank different phylogenies according to the score (the preference according to the criterion). Purely algorithmic methods do not determine the strength of support for that tree (Swofford et al. 1996).

The Parsimony Method

In parsimony algorithms a phylogenetic hypothesis is derived from the observed synapomorphies on the basis of their most parsimonious hierarchical arrangement (Vogler & DeSalle 1994). Each base position represents a character and character changes are analysed to find the shortest tree, which is called most-parsimonious tree (MPT). The principle of parsimony in science maintains that simpler hypotheses are preferable to more complicated ones. The estimation of trees under the criterion of parsimony means that *ad hoc* hypotheses are avoided. However, this is not always possible, and assumptions of homoplasy must be invoked (Swofford et al. 1996). Generally spoken, parsimony methods try to select trees that minimize the total tree length (the number of evolutionary steps, in this case base substitutions). Uninformative sites (autapomorphies and non-variable characters) are ignored for the analysis. Only informative sites (synapomorphies and homoplasies) are regarded. Thus only a fraction of the data is used – which makes it more effective, if the number of informative sites is large (Li 1997). The simplest parsimony methods are the Fitch and Wagner Parsimony. These methods impose no or minimal constraints on permissible changes in character state. The Fitch Parsimony allows unordered multistate characters, direct transformation from one state to the other, and either direction is equally probable, which is the case in nucleotide sequences (Fitch 1971). In consequence, it is possible to root the tree at any point without changing its length (Swofford et al. 1996). The parsimony approach can be misleading, if the rate of evolution has been accelerated in peripheral branches or if the branching events of the central branches occurred in a short period of time (which causes a low number of synapomorphies). In such a case, patterns that support the true tree will occur only rarely and an incorrect tree might be supported. A higher amount of characters analysed will then support the incorrect tree more and more, which is called “positively misleading” (Felsenstein 1978). Different methods have been developed to find the shortest tree among the high number of possible trees. For data sets of moderate size the simplest approach is to evaluate every possible tree (Swofford et al. 1996). This exhaustive search is feasible only for few taxa, as the number of possible topologies rises exponentially for each taxon added (> 2 Million possible topologies for ten taxa). Another exact algorithm for identifying all optimal trees is the branch-and-bound method introduced to phylogenetic inference by Hendy & Penny (1982). Initially three taxa are connected and then taxa are added sequentially. Parts of the search tree that contain only suboptimal solutions are eliminated and the number of evaluated trees thus reduced. In this study, the calculation was executed with the computer programme PAUP 3.1.1 (Swofford 1993) on a Power MacIntosh 6100/60 using the branch-and-bound method. The initial upper bound was computed stepwise.

Weighting

For functional genes the substitution rate usually varies among sites (Li 1997). For protein genes this can be explained by the degenerate code, for RNA genes by the different functions of different regions of the RNA. Therefore a different weighting of substitutions can be helpful for inferring gene trees. In this study different weighting schemes were conducted. For estimating the skewness, transversions were weighted 2x, 5x or 10x. Different weighting schemes (higher weights for the transversions or for the different codon positions) were also applied to the parsimony analysis, but this did not influence the results and it is therefore not presented here.

Outgroup Comparison

Parsimony methods do not require a prior determination of the character polarities (Swofford et al. 1996). The estimation of a character polarity can be difficult, but it is not meaningful for the parsimony methods. However, a phylogenetic analysis requires the output of a rooted tree. The character polarity can be inferred by the inclusion of sequences from an outgroup, if the outgroup is chosen in a way so that the ingroup is monophyletic. From this point of view it is necessary to choose an outgroup which clearly represents a different lineage. The data of the outgroup imply the plesiomorphous state, whereas the ingroup data should be apomorphous. Hence, it is helpful to choose more than one outgroup. The location at which the outgroup branches from the unrooted tree implies that there is a root with respect to the ingroup taxa. Since a hypothetical ancestor is implied by the polarity assignments, a rooted tree is gained from an analysis of polarized characters (Swofford et al. 1996). In this study, two *Odontomelus* species (Pargaini sensu Popov in press) were used as outgroups to the Phlaeobini and the genus *Parodontomelus* has been used as an outgroup to *Afrophlaeoba*. These genera proved to be suitable, when literature mtDNA data of the Oedipodinae *Locusta migratoria* were included (Flook et al. 1995). The phylogenetic consequences of the outgroup choice were evaluated by first employing all outgroups simultaneously and then assigning each outgroup separately while deleting others.

Bootstrapping

The bootstrap test is a method to gain information on an unknown distribution by nonparametric resampling (sampling and replacement) from the data matrix. It was introduced into phylogenetic analyses by Felsenstein (1985). New data matrices of the same structure (in terms of the number of taxa and characters) are composed of the sampled data. The method assumes that the original sample represents the original sample space. Hence, the sample has to be large enough. The bootstrap value represents the proportion of bootstrap replicates in which a branch appears. Usually the technique gives underestimates of the confidence level (Li 1997), but this error can be reduced by an increasing number of replications. In this study, 1000 replications were performed, using the computer programme PAUP 3.1.1 (Swofford 1993) on a Power MacIntosh 6100/60 (default parameters).

Bremer Support

The decay index or Bremer support indicates, which branches are also supported in longer trees than the MPT (Bremer 1994). The difference in tree lengths between the shortest tree that contains a group and the shortest tree that lacks the group was calculated by the computer programme PAUP 3.1.1 (Swofford 1993) on a Power MacIntosh 6100/60, by keeping all trees that are one step longer and examining the strict consensus tree for the stability of groups.

Maximum Likelihood

Maximum likelihood was introduced in phylogenetic inference by Cavalli-Sforza & Edwards (1967). Felsenstein (1981) applied it to nucleotide-based phylogenetics. The method estimates the hypothesis, maximizing the probability of observing the obtained data. This requires a model of evolutionary change (see below), e. g. that the rate of substitution is equal from one nucleotide to the other and that the expected number of substitutions of a branch is given by the substitution rate and the branch length (Swofford et al. 1996). The influence of the branch length is an important difference to the parsimony method. Uninformative sites for parsimony methods can become informative in the maximum likelihood method, since it considers that changes are more likely along long branches than along short ones. This explains the higher consistency of the method compared to parsimony. Moreover, it is less affected by sampling errors (Swofford et al. 1996). A problem in the application of maximum likelihood is the assumption of the models of evolutionary change that every site evolves at the same rate. Rate heterogeneity across sites can have severe consequences on the results, and thus the maximum likelihood method can be "positively misleading" as well (Felsenstein 1978). Therefore, it is suggested to incorporate a rate heterogeneity into likelihood analyses, e. g. by assuming different rates for the first, second and third positions of a protein-coding gene (Swofford et al. 1996). A new approach to phylogenetic inference combines the maximum likelihood method with the neighbor joining method described below (Ota & Li 2000).

Distance Methods

Distance methods use evolutionary distances, based on the percentage of substitutions between the different sequences. Due to back substitutions the observed K (the number of substitutions per site) is usually not identical with the "real" K. Therefore, different models of evolution for estimating the number of substitutions have been developed. The p-distance is the observed number of substitutions per site. The Jukes-Cantor-distance (J-C-distance) calculates corrections for back substitutions. The Kimura-2-Parameter-distance considers the different substitution rates of transitions and transversions. The Tajima-Nei-distance corrects different base frequencies. The Tamura distance considers both, different substitution rates of transitions and transversions and different base frequencies. The Tamura-Nei-distance additionally corrects different substitution rates of the two

different kinds of transitions (G-A, T-C). Since additional assumptions can cause estimation errors the effect of errors is stronger in models with a larger number of parameters (Li 1997). The distance used for the inference of phylogenies should thus depend on its magnitude. If the J-C-distance is ≤ 0.05, the estimation should be simple (e. g. p-distance, J-C-distance). Since the interspecific distances of the data observed in this study were ≤ 0.05, multiple substitutions were corrected by the Jukes-Cantor-distance. The J-C-distance assumes that equilibrium frequencies of all bases are the same and that all substitutions occur at the same rate (Swofford et al. 1996):

$$K = -\left(\frac{3}{4}\right)\ln\left(1-\frac{4p}{3}\right)$$

UPGMA

The unweighted pair-group method with arithmetic mean (UPGMA) first groups the two sequences with the shortest distance. Afterwards the mean distance of these two sequences is taken as a new value (composite taxon) and again the two sequences with the smallest distance connected. A presumption for this method is that all lineages have the same evolutionary rate. Since this is usually not the case, it is more appropriate to use neighbor joining, which corrects different rates of evolution.

Neighbor-Joining

The neighbor-joining method corrects the rate heterogeneity between the branches. It uses the minimum evolution principle and searches for the lowest sum of squares. Thus it is much more affected by the accuracy of the estimated distance. The method performs well if the sequences are long and the distances small (Li 1997), which is the case for the data presented here. The principle of this method is to find neighbors that may minimize the total length of the tree sequentially (Li 1997). It starts with a so-called “bush” – a starlike tree and chooses two taxa, which it then regards as new single unit by calculating the mean. The calculation was performed by the computer program MEGA 1.02 (Kumar et al. 1993).

4.3 Results

4.3.1 Alignments and Base Content

The following alignments represent the DNA sequences for the three gene parts analysed from all taxa studied, including the outgroups. For each sequence the base content, the number of variable sites and the AT-content is given. For protein coding sequences the number of variable positions of the transcribed amino acid sequences is given as well as the proportion of nonsynonymous substitutions.

12S rRNA

A 355-357 bp segment of 12S rRNA was collected from the specimens, which is shown in figure 5. Both strands of each sequence have been analysed and inaccuracies corrected. Fifty-one positions (14.3%) proved to be variable among all taxa, including three indels and twelve sites with transversions. Twelve sites were variable within the genus *Afrophlaeoba* (3.4%), exclusively containing transitions. The AT-content of the data varies from 72.0% to 74.1% (table 9).

Fig. 5: Alignment of the sequenced part of the 12S rRNA – 355-357 bp

```
12S rRNA                                                                  60
A_usam1    TTTAAATGTT AATTAAATTT ACCTGGGTAT TATTAGTTAA GATCTTTAAA CCCAAAGAAT
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... ....G..... .........C .......... .......... ..........
A_euthy    .......... .......... .........C .......... .......... ..........
A_longi1   .......... ....G..... .........C .......... .......... .T........
A_longi2   .......... ....G..... .........C .......... .......... .T........
P_arach    .....G.... ....TT.... G......... .......... .......... ....T.....
P_luci1    .....G.... ....TG.... G......... .......... .......... ..........
P_luci2    .....G.... ....TG.... G......... .......... .......... ..........
O_phloiod  .......... ....G.GC.. .........G .......... .......... ..........
O_brach    .......... ....G.GC.. .........G .........G .......... ..........

12S rRNA                                                                 120
A_usam1    TTGGCGGTAT TTTATTCCAT TCAGAGGAAC CTACCCTGTA ATTGATAATA CACGATTGAC
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .......... .......... .......... .......... .......A..
A_euthy    .......... .......... .......... .......A.. .......... .......A..
A_longi1   .......... .......... .......... .......A.. .......... .......A..
A_longi2   .......... .......... .......... .......... .......... .......A..
P_arach    .......... C.......G. .......... .......A.. .......... .......A..
P_luci1    .......... C......... .......... ...T.T.A.. .......... .......A..
P_luci2    .......... C......... .......... ...T.T.A.. .......... .......A..
O_phloiod  .......... C......... .......... ......CA.. .......... .......T..
O_brach    .......... C......... .......... ...T..CA.. .......... .......T..

12S rRNA                                                                 180
A_usam1    TTTACTTAAT TTATTTGTTT GTATATCTCC GTTATAGGAA GATCTTTTTG GAGTTATAAT
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .......... .......... ......A... A......... ..........
A_euthy    .......... .......... .......... ......A... .......... ..........
A_longi1   .......... .......... .......... ......A... A......... ..........
A_longi2   .......... .......... .......... ......A... A......... ..........
P_arach    .......... .......... .......... ......A... A......... ......A...
P_luci1    .......... .......... .......... ......A... A......... ....A.....
P_luci2    .......... .......... .......... ......A... A......... ....A.....
O_phloiod  ........T. .......... .......... ......A... A......... ..........
O_brach    .......G.. .......... .......... ......A... A......... ..........
```

```
12S rRNA                                                                  240
A_usam1    TTTCTTGATT TATAATTTTA GATTATTTCA GGTCAAGGTG CAGCTTATGA TTAAGAAGAG
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .......... .......... .......... .........A ..........
A_euthy    .......... .......... .......... .......... .........A ..........
A_longi1   .......... .......... A......... .......... .....C.... ..........
A_longi2   .......... .......... A......... .......... .....C.... ..........
P_arach    .......... .......... A......... .......... .........AT ..........
P_luci1    .......... .......... A......... .......... ......G.AT ..........
P_luci2    .......... .......... A......... .......... ......G.AT ..........
O_phloiod  ......A... ...G.....G A.A....... .......... .........A. ......G...
O_brach    .......... ...G...... A.A....... .......... .........A. ......G...
```

```
12S rRNA                                                                  300
A_usam1    GTGGGTTACA ATAGATTT-T -TCTATAATG GATTTAATTA TGAAATATTT TTAATGAAAG
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .......... ........C. .......... .......... ........G.
A_euthy    .......... .......... ........C. .......... .......... ........G.
A_longi1   .......... .......... ........C. .......... .......... ........G.
A_longi2   .......... .......... ........C. .......... .......... ........G.
P_arach    .......... .........C. ..T-....C. A....G.... .......... .C......G.
P_luci1    .......... .........C. ..T.....C. .....G.... .......... .C......G.
P_luci2    .......... .........C. ..T.....C. .....G.... .......... .C......G.
O_phloiod  .......... ...A....T. A.T....... ........GG ......TC.A ........G.
O_brach    .......... ...A....T. AAT....... ........GG ..G...TC.A ........G.
```

```
12S rRNA                                                                   357
A_usam1    TGGATTTGAT AGTAATTTAA TTTATTTAAT TTAATTGATA TTGGCTCTGA GGTGTGT
A_usam2    .......... .......... .......... .......... .......... .......
A_nguru    .......... .......... .......... .......... .......... .......
A_euthy    .......... .......... .......... .......... .......... .......
A_longi1   .......... .......... .......... .......... .......... .......
A_longi2   .......... .......... .......... .......... .......... .......
P_arach    .......... .......... .......... .......... .......... .......
P_luci1    .......... .......... .......... .......... .......... .......
P_luci2    .......... .......... .......... .......... .......... .......
O_phloiod  .......... .......... .......... .......... .......... .......
O_brach    .......... .......... .......... .......... .....C.... .......
```

Tab. 9: Sequence length and nucleotide content of the sequenced part of the 12S rRNA gene for each specimen examined

Specimen	Sequence length	G	C	A	T	G & C	A & T
A_usam1	355	61 (17.2%)	32 (9.0%)	109 (30.7%)	153 (43.1%)	93 (26,2%)	262 (73,8%)
A_usam2	355	61 (17.2%)	32 (9.0%)	109 (30.7%)	153 (43.1%)	93 (26,2%)	262 (73,8%)
A_nguru	355	59 (16.6%)	34 (9.6%)	111 (31.3%)	151 (42.5%)	93 (26,2%)	262 (73,8%)
A_euthy	355	58 (16.3%)	34 (9.6%)	112 (31.5%)	151 (42.5%)	92 (25.9%)	263 (74.1%)
A_longi1	355	58 (16.3%)	34 (9.6%)	112 (31.5%)	151 (42.5%)	92 (25.9%)	263 (74.1%)
A_longi2	355	59 (16.6%)	34 (9.6%)	111 (31.3%)	151 (42.5%)	93 (26,2%)	262 (73,8%)
P_arach	355	59 (16.6%)	35 (9.9%)	108 (30.4%)	153 (43.1%)	94 (26.5%)	261 (73.5%)
P_luci1	356	61 (17.1%)	33 (9.3%)	108 (30.3%)	154 (43.3%)	94 (26.4%)	262 (73.6%)
P_luci2	356	61 (17.1%)	33 (9.3%)	108 (30.3%)	154 (43.3%)	94 (26.4%)	262 (73.6%)
O_phloiod	357	62 (17.4%)	35 (9.8%)	110 (30.8%)	150 (42.0%)	97 (27.2%)	260 (72.8%)
O_brach	357	65 (18.2%)	35 (9.8%)	109 (30.5%)	148 (41.5%)	100 (28.0%)	257 (72.0%)

ND1 (NADH-dehydrogenase subunit 1)

Figures 6 and 7 show the alignment of the sequenced part of the DNA flanked by the primers ND2 and NDS. They include a part of the 16S rRNA, the complete coding region for the tRNA of the amino acid Leucin (tRNA-Leu), a 4 bp long intergene of unknown function (figure 6) and a part of the NADH-dehydrogenase subunit 1 (ND1), from the start codon to the primer NDS (figure 7). Both strands of each sequence have been analysed and inaccuracies corrected. Due to the different nature

of the regions, they are usually presented separately here. For the analysis of ND1 only the protein-coding part was used. The total sequence length varies from 543 to 546 bp., including a part of the ND1 gene, which is 372 bp long in all specimens. Within the ND1 part 66 sites (17.7%) varied, 47 of which represented the third codon position (71% of all variable sites), while 13 variations (19.7%) and five variations (9.1%) were observed at the first respectively the second position. Within the rest of the sequenced fragment 31 variable positions were found (17.6%). No indels were recorded for ND1, whereas ten indels had to be included in the 16S rRNA coding part. In the ND1 fragment transversions were found at 24 sites (6.5%), in the RNA-coding part eleven sites with transversions occurred (6.3%). Within the genus *Afrophlaeoba* six sites were variable within the ND1 part (1.6%), including only one transversion (0.3%). Within the RNA-coding part six sites were variable for *Afrophlaeoba* (3.4%), including two indels and one transversion (0.6%). The AT-content varied from 77.4% to 80.4% for the ND1 part and from 72.4% to 77.0% for the RNA part (table 10 and 11). If the ND1-coding part is transcribed to the amino acid sequence (124 amino acids), 24 positions are variable (19.4%) within the complete data set, but only three are variable within the genus *Afrophlaeoba* (2.4%) – and all of them concern *Afrophlaeoba euthynota*. The proportion of nonsynonymous substitutions within the genus *Afrophlaeoba* varies from 0.0 to 0.25, within *Parodontomelus* it is 0.48 and within *Odontomelus* 0.14. Between the genera the proportion of nonsynonymous substitutions ranges from 0.32 to 0.52.

Fig. 6: Alignment of a part of the 16S rRNA, tRNA-Leu and an intergene (IG) – 176 bp

```
16S rRNA                                                                    60
A_usam1    CGTGAGCCAG GTCGGTTTCT ATCCTAAGAT TAATTAATTT ATATTAGTAC GAAAGGACCA
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .......... .....G.... .......... .......... ..........
A_euthy    .......... .......... .......... .......... .......... ..........
A_longi1   .......... .......... .......... .......... .......... ..........
A_longi2   .......... .......... .......... .......... .......... ..........
P_arach    .......... .......... .....G.... ..T....... .......... ..........
P_luci1    .......... .......... .....G.... ..T....... .......... ..........
P_luci2    .......... .......... .....G.... ..T....... .......... ..........
O_phloiod  .......... .......... .......... AT..GT.... .......... ..........
O_brach    .......... .......... .......... AG...T.... .......... ..........
```

```
16S rRNA                                                          tRNA-Leu  120
A_usam1    TATGAATGAA ATATTTTTTA TA----TTTT GATTAAAATT AATTATT-AC TATTTTGACA
A_usam2    .......... .......... .......... .......... .......... ..........
A_nguru    .......... .........T .......... .......... .......... ..........
A_euthy    .......... .........T ....TA.... .......... ....G..... ..........
A_longi1   .....G.... .........T .......... .......... .......... ..........
A_longi2   .....G.... .........T .......... .......... .......... ..........
P_arach    ...A...... ...A.....T .T..TA.... .......... .......T.. ..........
P_luci1    ...AG..... ...A.....T --...A.... .......... .......T.. ..........
P_luci2    ...AG..... ...A.....T --...A.... .......... .......T.. ..........
O_phloiod  ...AGG..G. ..G....-CT .TATTAA... .......... ....-..T.. ..........
O_brach    ...AGG..G. ........CT .TATTAAC.- .TC....... ....-..... ..........
```

```
tRNA-Leu                                                                IG 176
A_usam1    GATTAATGTG TTGAATTTAG AATTCATTAA TGTAGATTTT TCTACAAATA GTATTG
A_usam2    .......... .......... .......... .......... .......... ......
A_nguru    .......... .......... .......... .......... .......... ......
A_euthy    .......... .......... .......... .......... .......... ......
A_longi1   .......... .......... .......... .......... .......... ......
A_longi2   .......... .......... .......... .......... .......... ......
P_arach    .......... .......... .......... .......... .......... ......
P_luci1    .......... .......... .......... .......... .......G.. ..T...
P_luci2    .......... .......... .......... .......... .......G.. ..T...
O_phloiod  .....G.... ...G...... ........T. .......... .......... ......
O_brach    .......... ...G...... ........C. .......... .......... ......
```

Fig. 7: Alignment of the sequenced part of the NADH-dehydrogenase subunit 1 (ND1, 372 bp)

```
ND1                                                                      51
A_usam1    ATA TTT TAT GAT TTA ATT ATG TTT ATT TTA AGT TTT ATT CTT TTA ATT ATT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach    ... ..A ... ... ..G G.. ..A ..C ... ... .A. ... ... ... ... ... ...
P_luci1    ... ..A ... ... ... ... ..A ... ... ... .A. ... ..C ... ... ... ...
P_luci2    ... ..A ... ... ... ... ..A ... ... ... .A. ... ..C ... ... ... ...
O_phloiod  ... ... ... ... ... T.. ... ... ... ..G .A. ... C.. ... ... ... ...
O_brach    ... ... ... ... ... T.. ... ... ... ... .A. ... C.. ... ... ... ...
```

```
ND1                                                                     102
A_usam1    TGT GTT TTA ATC AGT GTT GCT TTT TTA ACT TTA TTT GAA CGT AAA GTA TTA
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C..
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C..
A_longi2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C..
P_arach    ... ... ... ..T ... ... ... ... ... ... ... ..G ... ... ..G ... ...
P_luci1    ... ... ... ..T ... ... ... ... ... ... ... ..A ... ..A ..G ... ...
P_luci2    ... ... ... ..T ... ... ... ... ... ... ... ..A ... ..A ..G ... ...
O_phloiod  ... ... ... ..T ... ... ... ... ... ... ... ..A ... ... ..G ..T ...
O_brach    ... ... ... ..T ... ... ... ... ... ... ... ..A ... ... ..G ..T ..G
```

```
ND1                                                                     153
A_usam1    GGT TAT ATT CAA ATT CGA AAA GGT CCA AAT AAA GTT GGA TTT TTA GGT ATT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach    ... ... ... ... ... ... ... ... ... ... ..G ... ... ... .C. ... ...
P_luci1    ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ...
P_luci2    ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ...
O_phloiod  ... ... ... ..G ... ..T ... ... ... ... ... ... ..T ... G.T ... ...
O_brach    ..A ... ... ..G ..C ..T ... ... ... ... ... ... ..T ... G.T ... ...
```

```
ND1                                                                     204
A_usam1    CCT CAA CCT TTT AGT GAT GCT ATT AAA TTG ATT TGT AAG GAG CAA CCA ATT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach    .T. ... ... ... ... ... ... ... ... ..A G.. ... ... ... ... ... ..C
P_luci1    ... ... ... ... ... ... ... ... ... ..A ... .A. ..A ... ... ... ...
P_luci2    ... ... ... ... ... ... ... ... ... ..A ... .A. ..A ... ... ... ...
O_phloiod  ... ... ... ..C ... ... ... ... ... ..A ... ... ... ..A ..G ... ...
O_brach    ... ..G ... ... ... ... ... ... ..G ..A ... ... ..A ... ..G ... ...
```

```
ND1                                                                      255
A_usam1     CCT TTA ATA TCG AAT TAT TTA CTT TAT TAT TTT TCT CCT GTT TTT AAT TTA
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ..A ... ... ..G ... ... ... ... ... ... ... ... ... ...
A_euthy     ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1    ... ... ... ..A ... ... ..G ... ... ... ... ... ... ... ... ... ...
A_longi2    ... ... ... ..A ... ... ..G ... ... ... ... ... ... ... ... ... ...
P_arach     ... ... ... ... ..C ... ... A.. ... ... ... ... ... A.. ..A ... ...
P_luci1     ... ... ... ..A ..C ... ... G.. ... ... ... ... ... A.. ... ... ...
P_luci2     ... ... ... ..A ..C ... ... G.. ... ... ... ... ... A.. ... ... ...
O_phloiod   ... A.T ... ..A ... ... ... T.A ... ... ... ..A ... A.. ... ... ...
O_brach     ... A.T ... ..A ..C ... ... ..A ... ... ... ... ... A.. ... ... ...
```

```
ND1                                                                      306
A_usam1     ATA ATT TCT TTA GTT ATT TGG GTT ATT TTT CCT TAT TTA ACT TAT ATA TGT
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy     ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ...
A_longi1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach     ... ... ... ... ... ... ..A A.. ... ... ..A ... ... ... ... ... ...
P_luci1     ..G ... ... ... A.. ... ..A A.. ... ... ..A ... ... ... ... ... ...
P_luci2     ..G ... ... ... A.. ... ..A A.. ... ... ..A ... ... ... ... ... ...
O_phloiod   ..G ... ... ..G A.. G.. ... ... ... ... ... ... ... ... ... ... ...
O_brach     ... ... ... ... ... G.. ..A ... ... ... ... ... ..G ... ... ... ...
```

```
ND1                                                                      357
A_usam1     TCT TTT TCT TAT AGA TTT TTA TTT TTT TTA TGT TGT ACT AGA TTA GGT GTT
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy     ... ... .A. ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach     ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ..G
P_luci1     ... ... ... ... G.G ... ... ... ... ... ... ... ... ... ..T A.. ..A
P_luci2     ... ... ... ... G.G ... ... ... ... ... ... ... ... ... ..T A.. ..A
O_phloiod   ... ... ... ... G.T ... ..G ... ... ... ... ... ... ... ..G A.. ...
O_brach     ... ... ... ... G.T ... ... ... ... ... ... ... ... ... ..T ... ...
```

```
ND1                          372
A_usam1     TAT ACA GTT ATA ATT
A_usam2     ... ... ... ... ...
A_nguru     ... ... ... ... ...
A_euthy     ... ... ... ... ...
A_longi1    ... ... ... ... ...
A_longi2    ... ... ... ... ...
P_arach     ... .T. ... ... ...
P_luci1     ..C ... ... ... ...
P_luci2     ..C ... ... ... ...
O_phloiod   ... ..T ..A ... ...
O_brach     ... ..T ..G ... ...
```

Tab. 10: Sequence length and nucleotide content of the sequenced part of the 16S rRNA and tRNA-Leu genes for each specimen examined

Specimen	Sequence length	G	C	A	T	G & C	A & T
A_usam1	171 bp	25 (14.6%)	15 (8.8%)	59 (34.5%)	72 (42.1%)	40 (23.4%)	131 (76.6%)
A_usam2	171 bp	25 (14.6%)	15 (8.8%)	59 (34.5%)	72 (42.1%)	40 (23.4%)	131 (76.6%)
A_nguru	171 bp	26 (15.2%)	15 (8.8%)	57 (33.3%)	73 (42.7%)	41 (24.0%)	130 (76.0%)
A_euthy	173 bp	26 (15.0%)	15 (8.7%)	58 (33.5%)	74 (42.8%)	41 (23.7%)	132 (76.3%)
A_longi1	171 bp	26 (15.2%)	15 (8.8%)	57 (33.3%)	73 (42.7%)	41 (24.0%)	130 (76.0%)
A_longi2	171 bp	26 (15.2%)	15 (8.8%)	57 (33.3%)	73 (42.7%)	41 (24.0%)	130 (76.0%)
P_arach	174 bp	25 (14.4%)	15 (8.6%)	60 (34.5%)	74 (42.5%)	40 (23.0%)	134 (77.0%)
P_luci1	171 bp	27 (15,8%)	15 (8.8%)	55 (32.2%)	74 (43.3%)	42 (24.6%)	129 (75.4%)
P_luci2	171 bp	27 (15,8%)	15 (8.8%)	55 (32.2%)	74 (43.3%)	42 (24.6%)	129 (75.4%)
O_phloiod	174 bp	31 (17.8%)	16 (9.2%)	52 (29.9%)	75 (43.1%)	47 (27.0%)	127 (73.0%)
O_brach	174 bp	29 (16.7%)	19 (10.9%)	53 (30.5%)	73 (42.0%)	48 (27.6%)	126 (72.4%)

Tab. 11: Sequence length and nucleotide content of the sequenced part of the ND1 gene for each specimen examined

Specimen	Sequence length	G	C	A	T	G & C	A & T
A_usam1	372 bp	44 (11.8%)	33 (8.9%)	100 (26.9%)	195 (52.4%)	77 (20.7%)	295 (79.3%)
A_usam2	372 bp	44 (11.8%)	33 (8.9%)	100 (26.9%)	195 (52.4%)	77 (20.7%)	295 (79.3%)
A_nguru	372 bp	44 (11.8%)	34 (9.1%)	100 (26.9%)	194 (52.2%)	78 (21.0%)	294 (79.0%)
A_euthy	372 bp	45 (12.1%)	32 (8.6%)	100 (26.9%)	195 (52.4%)	77 (20.7%)	295 (79.3%)
A_longi1	372 bp	44 (11.8%)	34 (9.1%)	100 (26.9%)	194 (52.2%)	78 (21.0%)	294 (79.0%)
A_longi2	372 bp	44 (11.8%)	34 (9.1%)	100 (26.9%)	194 (52.2%)	78 (21.0%)	294 (79.0%)
P_arach	372 bp	46 (12.4%)	33 (8.9%)	104 (28.0%)	189 (50.8%)	79 (21.2%)	293 (78.8%)
P_luci1	372 bp	39 (10.5%)	34 (9.1%)	110 (29.6%)	189 (50.8%)	73 (19.6%)	299 (80.4%)
P_luci2	372 bp	39 (10.5%)	34 (9.1%)	110 (29.6%)	189 (50.8%)	73 (19.6%)	299 (80.4%)
O_phloiod	372 bp	48 (12.9%)	33 (8.9%)	93 (25.0%)	198 (53.2%)	81 (21.8%)	291 (78.2%)
O_brach	372 bp	49 (13.2%)	35 (9.4%)	91 (24.5%)	197 (53.0%)	84 (22.6%)	288 (77.4%)

ND5 (NADH-dehydrogenase subunit 5)

The sequenced parts of the ND5 gene are presented in figure 8. Both strands of each sequence were analysed and inaccuracies corrected. To improve the quality of the data, two additional primers were developed, aligning at each strand at approximately bp 400. Subsequently it is called here ND5 400 and ND5 400r. The total sequence length of the ND5 alignment is 1059 bp. Neither deletions nor insertions were required for multiple alignment. 243 sites were variable among all taxa (22.9%), including 69 variable sites at the first codon position (28.5% of all variable sites), 36 at the second (14.9%) and 137 at the third position (56.6%). Transversions occurred at 114 sites of the sequenced part (10.8%). Within the genus *Afrophlaeoba* 49 positions were variable (4.6%), including 12 transversions (1.1%). The AT-content of the ND5 sequence varies from 75.6% to 78.8% (table 12). In the amino acid sequence of ND5 93 variations (26.3%) can be inferred from the sequences (353 amino acids), but only 13 within the genus *Afrophlaeoba* (3.6%). The proportion of nonsynonymous substitutions within the genus *Afrophlaeoba* varies from 0.2 to 0.39, within *Parodontomelus* from 0.52 to 0.53 and within *Odontomelus* it is 0.38. Between the genera the proportion of nonsynonymous substitutions ranges from 0.51 to 0.65.

Fig. 8: Alignment of a part of the NADH-dehydrogenase subunit 5 (ND5) – 1059 bp

```
ND5                                                                   51
A_usam1     TCT TCT ACT CTT GTT ACT GCT GGT GTT TAT TTA TTA ATT CGT TTT AGT CCA
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
A_euthy     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach     ... ... ... ... ... ..A T.C ... ... ... ... ... ... ... ... ... ...
P_luci1     ... ... ... ... ... ..A ..C ... ... ... ... ... ... ... ... ... ...
P_luci2     ... ... ... ... ... ..A ..C ... ... ... ... ... ... ... ... ... ...
O_phloiod   ... ... ... ... ... ... ... ... ... ... ... ..G ... ..G ... ... ..T
O_brach     ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ..T
```

```
ND5                                                                        102
A_usam1     ATA TTG GAT TTA TAT AAT TAT GGT TGA TTT TTA TTG TTA GTT GGT TGT ATA
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ..G ... ... ... ... ... ... ... ... ... A.. ... ... ...
A_euthy     ... ..A ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1    ... ... ... ..G ... ... ... ... ... ... ... ... ... A.. ... ... ...
A_longi2    ... ... ... ..G ... ... ... ... ... ... ... ... ... A.. ... ... ...
P_arach     ..G ..A A.. ..G ... G.. ... ... ..G ... ... ..A ..G A.. ... ... ...
P_luci1     ... ..A A.. ... ... ... ... ... ... ... ... ..A ... A.. ... ... ...
P_luci2     ... ..A A.. ... ... ... ... ... ... ... ... ..A ... A.. ... ... ...
O_phloiod   ... ..A A.. A.T ... T.. .T. ... ..G ... ..G ..A A.. A.. ... ... ...
O_brach     ... ..A ... A.T ... GG. .T. ... ..G ... ... ..A A.. A.. ... ... ...
```

```
ND5                                                                        153
A_usam1     ACT ATA TTT ATA TCT GGT CTT GGT GCT AAT TTT GAG TTT GAC TTA AAA AAG
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
A_euthy     ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1    ..A ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
A_longi2    ..A ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
P_arach     ..G ... ... ... G.. ... ... ... ... ... ... ... ... ..T ... ... ..A
P_luci1     ... ... ... ... G.. ... ... ... ..A ... ... ... ... ..T ... ... ..A
P_luci2     ... ... ... ... G.. ... ... ... ..A ... ... ... ... ..T ... ... ..A
O_phloiod   ..A ..G ... ..G G.. ... ... ... ..A ... ... ..A ... ..T ..G ..G ..A
O_brach     ..G ... ..C ... G.. ... ... ... ... ... ... ..A ... ..T ... ... ..A
```

```
ND5                                                                        204
A_usam1     ATT ATT GCT CTT TCT ACT TTA AGT CAA CTT GGT TTA ATA ATG AGA ATT TTA
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ..A ... ... ... ... ... ..A ... ... ...
A_euthy     ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ...
A_longi1    ... ... ... ... ... ... ... ..A ... ... ... ... ... ..A ... ... ...
A_longi2    ... ... ... ... ... ... ... ..A ... ... ... ... ... ..A ... ... ...
P_arach     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_luci1     ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ...
P_luci2     ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ...
O_phloiod   ... ... ... T.A ... ... ... ... ..G ... ... ... ... G.. ... ... ...
O_brach     ... ... ... T.A ... ... ... ... ..G ... ... ... ... ..A ... ... ..G
```

```
ND5                                                                        255
A_usam1     TCT ATA GGT TAT TCT AAT CTT GCT TTT TTT CAT TTA TTA ACT CAT GCT TTG
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
A_euthy     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
A_longi1    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
A_longi2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A
P_arach     ... ... ..A ... CTA ... ... ... ... ... ... ... ... T.. ... ... ..A
P_luci1     ... ... ... ... ... ..G ... ... ... ... ... A.. ... T.. ... ... ..A
P_luci2     ... ... ... ... ... ..G ... ... ... ... ... A.. ... T.. ... ... ..A
O_phloiod   ..A ... ... ... C.A .GG ... ... ... ... ... ..G ... G.. ... ... ..A
O_brach     ..A ... ... ... ..A ..G ... ... ... ... ... ... ... G.. ... ... ..A
```

```
ND5                                                                        306
A_usam1     TTT AAG GCA TTA TTA TTT ATA TGT GCA GGT TCA ATA ATT CAT AAT TTA AAG
A_usam2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru     ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
A_euthy     ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
A_longi1    ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
A_longi2    ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ...
P_arach     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_luci1     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_luci2     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
O_phloiod   ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..T
O_brach     ... ..A ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ..T
```

ND5																	357
A_usam1	GAT	TCT	CAG	GAT	ATT	CGT	TTT	ATA	GGT	TCT	ATT	GTT	AAT	TTT	ATG	CCT	TTA
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...
A_euthy	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...
A_longi2	...	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...
P_arach	...	...	..A	...	...	...	...	..G	...	..A	...	...	...	...	...	...	...
P_luci1	...	...	...	...	...	...	...	...	...	..A	...	...	...	..C	..A	...	...
P_luci2	...	...	...	...	...	...	...	...	...	..A	...	...	...	..C	..A	...	...
O_phloiod	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	..A	..A	..G
O_brach	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	..A	..A	..G

ND5																	408
A_usam1	ACT	TCT	GTT	TGT	TTT	AGT	GTT	TCT	AGA	TTA	TCT	TTA	TGT	GGT	ATG	CCT	TTT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	.A.	...	...	...	...	..A	...	...	...	...	...	...
A_euthy	...	...	...	...	...	.A.	...	...	...	...	..A	..G	...	...	...	...	...
A_longi1	...	...	...	...	...	.A.	...	...	...	...	..A	...	...	...	..A	...	...
A_longi2	...	...	...	...	...	.A.	...	...	...	...	..A	...	...	...	..A	...	...
P_arach	...	...	A..	...	...	.A.	..C	..A	...	A..	..A	...	...	...	..A	...	...
P_luci1	...	...	A..	...	...	.A.	...	..A	...	A..	..A	...	...	...	..A	...	...
P_luci2	...	...	A..	...	...	.A.	...	..A	...	A..	..A	...	...	...	..A	...	...
O_phloiod	...	...	...	...	...	.A.	...	...	..T	...	...	...	...	..A	..A	..A	...
O_brach	...	...	...	...	...	.A.	...	...	..C	..G	...	...	...	...	..A	..A	...

ND5																	459
A_usam1	TTA	GCT	GGT	TTT	TAT	TCA	AAG	GAT	TTA	ATT	CTT	GAG	ATA	GTT	TGT	TTA	AGA
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	...	...
A_euthy	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	..G	...
A_longi2	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	..G	...
P_arach	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...
P_luci1	...	..G	...	...	...	...	.G.	...	...	...	...	..T	...	...	...	..T	...
P_luci2	...	..G	...	...	...	...	.G.	...	...	...	...	..T	...	...	...	..T	...
O_phloiod	...	...	...	...	...	...	...	...	...	...	...	...	..G	..C	...	A.G	...
O_brach	..G	...	..A	...	...	...	...	...	...	...	...	...	..G	...	...	...	...

ND5																	510
A_usam1	TGG	GTT	AAT	TAT	TTT	ATT	TAT	TTT	TTA	TTT	TTT	TTT	TCA	ACT	GGT	TTA	ACA
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_euthy	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P_arach	...	...	...	.T.	...	...	.T.	...	...	...	...	...	..T	...	...	...	...
P_luci1	...	...	...	...	..A	...	.T.	A..	..G	...	...	...	..T	...	...	...	...
P_luci2	...	...	...	...	..A	...	.T.	A..	..G	...	...	...	..T	...	...	...	...
O_phloiod	..A	A..	...	.G.	..A	...	.T.	...	...	...	..C	...	..T	...	...	...	..G
O_brach	..A	A..	...	.G.	..A	...	.T.	...	...	...	..C	...	..T	...	...	...	...

ND5																	561
A_usam1	GCT	TCA	TAT	TCT	TTT	CGT	TTA	TTT	TAT	TAT	TCT	ATA	TCT	GGT	GAT	TAT	AAT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C
A_euthy	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C
A_longi2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C
P_arach	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...
P_luci1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...
P_luci2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...
O_phloiod	...	..T	...	...	...	...	C..	...	...	...	...	...	...	...	...	A..	...
O_brach	...	..T	...	...	...	...	...	...	...	...	..A	...	...	...	...	A..	...

ND5																	612
A_usam1	TTT	AAT	TCT	AGT	TTT	TCT	TTT	AGT	GAT	GAT	GGT	TAT	TAT	ATT	TCT	TTC	GGT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	.A.	...	...	.A.	...	...	...	...	..T	...
A_euthy	.A.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	..T	...
A_longi2	...	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	..T	...
P_arach	...	T.C	...	...	C..	...	...	.A.	...	A.G	...	...	...	...	...	..T	...
P_luci1	...	T..	...	...	...	...	...	GA.	...	A.G	...	...	...	...	...	..T	...
P_luci2	...	T..	...	...	...	...	...	GA.	...	A.G	...	...	...	...	...	..T	...
O_phloiod	..C	T..	C..	G..	...	.G.	...	.A.	...	A..	...	...	...	...	..A	..T	...
O_brach	...	T..	C.A	.T.	...	.G.	...	GA.	...	A.G	...	...	...	...	..A	..T	...

ND5																	663
A_usam1	ATA	ATT	TCT	TTA	CTT	TTT	ATT	GCT	GTT	TTT	GGT	GGG	AGA	ATG	TTA	TCT	TGA
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	A..	...	...	...	...	...	..A	...	...	...
A_euthy	...	...	...	...	...	...	...	A..	...	...	...	...	...	..A	...	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	..A	...	..A	...	...	...
A_longi2	...	...	...	...	...	...	...	...	...	...	...	..A	...	..A	...	...	...
P_arach	...	...	G..	...	T.G	...	G..	...	...	...	...	..A	...	T.T	...	...	...
P_luci1	...	G..	...	...	T.G	...	...	...	...	...	...	..A	...	T.T	...	...	...
P_luci2	...	...	...	...	T.G	...	...	...	...	...	...	..A	...	T.T	...	...	...
O_phloiod	...	...	G..	...	T.A	...	G..	...	..A	...	..G	..T	...	T.T	..G	...	...
O_brach	...	...	G..	..G	T.G	...	G..	...	..A	...	...	..T	...	T.T	...	...	...

ND5																	714
A_usam1	TTA	ATT	CTT	CCT	ATT	CCT	TAT	GTA	ATT	GTT	TTA	CCT	ATT	TAT	TTG	AAG	TTT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A.A	...	...
A_euthy	...	...	...	..A	...	...	...	A..	...	...	...	...	G..	...	..A	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...	...
A_longi2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...	...
P_arach	...	...	T..	...	...	...	...	A..	...	...	...	..A	.A.	..C	..A	...	...
P_luci1	...	...	T..	...	...	...	...	A..	...	...	...	..A	.A.	...	..A	...	...
P_luci2	...	...	T..	...	...	...	...	A..	...	...	...	..A	.A.	...	..A	...	...
O_phloiod	...	...	T..	...	...	...	...	A..	...	...	...	...	.G.	...	..A	...	...
O_brach	...	...	T.C	...	...	...	...	A..	...	...	...	...	.G.	...	..A	...	...

ND5																	765
A_usam1	ATA	ACT	ATT	ATT	GTT	GTT	ATA	ATA	GGT	TCG	TAT	TTA	GGT	TAT	ATT	ATC	TTT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	G..	...	...	...	..G	...	...	..A	...	...	...	...	...	...	...
A_euthy	...	...	...	...	...	...	...	...	...	..A	...	A..	...	...	...	...	...
A_longi1	...	...	G..	...	...	...	..G	...	...	..A	...	...	...	...	...	...	...
A_longi2	...	...	G..	...	...	...	..G	...	...	..A	...	...	...	...	...	...	...
P_arach	...	..A	...	T..	...	...	...	T..	...	..A	...	...	...	...	..A	..A	.C.
P_luci1	...	..A	...	G..	...	..C	...	T..	...	..A	...	...	...	...	..A	..G	.C.
P_luci2	...	..A	...	G..	...	..C	...	T..	...	..A	...	...	...	...	..A	..G	.C.
O_phloiod	T..	..A	...	T..	AC.	...	T..	T..	...	..A	...	..T	...	...	T..	..T	.C.
O_brach	T..	..A	..A	...	AC.	...	T..	T..	...	..A	...	A.T	...	...	T..	..T	.C.

ND5																	816
A_usam1	AAT	TTA	ATT	TCT	TTT	AAT	TAT	TTA	TTT	TCT	TTA	AGT	ATA	TTA	CCT	ATT	TTT
A_usam2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_nguru	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C
A_euthy	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
A_longi2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P_arach	...	A.G	GA.	.T.	..A	.G.	A..	...	...	...	...	G..	...	...	T..	T..	G..
P_luci1	...	A..	.A.	.T.	..A	.G.	A..	...	...	...	...	GA.	...	...	T..	TA.	G..
P_luci2	...	A..	.A.	.T.	..A	.G.	A..	...	...	...	...	GA.	...	...	T..	TA.	G..
O_phloiod	...	A.T	G..	.T.	...	T..	G.C	C.T	...	...	...	.AA	..T	..G	T..	T..	G..
O_brach	.G.	G.T	GA.	.T.	.A.	T..	G..	C.T	...	...	...	.AA	..G	..G	T..	T..	G..

```
ND5                                                                    867
A_usam1    AGA TTT TTT GGT TCT ATA TGA TTT ATA CCT TTT CTT TCA ACT ATT TTT ATT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... ... ... ..G ... .G. ... ...
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ...
A_longi2   ... ... ... ... ... ... ... ... ... ... ... ... ... ... .G. ... ...
P_arach    ... ... ... ... ... ... ... ... ... ... ... ... ..T ... .G. ... ...
P_luci1    .A. ... ... ... ... ... ... ... ... ... ... ... ..T ... .G. ... ...
P_luci2    .A. ... ... ... ... ... ... ... ... ... ... ... ..T ... .G. ... ...
O_phloiod  ... ... G.. ... ... ... ..G ... ..G ... ..C ... ... ..A .GA ... ...
O_brach    ... ... G.. ... ... ... ... ... ... ... ... ... ... ..A .A. ... G..
```

```
ND5                                                                    918
A_usam1    AGA TAT TTT CCT TTA AGG ATT GGA TAT TAT TCT TCG AAG ATT TTA GAT TAT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ... ...
A_nguru    ... ... ... ... ... ... ... ..T ... ... ..A ..A ... .C. ... ... ...
A_euthy    ... ... ... ... ... ... ... ..T ... ... ..A ..A ... .C. ... ... ...
A_longi1   ... ... ... ... ... ... ... ..T ... ... ..A ..A ... .C. ... ... ...
A_longi2   ... ... ... ... ... ... ... ..T ... ... ..A ..A ... .C. ... ... ...
P_arach    ... ... ... A.. ... .A. ... ..T ... A.. ..A .TT ..A .C. ... ... ...
P_luci1    ... ... ... T.. ... .A. G.. ..T ... A.. ..A .TT ..A .C. ... ... ...
P_luci2    ... ... ... T.. ... .A. G.. ..T ... A.. ..A .TT ..A .C. ... ... ...
O_phloiod  ... ... ... ... ... .A. T.G ..T ... ... ..A ..A ... TC. ..T ... ...
O_brach    ... ..C ... ... ... .A. T.G ..T ... ... ..A .TA ... TC. ..T ... .G.
```

```
ND5                                                                    969
A_usam1    GGT TGG GGT GAA ATA TTA GGT GGT CAG GGT TTA TAT AGA TTA TTT GTT TAT
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_euthy    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_longi2   ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P_arach    ... ..A ... ... T.. ... ... ..A ... ... ... ... ... ... ... ... ...
P_luci1    ... ..A ... ... T.. ..T ... ..A ... ... ... ... ... ... ... ... ...
P_luci2    ... ..A ... ... T.. ..T ... ..A ... ... ... ... ... ... ... ... ...
O_phloiod  ... ... ... ... T.T ..T ... ... ..A ... ... ... .AT ..G ... A.. ...
O_brach    ... ..A ... ... T.T ..T ... ... ..A ..C ..G ... .AT ... ... A.. ...
```

```
ND5                                                                   1020
A_usam1    ATA ATT AAA TAT ATT CAG GAT TGA TAT GAT TTT AAT TTT AAG ATT TAT TTG
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ..A .G. ... ... ... ... ... ... ... ... ... ...
A_euthy    G.G ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ...
A_longi1   ... ... ... ... ... ..A .G. ... ... ... ... ... ... ... ... ... ...
A_longi2   ... ... ... ... ... ..A .G. ... ... ... ... ... ... ... ... ... ...
P_arach    ... ... ... ... ... ..A ... ... ... ... ..A ... ... .G. ... ... ...
P_luci1    ... ... ... ... ... ..A ... ... ... ... ..A ... ... .G. ... ... ...
P_luci2    ... ... ... ... ... ..A ... ... ... ... ..A ... ... .G. ... ... ...
O_phloiod  T.T ... GGT ... G.. ... TC. ... ... ... .CA ..C ... ... ... ... ..A
O_brach    T.T ... ..T ... ... ... TG. ... ... ... .C. ... ... ... ... ... ..A
```

```
ND5                                                    1059
A_usam1    TTG ACT TTT ATT TTT TGA ATG TTT ATT TTA GTT TTA CTG
A_usam2    ... ... ... ... ... ... ... ... ... ... ... ... ...
A_nguru    ... ... ... ... ... ... ... ... ... ... A.. ... ..A
A_euthy    ..A ... ... ... ... ... ... ... ... ... A.. ... ...
A_longi1   ... ... ... ... ... ... ... ... ... ... A.. ... ..A
A_longi2   ... ... ... ... ... ... ... ... ... ... A.. ... ..A
P_arach    ..A .T. ... ... ... ..G ... ... ... ... A.. ... T.A
P_luci1    ..A .T. ... ... ... ..G ... ... ... ... A.. ... T.A
P_luci2    ..A .T. ... ... ... ..G ... ... ... ... A.. ... T.A
O_phloiod  ..A ... ... ... ... ... ... ... G.. ... ..G AG. T.A
O_brach    ..A ... ... ... ... ... ... ... ... ..G ... AG. T.A
```

Tab. 12: Sequence length and nucleotide content of the sequenced part of the ND5 gene for each specimen examined

Specimen	Sequence length	G	C	A	T	G & C	A & T
A_usam1	1059 bp	150 (14.2%)	92 (8.7%)	255 (24.1%)	562 (53.1%)	242 (22.9%)	817 (77.1%)
A_usam2	1059 bp	150 (14.2%)	93 (8.8%)	255 (24.1%)	561 (53.0%)	243 (22.9%)	816 (77.1%)
A_nguru	1059 bp	145 (13.7%)	93 (8.8%)	264 (24.9%)	557 (52.6%)	238 (22.5%)	821 (77.5%)
A_euthy	1059 bp	143 (13.5%)	94 (8.9%)	266 (25.1%)	556 (52.5%)	237 (22.4%)	822 (77.6%)
A_longi1	1059 bp	145 (13.7%)	92 (8.7%)	265 (25.0%)	557 (52.6%)	237 (22.4%)	822 (77.6%)
A_longi2	1059 bp	145 (13.7%)	92 (8.7%)	265 (25.0%)	557 (52.6%)	237 (22.4%)	822 (77.6%)
P_arach	1059 bp	144 (13.6%)	88 (8.3%)	274 (29.9%)	553 (52.2%)	232 (21.9%)	827 (78.1%)
P_luci1	1059 bp	139 (13.1%)	86 (8.1%)	282 (26.6%)	552 (52.1%)	225 (21.2%)	834 (78.8%)
P_luci2	1059 bp	138 (13.0%)	86 (8.1%)	283 (26.7%)	552 (52.1%)	224 (21.2%)	835 (78.8%)
O_phloiod	1059 bp	161 (15.2%)	97 (9.2%)	244 (23.0%)	557 (52.6%)	258 (24.4%)	801 (75.6%)
O_brach	1059 bp	155 (14.6%)	94 (8.9%)	254 (24.0%)	556 (52.5%)	249 (23.5%)	810 (76.5%)

A-T Bias

Systematic error of phylogenetic analyses can result from models assuming the same equilibrium base frequencies in all lineages (Swofford et al. 1996). The A-T bias for the complete data set varies in a comparative narrow range from 75.3% to 77.9%. The variability is even lower within the genera: For *Afrophlaeoba* the A-T bias ranges from 76.8% to 77.2%, for *Parodontomelus* it varies from 77.3% to 77.9% and for *Odontomelus* from 75.3% to 75.5%. The complete variation between taxa and genes ranges from 72.0% to 80.4%, but within a single gene fragment the variability is lower. The high A-T bias is rather typical for insect mtDNA (Li 1997), although in the leaf beetle genus *Neochlamisus* a low A-T bias (63%) has been recorded (Funk 1999).

4.3.2 Paralogous or Orthologous?

It is of high interest for phylogenetic inference, whether the sequences under study are orthologous (at the same locality of the genome) or paralogous (copied to another part of the genome). Since Bensasson et al. (2000) found a high number of pseudogenes in the grasshopper genus *Podisma*, it has to be discussed, whether pseudogenes are also present in African Acrididae. A transcription of the ND1 and ND5 data sets to amino acid sequences resulted in low numbers of amino acid substitutions (16 of 477 in *Afrophlaeoba*). The absence of indels and the high proportion of silent substitutions support the hypothesis that the compared fragments are orthologous. The 12S rRNA specifying gene was compared with the highly conserved regions identified by Hickson et al. (1996). All substitutions occurred at sites, which are known to be variable. Thus it can be concluded that the 12S rRNA data set is also orthologous.

4.3.3 Homoplastic Content

The homoplastic content of the data sets was estimated with two methods, the calculation of the skewness of one million random trees and the proportion of transitions among all substitutions observed. Since the homoplastic content proved to be low for all data sets, only the complete data set is presented in detail. The basic values, however, are given for all data sets.

Skewness

The tree length distribution and skewness was calculated unweighted and weighted for each data set (12S rRNA, 16S rRNA + tRNA-Leu, ND1, ND5) and for the combined data set (table 13). The distribution of random tree lengths is skewed left for all data sets, indicating a low homoplastic content. The value even decreases, if the transversions are weighted higher than the transitions. For the combined data set, which was used for the phylogenetic analysis, the g1 value is -0.984876, indicating a rather low homoplastic content. Since the inclusion of two data sets of one species might increase the skewed left character of a distribution, the calculation was also performed excluding A_usam2, A_longi2 and P_luci2. This exclusion did not increase the skewness as well. The tree length distribution of the combined data set is presented in figure 9.

Tab 13: Skewness (g1) for one million random trees calculated from the different gene parts.

Gene fragment	Weighting	Mean	Standard deviation	g1
12S rRNA	none	109.1	6.9	-1.40
	transversions 2x	133.3	8.4	-1.63
	transversions 5x	205.7	13.4	-2.01
	transversions 10x	326.6	22.0	-2.22
16S rRNA / tRNA-Leu	none	51.1	3.3	-1.49
	transversions 2x	74.0	4.8	-1.70
	transversions 5x	142.8	9.6	-1.84
	transversions 10x	257.4	17.7	-1.88
ND1	none	143.3	10.4	-0.91
	transversions 2x	192.3	13.9	-1.16
	transversions 5x	339.4	25.4	-1.63
	transversions 10x	584.3	45.3	-1.89
ND5	none	563.7	40.2	-0.89
	transversions 2x	829.1	63.4	-0.89
	transversions 5x	1624.3	133.1	-0.90
	transversions 10x	2949.7	250.4	-0.90
Combined data set	none	867.2	59.9	-0.98
	transversions 2x	1228.4	89.4	-1.05
	transversions 5x	2311.5	179.2	-1.14
	transversions 10x	4115.8	328.7	-1.16
Deletion of single taxa (combined data set)	without double species (2s)	707.8	50.5	-1.08
	without *Odontomelus*	485.1	49.9	-1.40
	without *Parodontomelus*	535.3	44.6	-2.52
	without 2s and *Odontomelus*	332.0	29.3	-1.95
	without 2s and *Parodontomelus*	486.0	51.4	-2.00

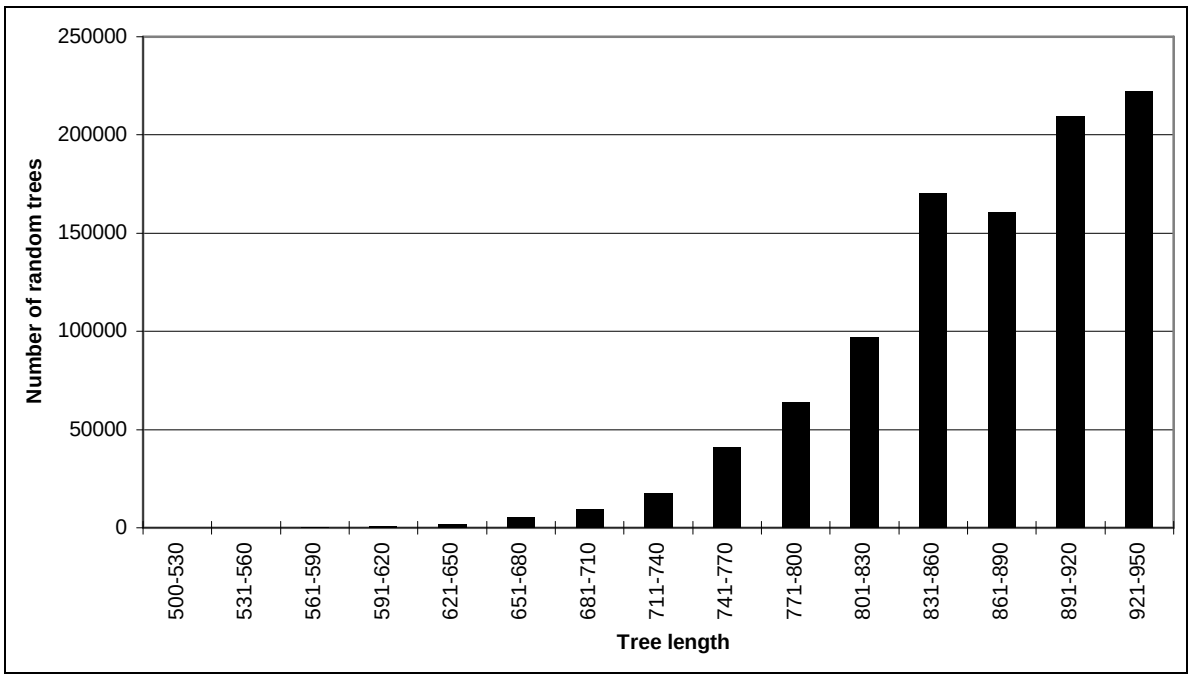


Fig. 9: Tree length distribution for the combined data set (unweighted), g1 = -0.984876

Proportion of Transitions

The proportion of transitions among all substitutions is shown for each sequence separately to emphasize the differences between the genes under study. Figure 10 and table 14 illustrate the proportion of transitions for the 12s rRNA data set. Within the genus *Afrophlaeoba* exclusively transitions have been found and the J-C-distances are quite low (0.009 to 0.032). One transition occurs between the two specimens of *A. longicornis* collected at Ilonga and Kilosa (J-C-distance 0.003), while the number of intrageneric transitions ranges from three to eleven. Although the J-C-distance within the other two genera (0.026) is quite similar to the distance within *Afrophlaeoba*, the proportion of transitions in *Odontomelus* (0.78) and *Parodontomelus* (0.56) is substantially lower. This is probably caused by stochastic effects due to the low number of variable sites within a genus (nine substitutions). Since the number of possible transversions is twice as high as the number of possible transitions, the proportion of transitions can decrease to 0.3, if multiple substitutions occurred over a long period of time. This trend is also visible in figure 10. While the J-C-distance within the Phlaeobini increases (0.047-0.062), the proportion of transitions decreases (0.69-0.86). Within the Acridinae the J-C-distance varies from 0.065 to 0.096 and the proportion of transitions decreases to 0.44-0.76. The high proportion of transitions within the genus *Afrophlaeoba* and the left skewed distribution indicate that the homoplastic content of the 12S rRNA is low and the data are suitable for a phylogenetic analysis. The low distances and the low number of informative sites suggest to combine the data set with the others.

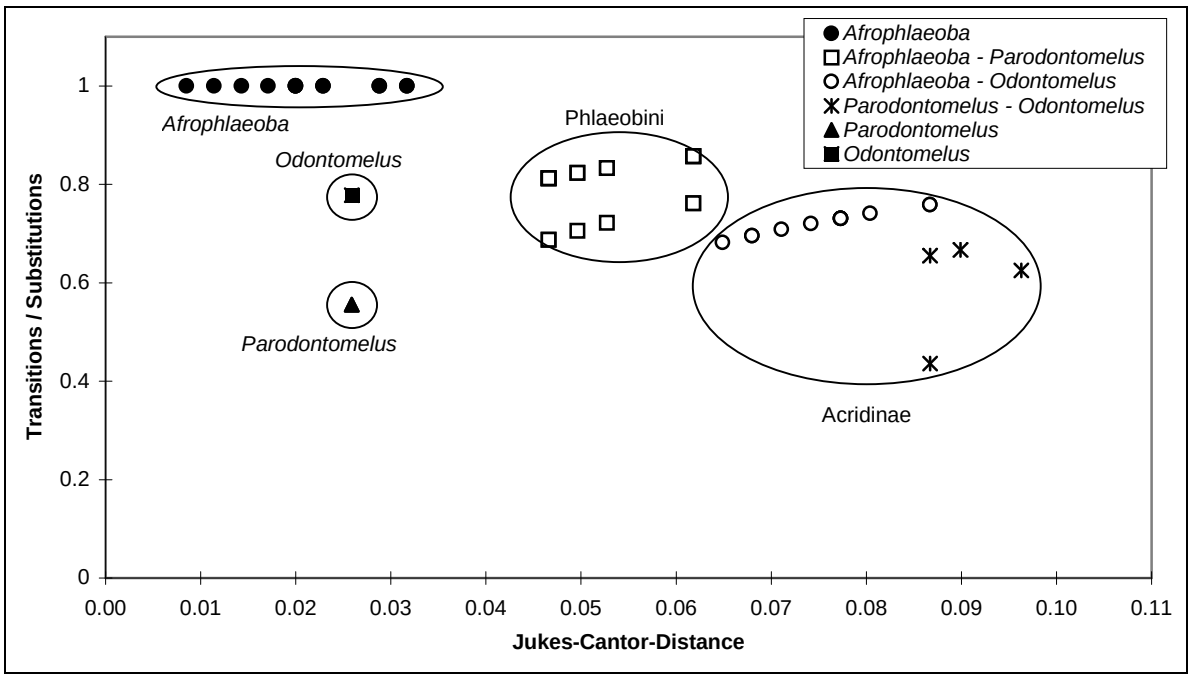


Fig. 10: Proportion of transitions of all substitutions for the 12S rRNA data set.

Tab. 14: Substitutions of the 12S rRNA fragment between each taxon studied. Upper right matrix: proportion of transitions, lower left matrix: absolute number of transitions and transversions (ns/nv).

	A_usam1	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod	O_brach
A_usam1			1	1	1	1	0.76	0.86	0.86	0.73	0.76
A_usam2	0/0		1	1	1	1	0.76	0.86	0.86	0.73	0.76
A_nguru	8/0	8/0		1	1	1	0.69	0.81	0.81	0.68	0.72
A_euthy	7/0	7/0	3/0		1	1	0.69	0.81	0.81	0.7	0.73
A_longi1	11/0	11/0	5/0	6/0		1	0.71	0.82	0.82	0.7	0.73
A_longi2	10/0	10/0	4/0	7/0	1/0		0.72	0.83	0.83	0.71	0.74
P_arach	16/5	16/5	11/5	11/5	12/5	13/5		0.56	0.56	0.44	0.63
P_luci1	18/3	18/3	13/3	13/3	14/3	15/3	5/4			0.66	0.67
P_luci2	18/3	18/3	13/3	13/3	14/3	15/3	5/4	0/0		0.66	0.67
O_phloiod	19/7	19/7	15/7	16/7	16/7	17/7	17/12	19/10	19/10		0.78
O_brach	22/7	22/7	18/7	19/7	19/7	20/7	20/12	20/10	20/10	7/2	

The trend of a decreasing proportion of transitions with increasing J-C-distance is also visible for the ND1 data set (figure 11, table 15), but in comparison to the 12S rRNA data set the J-C-distance within *Afrophlaeoba* is lower (0.008-0.014). The proportion of transitions ranges from 0.75 to 1, but the number of substitutions is low (3-5). No substitutions were found between *A. nguru* and *A. longicornis* or intraspecific. Within *Odontomelus* the J-C-distance is 0.059 and the proportion of transitions 0.86 (18 of 21 substitutions). In *Parodontomelus* twenty transitions were found among 23 substitutions (0.87) and the J-C-distance is 0.65. Among the Phlaeobini the pairwise J-C-distances ranges from 0.07 to 0.082 and the proportion of transition decreases (0.70-0.79). In the Acridinae the J-C-distance increases to 0.098-0.132 and the proportion of transitions varies from 0.46 to 0.58. This indicates that the homoplastic content of the data is low for the genus *Afrophlaeoba* and even for the Phlaeobini, but it increases in the case of the Acridinae.

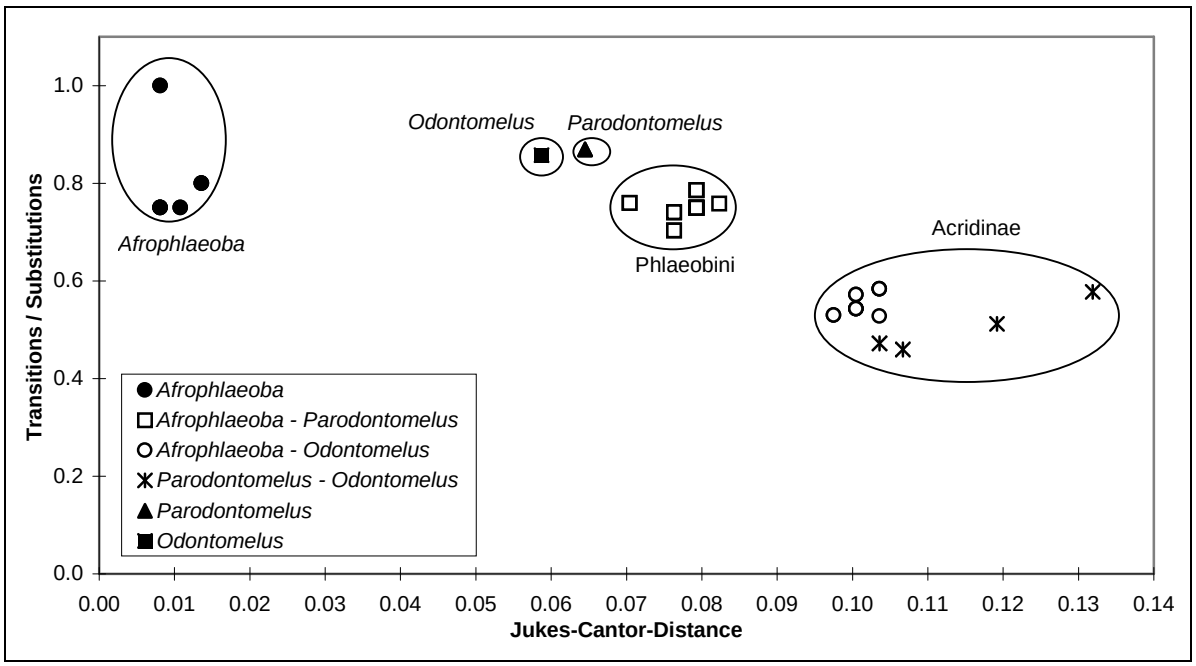


Fig. 11: Proportion of transitions of all substitutions for the ND1 data set.

Tab. 15: Substitutions of the ND1 fragment between each taxon studied. Upper right matrix: proportion of transitions, lower left matrix: absolute number of transitions and transversions (ns/nv).

	A_usam1	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod	O_brach
A_usam1			1	0.75	0.75	0.75	0.76	0.74	0.74	0.57	0.53
A_usam2	0/0		1	0.75	0.75	0.75	0.76	0.74	0.74	0.57	0.53
A_nguru	3/0	3/0		0.8			0.79	0.75	0.75	0.58	0.54
A_euthy	3/1	3/1	4/1		0.8	0.8	0.76	0.7	0.7	0.54	0.53
A_longi1	3/1	3/1	0/0	4/1			0.79	0.75	0.75	0.58	0.54
A_longi2	3/1	3/1	0/0	4/1	0/0		0.79	0.75	0.75	0.58	0.54
P_arach	19/6	19/6	22/6	21/7	22/6	22/6		0.87	0.87	0.58	0.51
P_luci1	20/7	20/7	21/7	19/8	21/7	21/7	20/3			0.46	0.47
P_luci2	20/7	20/7	21/7	19/8	21/7	21/7	20/3	0/0		0.46	0.47
O_phloiod	20/15	20/15	21/15	19/16	19/16	19/16	26/19	17/20	17/20		0.86
O_brach	18/16	18/16	19/16	19/17	19/17	19/17	21/20	17/19	17/19	18/3	

The proportion of transitions for the 16S rRNA and tRNA-Leu is shown in figure 12 and table 16. In this data set the proportion of transition decreases rapidly with the increasing J-C-distance, although the distances are generally small. This indicates that the fragment consists of large conserved regions and a small amount of rapidly evolving sites (Funk 1999). Within *Afrophlaeoba* the pairwise J-C-distance ranges from 0.006 to 0.012, which are the lowest distances measured. The proportion of transition is always 1, but the number of substitutions is only 1-2. No substitutions occur between *A. euthynota* and *A. usambarica* or within a species. In *Odontomelus* the J-C-distance is 0.026 and the proportion of transitions 0.63 (five of eight substitutions). In *Parodontomelus* the J-C-distance is 0.018 and the proportion of transitions 0.67 (two of three substitutions). Within the Phlaeobini the J-C-distance ranges from 0.018 to 0.05. The proportion of transitions varies from 0.33 to 0.63, which is probably caused by the low distances. In the Acridinae the J-C-distance ranges from 0.083 to 0.111 and the proportion of transitions from 0.47 to 0.71. Since only one informative site for the genus *Afrophlaeoba* can be found on this sequence, it doesn't contribute much to the phylogenetic inference.

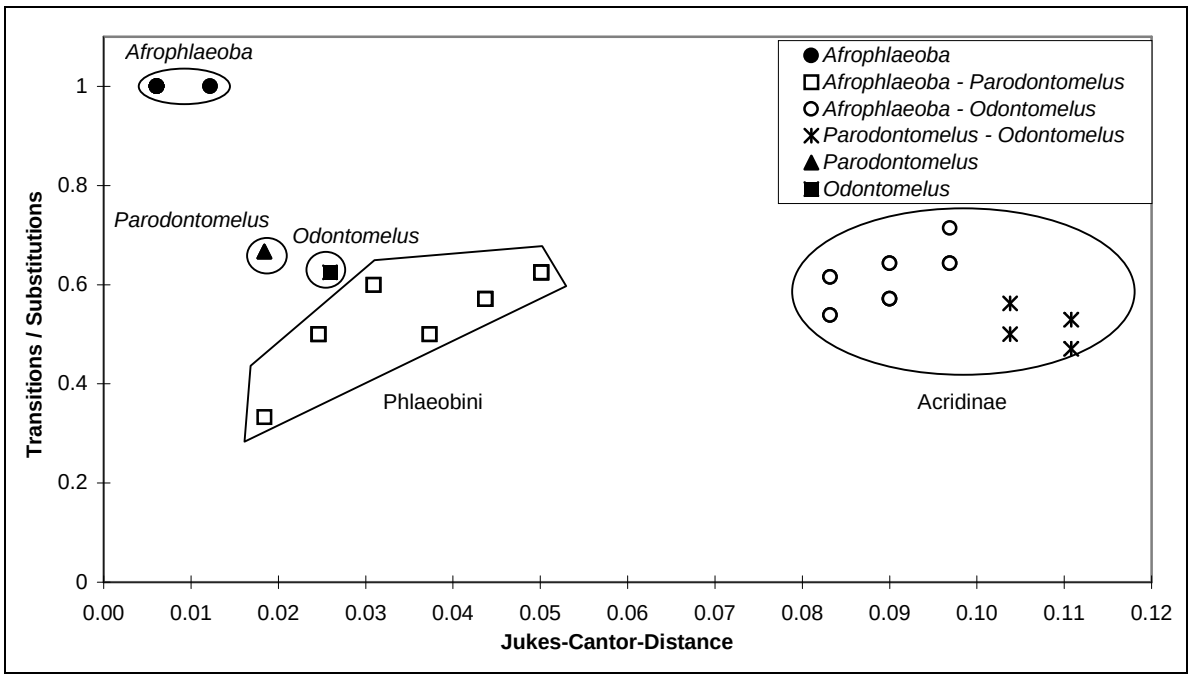


Fig. 12: Proportion of transitions of all substitutions for the 16S rRNA + tRNA-Leu data set.

Tab. 16: Substitutions of the 16S rRNA + tRNA-Leu fragment between each taxon studied. Upper right matrix: proportion of transitions, lower left matrix: absolute number of transitions and transversions (ns/nv).

	A_usam1	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod	O_brach
A_usam1			1		1	1	0.5	0.57	0.57	0.57	0.64
A_usam2	0/0		1		1	1	0.5	0.57	0.57	0.57	0.64
A_nguru	1/0	1/0		1	1	1	0.33	0.5	0.5	0.64	0.71
A_euthy	0/0	0/0	1/0		1	1	0.5	0.57	0.57	0.57	0.64
A_longi1	1/0	1/0	2/0	1/0			0.6	0.63	0.63	0.54	0.62
A_longi2	1/0	1/0	2/0	1/0	0/0		0.6	0.63	0.63	0.54	0.62
P_arach	2/2	2/2	1/2	2/2	3/2	3/2		0.67	0.67	0.5	0.56
P_luci1	4/3	4/3	3/3	4/3	5/3	5/3	2/1			0.47	0.53
P_luci2	4/3	4/3	3/3	4/3	5/3	5/3	2/1	0/0		0.47	0.53
O_phloiod	8/6	8/6	9/5	8/6	7/6	7/6	8/8	8/9	8/9		0.63
O_brach	9/5	9/5	10/4	9/5	8/6	8/6	9/7	9/8	9/8	5/3	

The ND5 data set proved to have the highest sequence divergences. Thus the proportion of transitions is also lower than in the 12S rRNA or ND1 data set (figure 13, table 17). An extreme low J-C-distance of 0.010 (10 substitutions) was found between *A. longicornis* and *A. nguru*, while the distances between all other species varied from 0.026 to 0.034 (27-35 substitutions). The proportion of transitions ranges from 0.70 to 0.79, but reaching 0.90 between *A. nguru* and *A. longicornis*. One transition occurred between the two specimens of *A. usambarica* and within *Parodontomelus luci* (J-C-distance 0.001). In the genus *Odontomelus* the J-C-distance was 0.075 and the proportion of transitions 0.71 (54 of 76 substitutions). Between the two *Parodontomelus* species the J-C-distance varied from 0.043 to 0.044 and the proportion of transitions from 0.61 to 0.62 (44 to 45 substitutions). The J-C-distance among all Phlaeobini studied amounted from 0.090 to 0.100 with a proportion of transitions varying from 0.48 to 0.55. In the Acridinae the J-C-distance was 0.140-0.165 and the proportion of transitions varied from 0.48 to 0.51. The higher distances and lower proportion of

transitions of the ND5 data set is consistent with a higher ratio of nonsynonymous substitutions (see above). Although the homoplastic content of the ND5 gene is higher than in 12S rRNA and ND1, the skewness and the proportion of transitions indicate that the quality of the data is high enough to include it in the analysis. Since the number of informative sites and the sequence length also is higher in ND5, this data set contributes most to the phylogenetic inference.

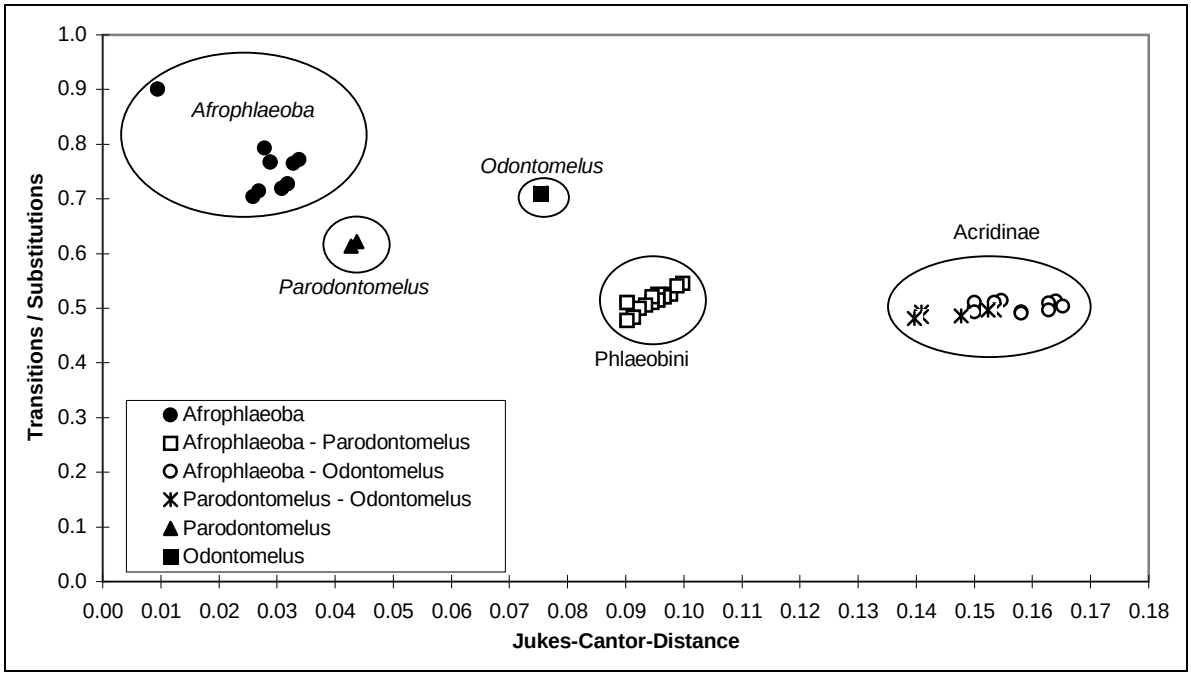


Fig. 13: Proportion of transitions of all substitutions for the ND5 data set.

Tab. 17: Substitutions of the ND5 fragment between each taxon studied. Upper right matrix: proportion of transitions, lower left matrix: absolute number of transitions and transversions (ns/nv):

	A_usam1	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod	O_brach
A_usam1		1	0.77	0.71	0.73	0.73	0.55	0.53	0.52	0.51	0.51
A_usam2	1/0		0.76	0.7	0.72	0.72	0.54	0.52	0.52	0.51	0.51
A_nguru	27/8	26/8		0.79	0.9	0.9	0.53	0.52	0.51	0.5	0.5
A_euthy	20/8	19/8	23/6		0.77	0.77	0.52	0.51	0.5	0.5	0.5
A_longi1	24/9	23/9	9/1	23/7			0.51	0.48	0.48	0.49	0.49
A_longi2	24/9	23/9	9/1	23/7	0/0		0.51	0.48	0.48	0.49	0.49
P_arach	54/45	53/45	50/45	49/45	46/44	46/44		0.62	0.61	0.49	0.49
P_luci1	51/46	50/46	49/46	47/46	44/47	44/47	28/17		1	0.5	0.49
P_luci2	50/46	49/46	48/46	46/46	43/47	43/47	27/17	1/0		0.5	0.48
O_phloiod	80/76	79/76	79/78	77/78	74/77	74/77	69/73	73/74	73/74		0.71
O_brach	76/72	75/72	73/78	72/72	71/73	71/73	67/69	66/70	65/70	54/76	

Within the genus *Afrophlaeoba* the proportion of transitions is high for all DNA sequences studied, indicating a low transition saturation. The variability and the number of informative sites is low for the 16s rRNA + tRNA-Leu, 12S rRNA and ND1 data sets, but higher for ND5. The skewness is convenient for all sequences and for the combined data set. Thus for the phylogenetic inference all sequences were added to a combined data set with 1964 characters.

4.3.4 Phylogenetic Inference

Since all DNA data sets contain only little homoplasy, the combined data set was used for phylogenetic inference. The calculation was performed with two methods, parsimony and neighbor joining. The topologies were rooted with the sequences of the two *Odontomelus* species.

4.3.4.1 Most-Parsimonious Tree

Due to the homoplastic content, alternative weighting schemes recovered the same MPT and thus the presentation of different weighting schemes is ignored here. The parsimony analysis recovered only one completely dichotomous MPT (figure 14), which is 487 steps long. The minimal number of homoplastic events within the MPT is 62. This means that 425 evolutionary steps (in terms of substitutions) support the branches of the MPT and are assumed to be apomorphies. The CI calculated from these data (425/487) is 0.873 (excluding uninformative characters = 0.830), the RI is 0.882 and the RC 0.770. These high indices confirm the low homoplastic content within the data set. A CI < 1 was found at 58 sites among 383 variable sites.

The MPT supports the hypothesis of a monopyhletic origin of the mitochondria of *Parodontomelus* and *Afrophlaeoba*. Within the genus *Afrophlaeoba* two groups can be distinguished. One branch connects *A. nguru* and *A. longicornis*, the other one connects *A. usambarica* and *A. euthynota*. The number of evolutionary steps within the genus *Afrophlaeoba* is comparatively low. Seven substitutions support the *usambarica-euthynota* group and eleven the *nguru-longicornis* group. On the *euthynota*-branch 17 substitutions have to be proposed including eight homoplastic events for the complete tree or five homoplasies respectively within the genus *Afrophlaeoba*. Three of those (ND5 sites 57, 685 and 1023) would branch *A. euthynota* basally (as an outgroup to the other *Afrophlaeoba* species), one (ND5 site 634) with *A. nguru* and one (12S rRNA site 98) with *A. longicornis*. Of course such branches would cause more homoplasies within other groups and thus enlarge the tree (the number of additional steps necessary to reject a branch is the decay index described below). On the *usambarica*-branch 23 substitutions are found, including eleven homoplasies for the complete tree and three within the genus *Afrophlaeoba*. One of those (ND5 site 289) would branch *A. usambarica* basally and two (12S rRNA site 229 and ND5 site 708) with *A. longicornis*. The *nguru*-branch contains six substitutions including two homoplasies, but only one within *Afrophlaeoba* (ND5 site 634 – the one grouping with *A. euthynota*). On the branch of *A. longicornis* ten substitutions are included in the most parsimonious tree, of which eight are homoplastic events for the complete tree and five for the genus *Afrophlaeoba*. Additionally to the two homoplasies (12S rRNA site 229, ND5 site 708) connecting *A. usambarica* and *A. longicornis*, three homoplasies (12S rRNA site 201, ND5 sites 402 and 648) would branch *A. longicornis* basally. A matrix summarizing the number of homoplasies between each taxon is given in table 18.

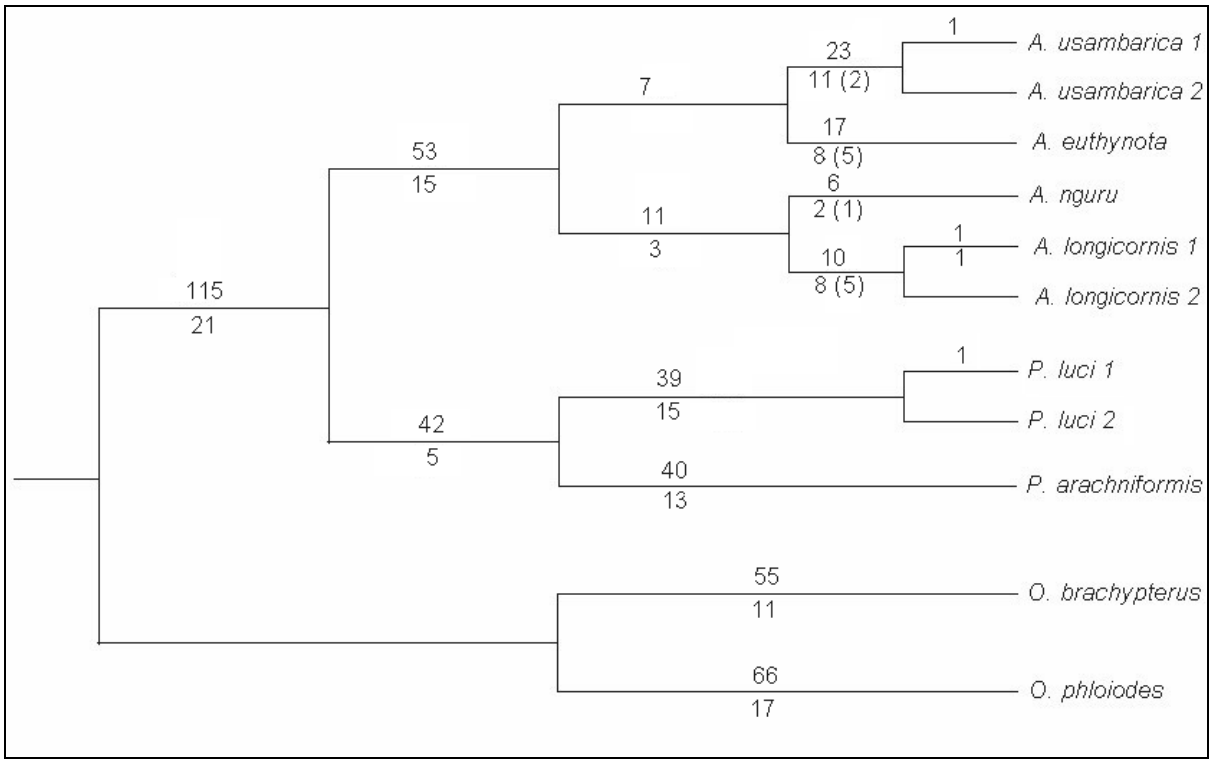


Fig. 14: MPT for the combined data set including all taxa. The numbers above the branches refer to the number of evolutionary steps (in terms of substitutions). The numbers below the branches refer to the number of homoplastic events among these substitutions. Figures in brackets give the number of homoplastic events within the genus *Afrophlaeoba*.

Tab. 18: Pairwise homoplasy matrix for the complete data set.

	A_usam1	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod
A_usam2	0									
A_nguru	0	0								
A_euthy	0	0	2							
A_longi1	4	4	0	2						
A_longi2	4	4	0	0	0					
P_arach	6	6	4	8	8	6				
P_luci1	2	2	2	10	6	4	0			
P_luci2	2	2	2	10	6	4	0	0		
O_phloiod	22	22	12	20	20	18	16	20	20	
O_brach	14	14	6	14	14	12	12	20	20	0

4.3.4.2 Reliability of the Branches

Figure 15 shows the consensus tree of 1000 bootstrap replicates. Its structure does not differ from the MPT. The bootstrap values, which are given above each branch, are exceptionally high. The distinct clades of *Parodontomelus* and *Afrophlaeoba* are well supported by the bootstrapping (bootstrap value: 100). The branch connecting *A. nguru* and *A. longicornis* also received strong bootstrap support (97). The weakest branch is connecting *A. usambarica* and *A. euthynota* with a bootstrap value of 80. An exclusion of single taxa did not influence the general structure of the tree, but had some influence on the bootstrap confidence of the *euthynota-usambarica* branch. A calculation including only one specimen per species recovered an identical tree with a higher bootstrap value of

90, a tree length of 486 and more or less similar homoplasy indices (CI: 0.874, RI: 0.823, RC: 0.720). An exclusion of *Odontomelus* from the data set and the use of *Parodontomelus* as outgroup lowered the bootstrap value of the *euthynota-usambarica* branch (70). If both *Odontomelus* species and the double specimens were removed from the data set, the bootstrap value increased again to 78 with a tree length of 250 and higher consistency indices (CI: 0.928, RI: 0.852, RC: 0.791). If the calculation was performed without *Parodontomelus*, the tree length was 351 with exceptionally high indices (CI: 0.940, RI: 0.913, RC: 0.858) but only minor differences in bootstrap confidence for the *euthynota-usambarica* group (81 or 83 excluding double specimens, respectively).

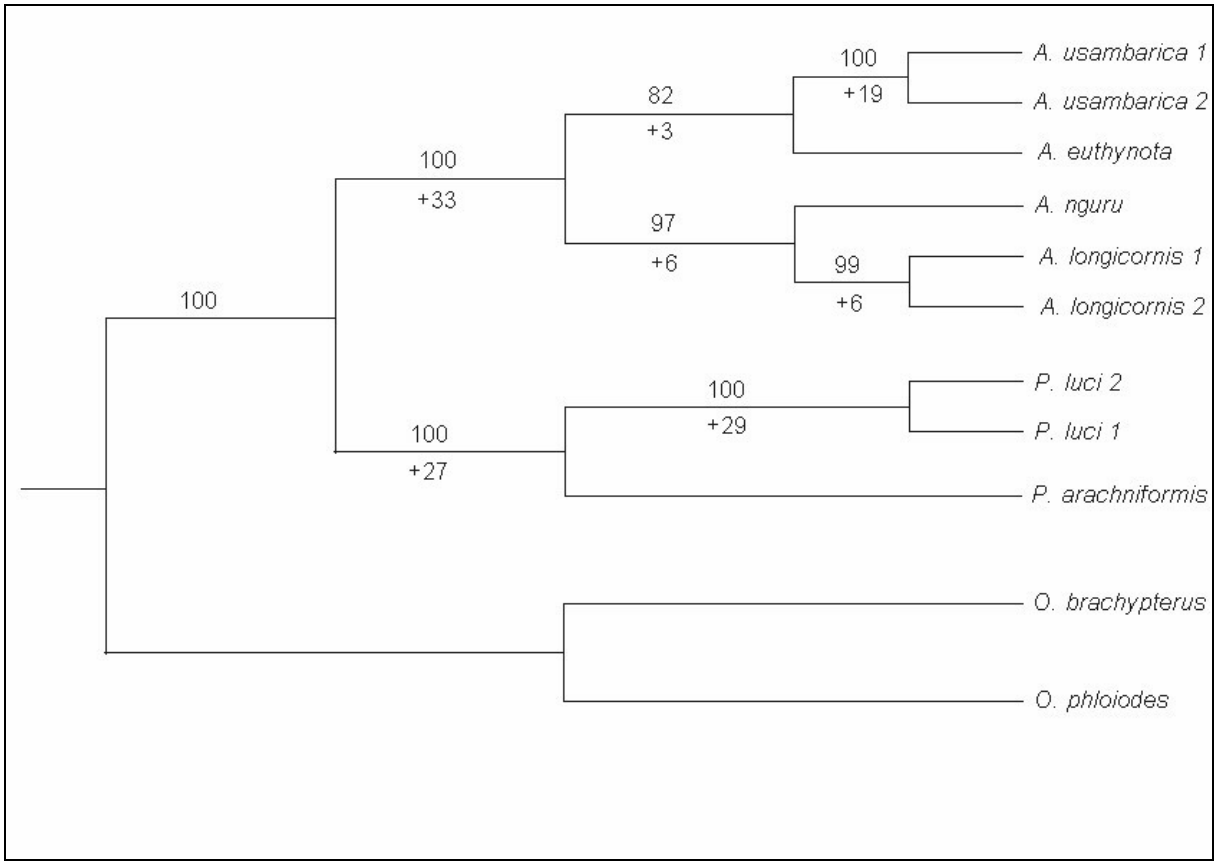


Fig. 15: Rooted consensus tree from 1000 bootstrap replicates, including the bootstrap values (above the branches) and the decay indices (beneath the branches).

The decay indices (Bremer Support) are given below the branches, confirming the weaker support for the *euthynota-usambarica* branch. Only three more evolutionary steps have to be assumed for another tree, branching *A. euthynota* basally to the other *Afrophlaeoba* species and five steps more to branch it with *A. longicornis* and *A. nguru*. The *nguru-longicornis* group disappears at a cost of six more evolutionary steps, while the other branches have higher decay indices and are well supported. The monophyly of *Afrophlaeoba* is supported by a decay index of 33 steps, the monophyly of *Parodontomelus* by a decay index of 27. The low decay indices within *Afrophlaeoba* are probably mainly a subject of the low number of substitutions (apomorphies) within these branches.

4.3.4.3 Neighbor Joining

The phenogram resulting from the neighbor joining analysis is given in figure 16. No structural differences can be found between the MPT and the phenogram. In contrast to the MPT, the phenogram includes information on the genetic distances (branch lengths) from which it was inferred. It is clearly visible that the distances between the two *Odontomelus* species and between the two *Parodontomelus* species are much higher than the distances within the genus *Afrophlaeoba*. Within *Afrophlaeoba* the two species *A. nguru* and *A. longicornis* have the lowest distances, while the distances between *A. usambarica* and *A. euthynota* are approximately the same as between *A. euthynota* and *A. nguru*. A matrix presenting the J-C-distances between all specimens is given in table 19.

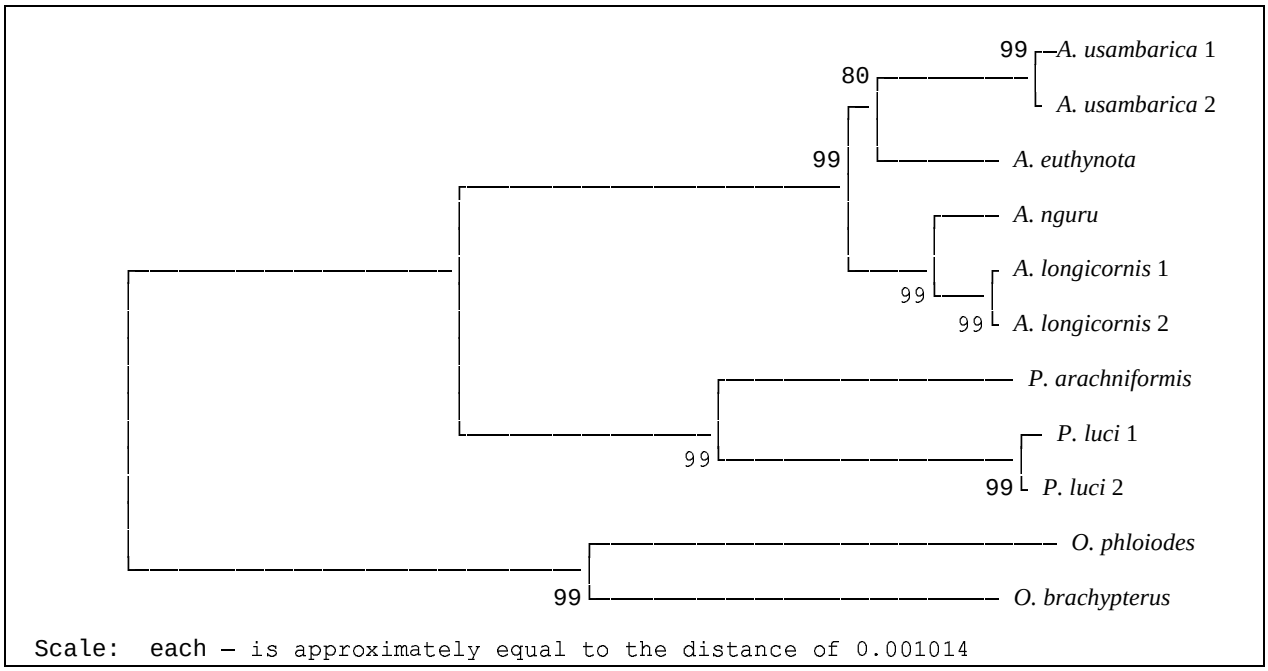


Fig. 16: Phenogram resulting from a neighbor joining analysis of the computer programme MEGA 1.02, including the confidence levels from the t-test.

Tab. 19: Pairwise J-C-distances between the taxa for the combined data set.

	A_usam2	A_nguru	A_euthy	A_longi1	A_longi2	P_arach	P_luci1	P_luci2	O_phloiod	O_brach
A_usam1	0.0005	0.0245	0.0203	0.0250	0.0245	0.0806	0.0823	0.0817	0.1289	0.1253
A_usam2		0.0240	0.0197	0.0245	0.0240	0.0800	0.0817	0.0812	0.1283	0.1247
A_nguru			0.0197	0.0088	0.0083	0.0766	0.0783	0.0777	0.1283	0.1235
A_euthy				0.0219	0.0224	0.0772	0.0772	0.0766	0.1265	0.1223
A_longi1					0.0005	0.0755	0.0777	0.0772	0.1241	0.1211
A_longi2						0.0760	0.0783	0.0777	0.1247	0.1217
P_arach							0.0422	0.0416	0.1295	0.1253
P_luci1								0.0005	0.1283	0.1217
P_luci2									0.1277	0.1211
O_phloiod										0.0609

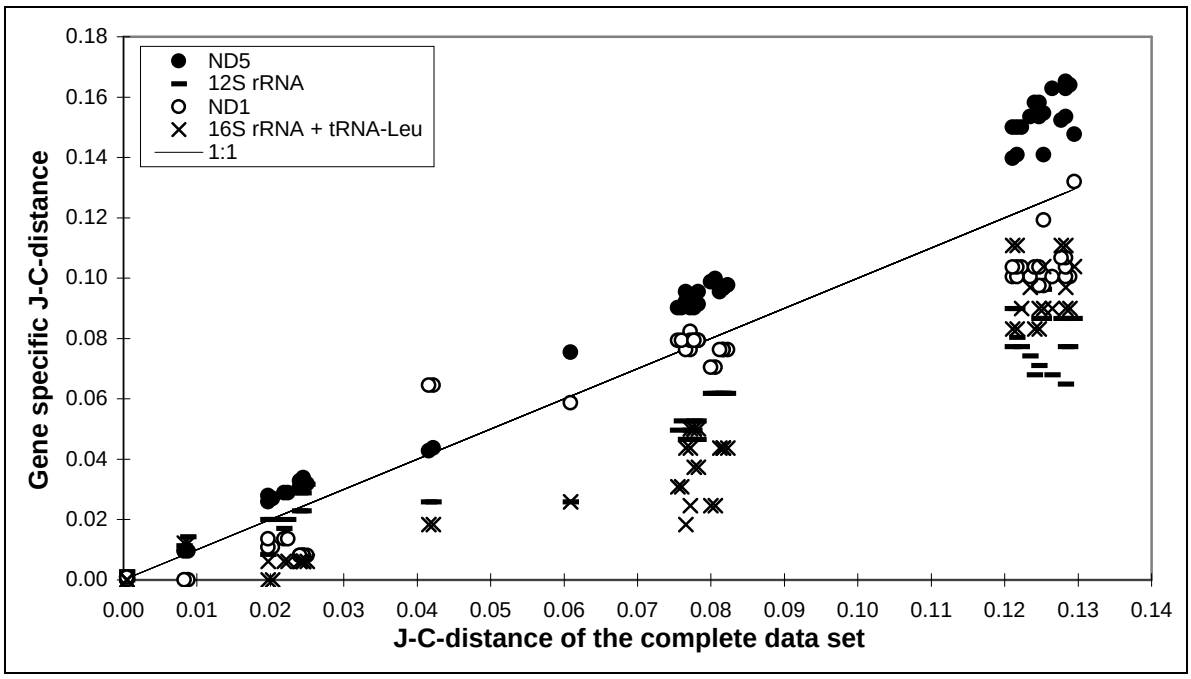


Fig 17: Substitution rates of the four different gene fragments in relation to the J-C-distances of the complete data set; the 1:1 line represents the case of equal rates to the complete data set, which is approximately given by the ND1 fragment.

4.3.5 Substitution Rates

The number of substitutions accumulates with time, but probably not in a linear way. As stated above, transitions are known to occur more often than transversions. Moreover, the three codon positions of protein coding genes evolve at different rates due to the degenerate code. In RNA specifying genes, conserved regions can be distinguished from regions evolving faster (Hickson et al. 1996). In protein coding genes some parts may code for amino acid sequences, which are highly conserved due to functional constraints, while others may represent more plastic parts of the protein. Some genes are higher conserved than others or they consist of larger parts of highly conserved sites. Therefore, it is not surprising that a comparison of the substitution rates of different genes will result in the observation of different rates. However, if the different taxa are compared, it is even visible that within some genera the rates of the different genes differ (figures 10-13). Figure 17 represents a plot of the J-C-distances of the different gene fragments against the total J-C-distance. On the abscissa the distances between different taxa can be observed. It starts with the very low intraspecific distances. Around 0.01 the species group *nguru-longicornis* is plotted, where no exchange between the ND1 genes have been found and the 12S rRNA distances are slightly higher than the ND5 distance. Between 0.02 and 0.03 the other *Afrophlaeoba* species are plotted, where 12S rRNA is slower than ND5, but still a little bit faster than ND1. For *Parodontomelus* (around 0.04) the ND1 gene proves to be faster than the ND5 gene, but both protein-coding genes are faster than the RNA specifying genes. At a J-C-distance of 0.06 the distances between the two species of *Odontomelus* are plotted. In this group ND5 is faster than ND1, and both are much faster than the RNA specifying genes. Around 0.08

the genus *Afrophlaeoba* is plotted against the genus *Parodontomelus*. Thus it represents the distances within the Phlaeobini. The ND5 gene is faster here than ND1, and 16S rRNA is the slowest fragment. The last group is the comparison of *Odontomelus* with the Phlaeobini (0.13). At this distance ND5 is still the fastest gene, but ND1 is only slightly faster than 16S rRNA. The slowest gene fragment is 12S rRNA. In summary it can be concluded that at lower overall distances (< 0.05) the rates of the different genes are highly variable as they are influenced by stochastic events. At higher rates it becomes obvious that RNA specifying genes evolve at slower rates than the protein coding genes. However, the number of highly variable sites is also limited and the high substitution rates within the ND1 gene reach saturation after some time, while the process of visible substitution might continue in the RNA specifying genes.

4.4 Discussion

4.4.1 Suitability of Genes and Primers

Initially, universal primers were chosen to amplify some gene fragments of the specimens. The amplification was successful for all of them. In some cases the annealing temperatures had to be adjusted to compensate for the lower affinities (if substitutions occurred in the primer region). The self-designed primers also fitted well and the transcription of the protein coding genes and the control of the conserved regions of the 12S rRNA gene suggest that all sequenced data are orthologous.

The suitability of the genes for the phylogenetic analysis has to be evaluated differently. The evolutionary distances within the genus *Afrophlaeoba* are comparatively low and thus genes with a higher evolutionary speed are more suitable for the analysis. Within the data presented here, 12S rRNA, 16S rRNA (incl. tRNA-Leu) and ND1 had very low numbers of substitutions within the genus *Afrophlaeoba* and therefore do not contribute much to the parsimony method. The distances within the ND5 fragment are higher and so is the number of informative sites. Thus the ND5 fragment contributes most to the parsimony analysis. If a MPT was produced by exclusively analysing the ND5 data, the result would not differ from the combined approach. The bootstrap values of the consensus tree were slightly lower for the *euthynota-usambarica* branch (74), since this branch already contains less covariance. According to Clary & Wolstenholme (1985) the ND5 gene is one of the fastest-evolving mitochondrial genes and long enough for statistical analysis and therefore especially useful in resolving the phylogenetic relationships within a genus with a low degree of diversification (Su et al. 1996). The reconstructed gene tree topologies proved almost entirely insensitive to the choice of the data set, the algorithm, the outgroup, and the weighting scheme and the values from the bootstrap analysis showed only minor variation, indicating the robustness of the recovered mitochondrial relationships.

4.4.2 Choice of the Outgroups

A preliminary parsimony analysis with the help of the DNA sequences of *Locusta migratoria* (Flook et al. 1995) showed that *Odontomelus* is well suited as an outgroup for the Phlaeobini and that *Parodontomelus* is well suited as an outgroup for the genus *Afrophlaeoba*. This confirms the taxonomic studies of Jago (1983) and Popov (in press). Although from the external appearance the species of *Afrophlaeoba* closely resemble those of the genus *Odontomelus*, they belong to a completely different tribe within the Acridinae. The convergent morphology of both species is expressed in the original description of *Afrophlaeoba usambarica* (RAMME, 1929) as *Odontomelus usambaricus*. Dirsh (1965) missed the epiphallic differences between *Odontomelus* and *Afrophlaeoba* by examining the genitalia of *Afrophlaeoba usambarica* as a typical *Odontomelus*, instead of the type species *Odontomelus brachypterus* (Jago 1983).

4.4.3 Phylogenetic Inference and Divergence Age

If a gene tree is to be used for inferring the history of closely related taxa, its topology must be sufficiently resolved and robust so that noteworthy phylogenetic patterns can be confidently documented (Funk 1999). The high bootstrap values of these branches and the low homoplasy indices demonstrate that the inferred gene trees are very consistent. Thus the gene tree is accepted here as hypothesis for a species tree. Of course, a gene tree inferred from mitochondrial DNA could only reflect the evolutionary history of the mitochondria. Limited intraspecific sampling leaves phylogenetic analyses prone to misinterpretations due to undetected introgressed haplotypes (Funk 1999). Since there is evidence for introgression of mitochondria for some insect species, including *Drosophila* (Ballard 2000) and *Carabus* (Düring & Brückner pers. comm.), it cannot be excluded that such events occur also in the group under study. However, introgression is based on gene flow and only possible, if a connection between two populations existed. The evolutionary history of the mitochondria will give some information about paths of gene flow even if it does not reflect the “real” phylogenetic situation. Thus it will not affect the main conclusions of this paper. Moreover, there is no reason to suggest an introgression event between the species of *Afrophlaeoba*, since there is no morphological evidence, which cast doubt on the hypothesis. According to Brower et al. (1996), sources of misleading haplotypes are sufficiently rare so that they pose no serious problem to the phylogenetic analyses.

Since the hypothesis of the existence of a molecular clock has first been proposed (Zuckerkandl & Pauling 1962), much evidence against the universal character of such a clock has been published (Li 1997). Hence, dating of divergence ages remains a difficult process. A molecular clock can be misleading at low distances, because some few substitutions will modify the computed time of divergence dramatically, and the influence of stochastic events will be higher (Prüser & Mossakowski 1998). Moreover, the variation of recent estimates for molecular clocks is very high. Many authors

suggested a rate of 0.02 per my for the ND1 gene (DeSalle et al. 1987, Brower 1994, Juan et al. 1995), but some authors calculated much higher rates (0.046 per my for *Dolichopoda* cave crickets, Venanzetti et al. 1993) or much lower rates (0.0039 to 0.0098 per my for Mediterranean *Carabus* species, Prüser & Mossakowski 1998). Additionally, it has to be taken in consideration that genetic sequences might diverge sooner than the organisms (Li 1997). Thus the estimates have to be adjusted to a sooner date. Unfortunately the details of the natural history of eastern Africa, especially the vegetational changes during and after the Pleistocene and the human effects on it remain matters of controversy (Howell 1993). Therefore, it is not possible to calibrate a molecular clock for the taxa under study by means of geological or well-known climatic events within the range of the species. Moreover, the sparse worldwide fossil evidence for Acrididae is of doubtful generic assignments (Storozhenko 1997). Finally, a molecular clock does certainly not work in a linear way, since different sites evolve at different rates and the rate of transitions differs from the rate of transversions. The proportion of homoplastic events will increase with time, obscuring the number of evolutionary steps. Since at present no other method for the dating of the divergence time is available, rough estimates are presented here, using medium evolutionary rates of 0.01-0.03 per my. Additionally, both, the minimum rates (Prüser & Mossakowski 1998) and the maximum rates for the ND1 fragment (Venanzetti et al. 1993) are considered as possible wider range. The complete data set was used for calculation, since it evolves at similar rates as the ND1 data set (figure 17).

The parsimony and neighbor joining analyses strongly supported a robust mtDNA genealogy that revealed the monophyly of the genera *Afrophlaeoba* and *Parodontomelus*. The latter genus was chosen as an outgroup and thus only two species were examined. Hence, a final conclusion to the monophyly of this genus is not possible and was not subject of this study. *Odontomelus* has an average J-C-distance of 12.5% to the species of *Afrophlaeoba* and *Parodontomelus*. Calculated from the medium rates this would result in divergence times of 4.17 my to 12.52 my between the Phlaeobini and Pargaini. If the two extreme rates mentioned above are used for calculation, the age would vary from 2.7 my to 32.1 my. This might illustrate the high variation due to different assumed molecular clocks. Since Acridinae are generally regarded as a comparatively young group (Rowell & Flook 1998) and they are specialized on grasses in their diet (Jago 1973), a time of divergence of 32.1 my seems to be too high, while a divergence time of 2.7 my would suggest that both tribes had a major radiation in the Pleistocene. Since the tribes are present in Southern Asia, Africa and Madagascar, such a high speed of radiation and dispersal is rather unlikely, too. The medium rates of 0.01-0.03 per my result in more convenient ages, but as long as the complete phylogeny of the two groups is not studied, this remains speculation. The high genetic distances between the two *Parodontomelus* species and the closer restriction to forests (chapter 7) suggest it to be a suitable genus for further phylogenetic analysis with a main interest in forest connections within the Eastern Arc and the Coastal Forests. Within the genus *Afrophlaeoba* the results suggest two groups, the *euthynota-usambarica* group and the *nguru-longicornis* group.

The *nguru-longicornis* Group

The sister relationship between *A. nguru* and *A. longicornis* is supported by high bootstrap values and confirms the morphological results (chapter 5). The average J-C-distance between both species is exceptionally low (0.00855), so that it is even worth discussing, whether both are conspecific. Su et al. (1998) presented 1-2% divergences within and 4.2% between different subspecies in the ground beetle *Damaster blaptoides*, based on ND5 sequences. Within the genera of Carabinae, Su et al. (1996) found pairwise sequence divergences from 2.7% to 4.9%. Prüser & Mossakowski (1998) found 0.57% to 4.6% divergence within *Carabus* species and 7.8% to 11.5% between species. However, according to the biological species concept (Mayr 1942) the species status cannot be assigned by studying genetic distances or morphology. A test of the potential to interbreed is experimentally difficult and less meaningful for the elucidation of the evolutionary history of the species complex, since the question of paths and conditions for gene flow is not dependent on the present taxonomic rank. At least the low distances of the two species suggest a relatively recent divergence. This is also supported by the high ratio of transitions in comparison to the other gropus of species (90% in the ND5 sequence in comparison to 70-79%). If medium evolutionary rates are considered, the estimates for the *longicornis-nguru* divergence vary from 285 thousand to 855 thousand years. Considering the extreme rates, the estimates may vary from 186 thousand years to 2.2 my. If the higher proportional error at lower rates is taken into account, a minimum time of separation cannot be given. At least the maximum time of divergence can be dated, indicating that the two species separated no sooner than the Pleistocene.

The *euthynota-usambarica* Group

The branch connecting *A. usambarica* and *A. euthynota* has the lowest bootstrap value (80) and is also more sensitive to the inclusion or exclusion of single taxa. This is probably caused by the higher number of homoplastic events, which have to be proposed for the *euthynota*-branch. If the J-C-distances are compared (table 19), it becomes obvious that *A. euthynota* has approximately the same distance to *A. nguru* than to *A. usambarica*. On the other hand there are seven apomorphic sites supporting the *euthynota-usambarica* group, while there is only one site supporting an *euthynota-nguru* group. A basal *euthynota*-branch could be obtained at a cost of only three steps on the tree. According to Hillis & Bull (1993) several studies suggest that for maximum parsimony bootstrap values ≥ 70% the probability of a clade being correct is at least 95%. Thus the MPT still remains the best hypothesis for the phylogenetic origin. It is also supported by the morphological data (see chapter 5). The similar distances between *A. nguru* and *A. euthynota* and between *A. usambarica* and *A. euthynota* suggest that *A. euthynota*, *A. usambarica* and the *nguru-longicornis* group radiated within a short period from their common ancestor. This may also cause the lower bootstrap value of the *euthynota-usambarica* group. The average J-C-distance between both species (0.0200) is more than twice as high as between *A. nguru* and *A. longicornis*, although it is still comparatively low for

the species level. The calculated medium divergence ages of the *euthynota-usambarica* group vary from 667 thousand years to 2 my, and 435,000 years to 5.13 my using the extreme rates. From the comparison with the *nguru-longicornis* group it can be concluded that a present connection between both species is unlikely to exist.

Basal Divergence of Both Species Groups

The distances between both species groups are only slightly higher than between *A. euthynota* and *A. usambarica* (average J-C-distance 0.0226). Medium estimates of divergence times vary from 753 thousand years to 2.26 my, while the extremes range from 491 thousand years to 5.8 my. The distance between *A. euthynota* and *A. nguru* is even as high as between *A. euthynota* and *A. usambarica*. Thus it is difficult to suggest any divergence time for the two groups prior to the divergence of the *euthynota-usambarica* group. It is even possible that the same event of habitat fragmentation caused the separation of three main groups: *A. usambarica*, *A. euthynota* and the *longicornis-nguru* group. The phylogeographic interpretation of those conclusions will be further discussed after the ecology has been dealt with.

Intraspecific Variability

The intraspecific variability was not subject of this study and, therefore, it is not possible to present general statements on the degree of intraspecific variation. Of three species, however, two specimens were included in the analysis. The intraspecific J-C-distance within all three species (*A. usambarica*, *A. longicornis*, *P. luci*) was 0.0005 – a distance based upon a single substitution. This occurs despite the fact, that the two colour morphs of *P. luci* were collected from the same locality, while the two *A. usambarica* specimens were collected at localities with a distance of 3 km and the two specimens of *A. longicornis* with a distance of 13.6 km separated by Mkondoa River. A calculation of the age of divergence of such a single substitution may illustrate the influence of stochastic events on calculated times of divergences. Using the medium rates, one substitution would be equal to 16,667 years to 50,000 years. In the high evolutionary rate of *Dolichopoda* one substitution would be equal to 10,869 years, while in the low evolutionary rate of *Carabus* one substitution would represent an age of 128,205 years. This illustrates that any stochastic substitution event leads to large changes in the time estimates.

5 Morphology

5.1 Introduction

Since the beginning of biological systematics the characters used in species descriptions have altered remarkably. The faunistic exploration of the African grasshopper fauna boomed at the end of the 19th and the beginning of the 20th century with a high number of descriptions by Stål (1876), Krauss (1877), I. Bolívar (1881), Karsch (1891), Brancsik (1892), C. Bolívar y Pieltain (1893), Burr (1899), Saussure (1899), Sjöstedt (1901), Giglio-Tos (1907), Karny (1907), Rehn (1914), Uvarov (1922) and Ramme (1929), to mention just the early works of the most important taxonomists. The early typological descriptions were mainly based upon external morphological characters, such as colour patterns and morphs. Systematic errors were common, due to convergent evolutionary processes, high intraspecific variation or cryptic species (Ramme 1929). With the beginning of research in communicative behaviour, new characters have proved to be of high taxonomic value (Faber 1928). Several species have been discovered due to their specific song patterns and some are difficult to identify unless they are singing. The high value of songs for prezygotic isolation forced taxonomists to include fieldwork and laboratory song analysis in their studies (Ragge & Reynolds 1998). In future, the use of molecular characters in taxonomy will probably increase (Hochkirch 1999). Tropical species, however, are still described mainly morphologically, since they are not available alive in the museums. In morphological descriptions the genitalia have become important characters, since they might represent barriers for hybridisation between species, and they are less influenced by the environment (Dirsh 1956). On this basis revisions of a high proportion of the East African Orthoptera have been published, including the Phlaeobini (Jago 1983, Popov in press). Species or genera, which appeared to be closely related, proved to belong to different groups after the genitalia were studied. However, the knowledge on intraspecific variability has remained low, since frequently only few specimens were available. Much of the confusion in “difficult” groups stems from an insufficient knowledge of the intraspecific variability (Blackith & Kevan 1967). Examinations of larger series of specimens are necessary to obtain better descriptions and to prove the stability of morphological characters. Present taxonomy is still mostly based on phenetic similarities rather than on phylogenetic analyses based on discrete characters in the sense of Hennig (1950). This is mainly caused by practical reasons, such as the low availability of discrete characters (Blackith & Kevan 1967).

The intention of the following analyses was to prove the applicability of the keys and descriptions of Jago (1983), to find appropriate characters for the identification of the rather homogenous species, to investigate the degree of geographical variation in morphometrics and to infer a phylogeny, if possible. For this purpose measurements of 27 body dimensions of 149 males of *Afrophlaeoba* and *P. arachniformis* were undertaken, and 66 qualitative characters were examined. The qualitative characters also included the structure of the inner genitalia.

5.2 Methods

5.2.1. Collection and Preparation of Specimens

Thirty males of each *Afrophlaeoba* species and of *Parodontomelus arachniformis* were collected during the second field trip to Tanzania (table 20). These specimens were dried at the locality and transferred into parchment bags. In the laboratory they were relaxed in a refreshment box with water saturated atmosphere and naphthaline (to avoid mould). The genitalia of the refreshed specimens were dissected under a stereo-dissecting microscope. For this purpose the abdomen was gripped with a horizontal forceps and the genital complex was squeezed out by horizontal pressure with the forceps. If the genitalia did not evert completely upon pressure from the forceps, they were out gently, using an entomological pin, with the point bent through 90°. Afterwards the genitalia were separated from the abdomen by cutting through the membranes around the edge of the genitalia with a pair of miniatur scissors. Finally the genitalia were transferred to an alcoholic glycerol solution (10% glycerol), for preservation. Afterwards the specimens were pinned and the antennae were directed in an approximately straight line to allow morphometric measurements. Additionally to the collected specimens, some body dimensions of the holotypes and paratypes at the Natural History Museum in London were measured with a stereo-dissecting microscope and an ocular micrometer.

Tab. 20: Date, localities and altitude, from which the specimens have been obtained.

Species	Date	Locality	Altitude
Afrophlaeoba euthynota	06.12. to 13.12.1997	Uluguru Mountains near Morogoro	800-1400 m
Afrophlaeoba usambarica	02.01. to 15.01.1998	East Usambara Mountains near Amani	600-1100 m
Parodontomelus arachniformis	06.01. to 18.01.1998	East Usambara Mountains near Amani	600-850 m
Afrophlaeoba nguru	30.01. to 02.02.1998	Nguru Mountains near Mhonda	500-900 m
Afrophlaeoba longicornis	08.02. to 13.02.1998	Rubeho Mountains near Kilosa	500-600 m

5.2.2 Body Dimensions Measured

A total of 149 insects were measured (*P. arachniformis*: 30, *A. euthynota*: 28, *A. longicornis*: 31, *A. nguru*: 30, *A. usambarica*: 30). The measured body dimensions were selected according to the keys and descriptions given by Jago (1983). Body parts, which are susceptible to shrinkage, (in dried insects) are not desirable for a measurement (Blackith & Reyment 1971). Thus the total body length was ignored, since the abdomen is sensitive to water loss. The characters under comparison have to be homologous in the sense of Remane (1952), but in the case of closely related organisms and quantitative measurements homologies are generally evident (Blackith & Reyment 1971). The measurements were performed with an apparatus constructed by D. Wienrich, a step-motor-controlled (translateur TL 17 CA, 2 axes, of Micro-Controle Electronique, Vitry-sur-Seine, France) stereo-

dissecting microscope. The following list presents the 27 body dimensions measured and their abbreviation (figures 17-19):

- Length of flagellum (measured at the inner edge of the right antenna) – FlagL
- Length of the flagellar segments 1+2, 3, 4, 5, 6 and 9 (always along the inner edge of those segments; the first two segments are often fused and were therefore measured together) – FS1+2L, FS3L etc.
- Apical width of the same flagellar segments – FS2W, FS3W etc.
- Length of fastigium of vertex (along the median carinula in front of the eyes) – VertL
- Width of fastigium of vertex (measured in front of the eyes) – VertW
- Length of head along the median carinula – HeadL
- Width of head at the broadest distance between the outer edges of the eyes – HeadW
- Interocular distance at the smallest distance – Intoc
- Length of prozona of pronotal disc along the median carina – Proz
- Length of metazona of pronotal disc along the median carina – Metaz
- Distance between lateral carinae of pronotum at the anterior margin – AntCar
- Distance between lateral carinae of pronotum at the posterior margin – PostCar
- Length of right tegmina from the lower base to the tip – TegL
- Maximal width of right tegmina – TegW
- Length of right cercus – Cerc
- Length of right hind femur – HFemL
- Maximal width of right hind femur – HFemW

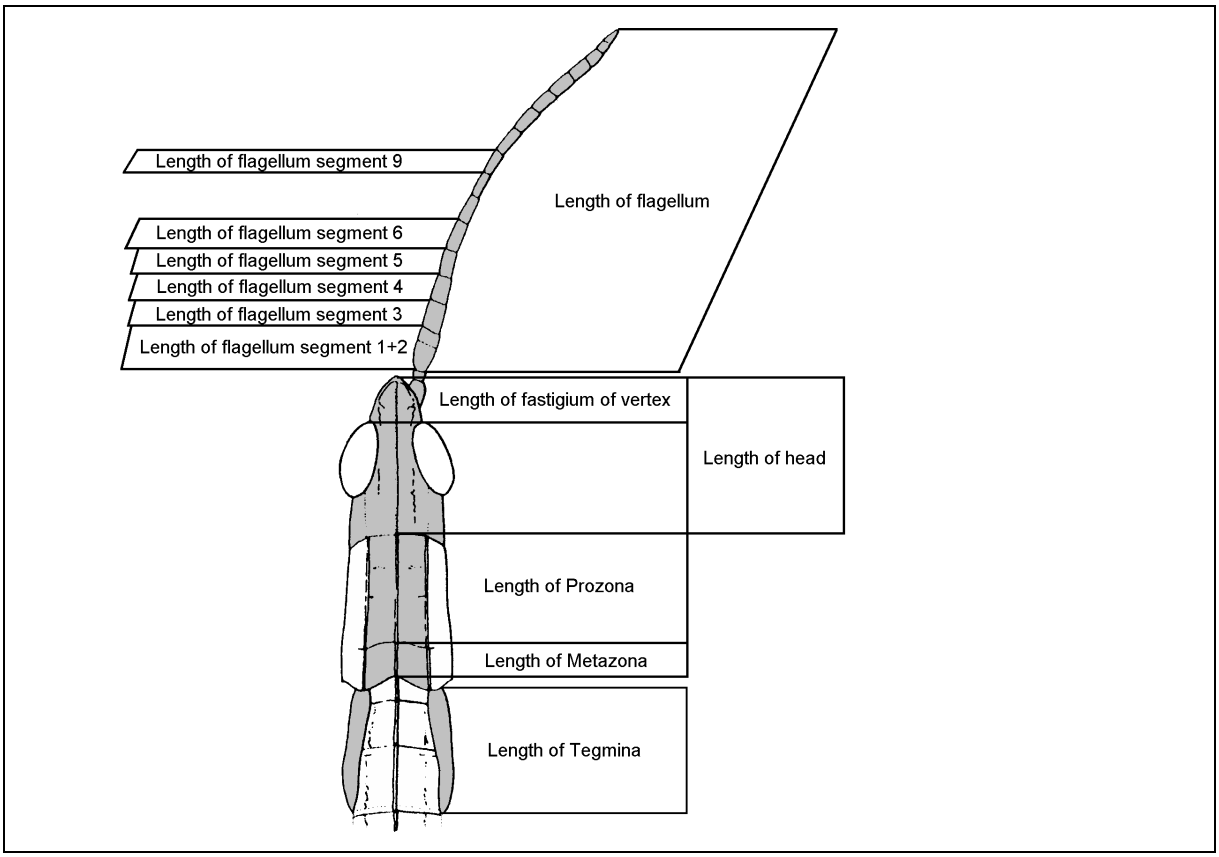


Fig. 17: Twelve of the body length dimensions measured (flagellum, head, pronotum, tegmina); figure changed after Jago (1983)

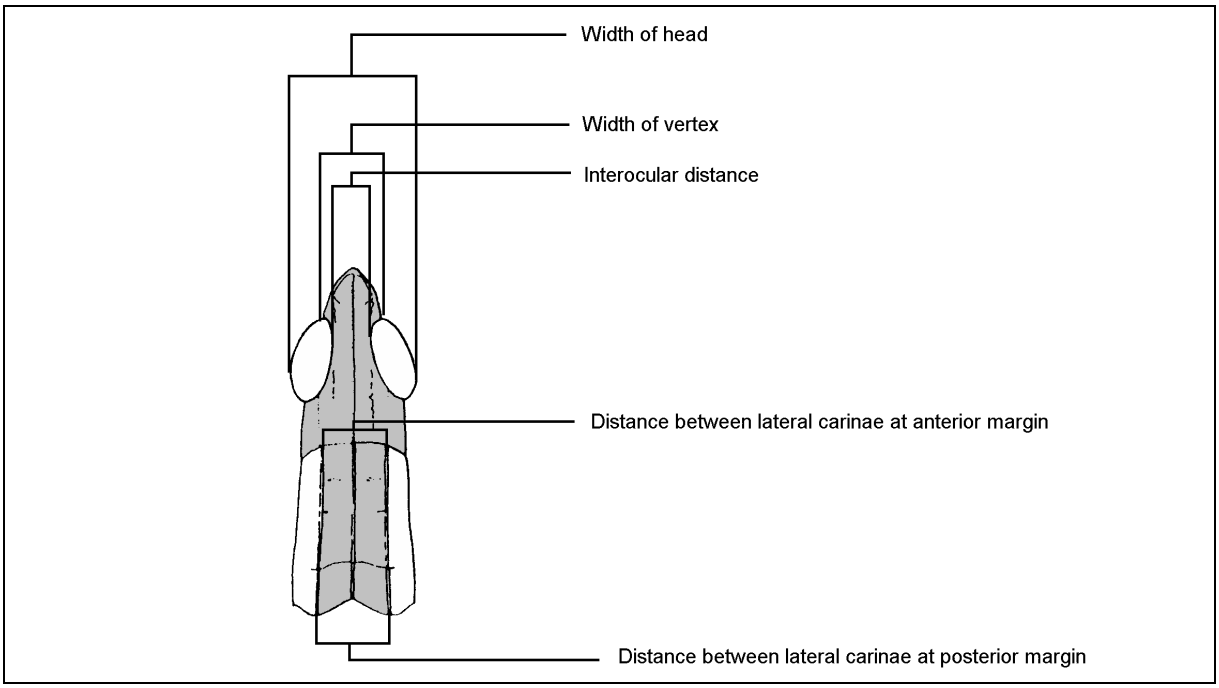


Fig. 18: Five of the body width dimensions measured (head, pronotum); figure changed after Jago (1983)

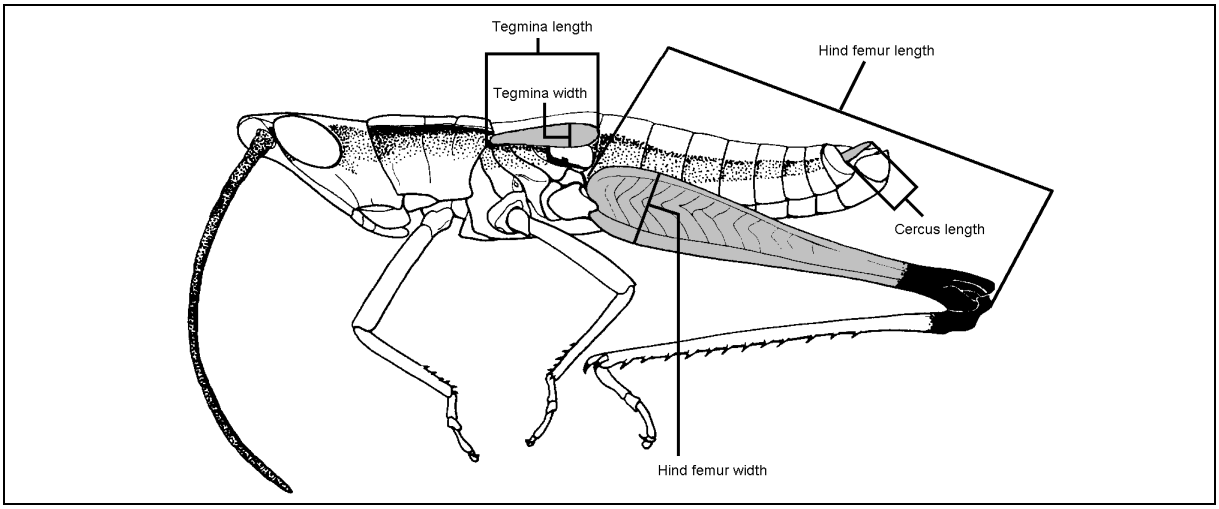


Fig. 19: Five of the body dimensions measured (tegmina, hind femur, cercus); figure changed after Dirsh (1965)

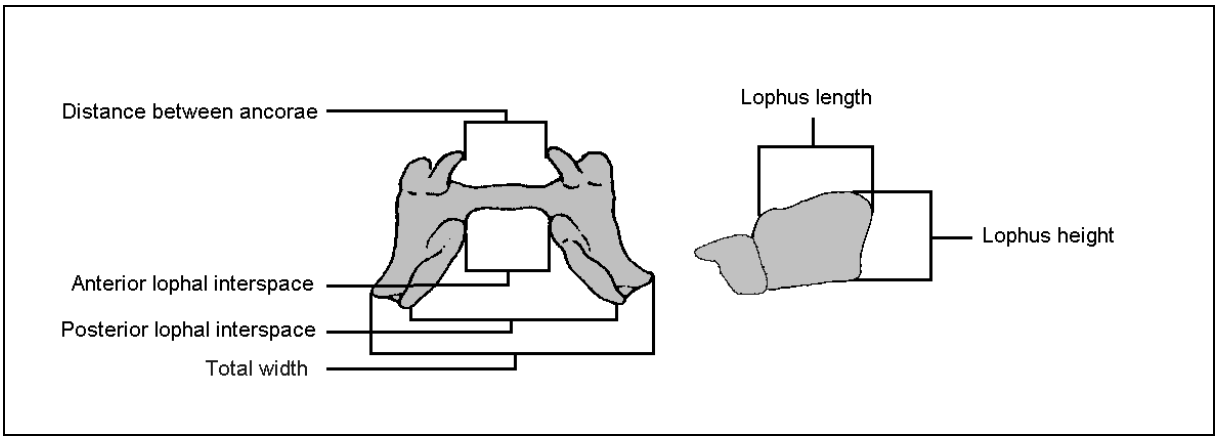


Fig. 20: Epiphallus and lophus of *A. usambarica*, illustrating the dimensions measured.

5.2.3 Qualitative Characters Examined

Sixty-six nonmetric characters were examined with a stereo-dissecting microscope. They were chosen partly according to the keys and descriptions of Jago (1983). Twenty males of each species were examined. The included parts of the body were the shape of the flagellum with its basal segments (six characters), the frontal ridge with carinulae (three characters), the fastigium verticis with carinulae (five characters), head, eyes and mouthparts (three characters), the pronotum with carinae, sulci and lateral lobes (twelve characters), the tegminae with its veins (seven characters), the abdominal tergites with carinae, supra-anal plate, cerci and subgenital plate (eleven characters), the legs with genicular lobes, carinae and posttibial spines (eleven characters) and the epiphallus with its lophi and ancorae (eight characters). For six males of each species some dimensions of the epiphallus (lophal interspace, distance between ancorae, total width, lophi length and height) were measured with a stereo-dissecting microscope and an ocular micrometer at 50 × magnification (figure 20).

5.2.4 Data Analysis

Discriminant Analysis

Multivariate morphometrics is mainly useful at the taxonomical level of species and below, since lower taxa may differ in only a few qualitative character dissimilarities (Blackith & Reyment 1971). Such missing differentiation may cause dendrograms to fail in separating groups, while it may be possible to construct a chart illustrating the degree of phenetic relationships between groups at different levels by the use of multivariate methods (Blackith & Reyment 1971). One of these methods is the discriminant analysis, which was performed with the computer programmes SAS (Luginbuhl & Schlotzhauer 1987) and SPSS 9.0.1. Specimens with missing data (usually missing flagellum) were ignored and thus 140 specimens were included in the analysis. This sample consisted of 28 *P. arachniformis*, 26 *A. euthynota*, 30 *A. longicornis*, 27 *A. nguru* and 29 *A. usambarica*. Since the sample size of the within group should always be higher than the number of variables, two characters had to be excluded. These characters were chosen according to their contribution to the discriminant functions, described by the Wilks' Lambda. A first analysis was made including the outgroup *P. arachniformis*, the second analysis excluded the outgroup, including 112 specimens. The reason for the exclusion was the great distance between *P. arachniformis* and the *Afrophlaeoba* species, which influenced the visibility of the intrageneric differences in a plot of the canonical variates.

The discriminant analysis is an ordination technique for displaying and describing the differences between group centroids (in this case for each species) by extracting the eigen vectors from the pooled variance-covariance matrix of the within-group (Slaney & Weinstein 1996). It tests the differences for significance and reveals measures of the contribution of each character to the discriminant function. While the characters have to be scaled metric, the groups can be nominally scaled. It also is possible

to forecast the group affiliation of an element of unknown identity, if the resolution is high enough. In contrast to cluster analyses the discriminant analysis does not create groups, but rather examines given groups (Backhaus et al. 2000). The method is quite robust even to considerable deviations from the theoretical requirement of homogeneity in the dispersion matrices (Blackith & Reyment 1971).

The first step of a discriminant analysis is the definition of the groups to be examined. In the present study the groups are defined by the different species or localities from which they were collected. The sample size has to be large enough and the number of groups should not exceed the number of variables (Backhaus et al. 2000). The canonical discriminant function is estimated computationally, which allows an optimal discrimination between the groups and the assessment of the discriminatory importance of the characters. The discriminant function is structured as follows:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + ... + b_j X_j$$

Y = discriminant variable
X_j = character variable j (j = 1,2,…,J)
b_j = discriminant coefficient for the character variable j
b_0 = constant term

It is scaled metric due to a linear combination of each variable. For each group a centroid can be calculated according to the equation:

$$\overline{Y}_g = \frac{1}{I_g} \sum_{i=1}^{I_g} Y_{gi}$$

Y = discriminant variable
g = group
i = element

The difference between two groups is defined simply by their residual. Each element and the centroids can be localized on a canonical axis (also referred to as canonical variate) to illustrate the differences. The variance within and between the groups is of high importance. The variance between the groups can be obtained from the squared deviation of the group centroids (multiplied by the sample size) from the grand total centroid (SS_b: sum of squares between). The variance within the groups is calculated by the squared deviation of each element from the centroid (SS_w: sum of squares within). The best discriminance between two groups is estimated by maximising the discriminant criterion (Γ), which is calculated by dividing SS_b/SS_w. This maximum discriminant criterion is referred to as eigen value. Geometrically spoken, the discriminant function is a plane within the space of variables. In a two-dimensional plot it is usually referred to as discriminant axis or canonical variate (Blackith & Reyment 1971, Backhaus et al. 2000). The number of discriminant functions depends upon the number of groups and variables (maximum: g-1 functions). The second discriminant function is calculated to explain a maximum proportion of the variance left. Since the first function is already maximised, the second function must have a smaller eigen value. The proportion of the eigen values represents a good measure for the relative importance of each function (Backhaus et al. 2000).

To evaluate the quality of a discriminant function, it is possible to compare the proportion of rightly grouped elements with a random distribution (classification). The effect of the same sample can be

ignored, if the total sample is divided in subsamples. Another measure of the quality is the canonical correlation coefficient:

$$c = \sqrt{\frac{\gamma}{1+\gamma}}$$

c = canonical correlation coefficient
γ = explained variance
1+γ = unexplained variance

The most frequently used criterion for evaluating the discriminance is Wilks' Lambda:

$$\Lambda = \frac{1}{1+\gamma}$$

Wilks' Lambda is an inverse measure, which means that smaller values represent a better discriminating power of a function and vice versa. It is possible to transform Wilks' Lambda into a probabilistic variable and make probability statements. This allows statistical significance testing by the χ^2 test or F statistics. In contrast to Wilks' Lambda these tests of significance evaluate the difference between the groups, but not the discriminating power of the function. The multivariate Wilks' Lambda is the product of the univariate lambdas (Backhaus et al. 2000).

The significance of each parameter measured is calculated by univariate F statistic, based on Wilks' Lambda. However, the discriminating power of the combined variables is often substantially higher than their sum due to multivariate interactions. A multivariate approach to evaluate the discriminating significance of a variable in a discriminant function is given by the discriminant coefficients, which are usually standardized in a way so that the pooled within-group variance is one. The discriminant coefficients are standardized by multiplying them with the pooled within-group variances. The amount of the standardized discriminant coefficient of each variable for each function is equivalent to the discriminating power within this function (Backhaus et al. 2000).

A stepwise discriminant analysis includes the character variable in each step, which maximizes the quality measures (respectively minimize Wilks' Lambda). Characters which do not contribute significantly to the discriminant functions, are excluded. This algorithm automatically sorts the variables according to their importance by including the most important characters first (Backhaus et al. 2000). A stepwise computation was performed using SPSS 9.0.1. to identify those body dimensions, which are minimizing Wilks' Lambda. The maximum significance of the F value for the inclusion of a variable was set to 0.01; the minimal significance for the exclusion was set to 0.15.

Phylogenetic Inference

A phylogenetic analysis of discrete data was not possible, due to the lack of dissimilarities in qualitative characters. The metric scale of morphometric data does not allow to derive different character states from them, which could be used for a conventional phylogenetic analysis based on synapomorphies. The discriminant analysis, however, reveals pairwise squared Mahalanobis distances between the five groups, which are found by multiplying the vector of coefficients that constitutes the

discriminant function by the vector of differences between the means for the groups. Because, it is based on the discriminant function, the Mahalanobis generalized distance allows each character to carry only its proper amount of information about the separation of groups, and eliminates the effects of correlation between the characters (Blackith & Reyment 1971). The discriminant technique might be an appropriate method for inferring phenetic affinities, since there can be no question of *a priori* weighting (Blackith & Reyment 1971). The roots of the pairwise Mahalanobis distances can be used to perform distance methods as they were described in chapter 4.2.6.3 to derive a phenogram (Zink et al. 1999). Such a phenogram is the result of a polythetic process based on the need to maximize the between-group variance in relation to the within-groups variance. It represents relationships based on phenetic similarity rather than on common descent (Blackith & Reyment 1971). In a clustering process more closely associated individuals or groups are brought together into a cluster, which is then considered to be differentiated from other associations forming separate clusters. The great strength and resilience of such divisive techniques may be influenced by convergence or divergence processes. Hence, differences frequently need to be distinguished from various aspects of environmentally induced variation. According to Blackith & Reyment (1971) the possible problems of convergent or divergent evolution can be minimized by including a multiplicity of characters, drawn from as wide a range of the parts of the organism as possible. According to Omland (1997) molecular and morphological evolution may be coupled in many cases, unless extraordinary body forms are induced by special evolutionary pressures (Wilson 1991). In morphologically closely related species, this does not seem to be the case. To estimate the similarity of the gene trees inferred in chapter 4 and the morphological phenograms in this chapter, the correlation between the genetic Jukes-Cantor-Distance and the roots of the Mahalanobis generalized distance is examined.

Univariate Analysis

SAS reveals simple statistics (mean, variance, standard deviation) for the total sample and for each species, which were used for the univariate pairwise analyses of each species and character. The morphometric data rendered normal distributions (χ^2 test), so Student's t-tests were performed to test on differences between the groups (Precht 1979). The highest P accepted was 0.05. The tests were performed for each body dimension and for some ratios. Only those characters were presented, which are either given in the keys and descriptions of Jago (1983) or which contribute much to the discrimination of species.

5.3 Results: Morphometrics

5.3.1 Canonical Discriminant Analysis including *P. arachniformis*

5.3.1.1 Choice of the Characters

Most of the measures are significantly correlated with each other (87% of the 368 possibilities, $P < 0.05$). Less correlated characters include the flagellar widths (in particular of segment 4 and 6) and the interocular distance. In comparison to those widths, the length measures are well correlated with each other. In some cases (interocular distance) the missing correlations are probably an outcome of the different proportions of *P. arachniformis* in comparison to the *Afrophlaeoba* species. The univariate F-statistics revealed significant differences between all 27 characters ($P < 0.0001$). Due to the lower sample of *A. euthynota* (26 specimens), caused by antennal loss, two variables with high Wilks' Lambda had to be excluded. Wilks' Lambda for each character is given in table 21 and the highest values are emphasized. Those two characters (widths of second and sixth flagellar segments) were excluded from the analysis.

Tab. 21: Wilks' Lambda, F statistics, degrees of freedom and significance for the 27 characters measured (df1 = 4, df2 = 135, * = significant, $P < 0.0001$), grey shaded areas represent the two characters with the highest Wilks' Lambda, which have been excluded from the analysis.

Character	FlagL	FS1+2L	FS2W	FS3L	FS3W	FS4L	FS4W	FS5L	FS5W	FS6L	FS6W	FS9L	FS9W	HeadL
Wilks' Lambda	0.313	0.355	0.788	0.317	0.778	0.436	0.729	0.28	0.722	0.245	0.837	0.3	0.682	0.451
F	74.15	61.45	9.103	72.79	9.657	43.72	12.52	86.65	12.98	103.76	6.569	78.88	15.77	41.02
Significance	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Tab. 21 (continued)

Character	HeadW	VertL	VertW	Intoc	AntCar	PostCar	Proz	Metaz	TegL	TegW	Cerc	HFemL	HFemW
Wilks' Lambda	0.22	0.304	0.418	0.506	0.563	0.195	0.366	0.383	0.626	0.121	0.455	0.244	0.2
F	119.71	77.34	47.03	32.97	26.14	139.35	58.52	54.47	20.17	244.82	40.43	104.30	135.14
Significance	*	*	*	*	*	*	*	*	*	*	*	*	*

5.3.1.2 Discriminating Power of the Analysis

Wilks' Lambda is exceptionally low ($\Lambda = 0.001$; $\chi^2 = 835.7$; df = 100; $P < 0.0001$). For CAN2 to CAN4 it is 0.046 ($\chi^2 = 381.4$; df = 72; $P < 0.0001$), for CAN3 to CAN4 0.200 ($\chi^2 = 199.7$; df = 46; $P < 0.0001$) and for CAN4 0.562 ($\chi^2 = 71.5$; df = 22; $P < 0.0001$). The low values of Wilks' Lambda illustrate the high discriminating power of the discriminant functions. The classification phase of the discriminant analysis assigned all but six specimens (95.7%) correctly. The wrong assignments included four *A. longicornis* grouped with *A. nguru*, one *A. euthynota* with *A. usambarica* and one *A. longicornis* with *A. usambarica*.

5.3.1.3 Pairwise Mahalanobis Distance and Discriminant Structure

The multivariate analysis revealed significant pairwise differences between all species (F statistics; $P < 0.0001$). The highest Mahalanobis distances were found between the outgroup *P. arachniformis* and the *Afrophlaeoba* species (table 22). Within *Afrophlaeoba* the lowest Mahalanobis distance occurred between *A. nguru* and *A. longicornis* and the highest distance between *A. nguru* and *A. euthynota*.

Tab. 22: Generalized Mahalanobis distances (D^2) between the species; *P. arachniformis* is shaded.

Species	*A. euthynota*	*A. longicornis*	*A. nguru*	*A. usambarica*
P. arachniformis	238.53	239.02	212.12	249.67
A. euthynota		23.52	30.16	20.82
A. longicornis			8.85	16.37
A. nguru				20.09

The great distance between *P. arachniformis* and all *Afrophlaeoba* species is also illustrated by the eigen value of the canonical variate 1 (CAN1, table 23). This first variate clearly discriminates *P. arachniformis* from the *Afrophlaeoba* species. The *P. arachniformis* data set forms a distinct group in the discriminatory topology on this axis (figure 21). CAN1 explains 86.5% of the differences, while the second function explains only 7.6%, the third function 4.1% and the fourth function 1.8% of the differences (table 23). All four functions, however, show significant differences between the five species (F statistics; $P < 0.0001$).

Tab. 23: Eigen values of the four functions; note the high eigen value of CAN1 (shaded).

CAN	Eigen value	Difference	Proportion	Cumulative
1	37.99	34.66	0.865	0.865
2	3.33	1.52	0.076	0.941
3	1.81	1.03	0.041	0.982
4	0.78	-	0.018	1.000

The group centroids of each species for the four canonical variates are given in table 24, illustrating again the high discrimination of *P. arachniformis* on CAN1. Figure 21 shows a plot of the first two canonical variates of the discriminatory topology, illustrating the great distance of *P. arachniformis* on the first function and the differences between *A. euthynota* and *A. nguru* on the second function.

Tab. 24: Group centroids for the five species on the four canonical variates; note the high distance between *P. arachniformis* and the *Afrophlaeoba* species on the CAN1.

Species	CAN1	CAN2	CAN3	CAN4
P. arachniformis	12.07	-0.10	0.10	0.09
A. euthynota	-3.00	-3.10	-1.34	-0.28
A. longicornis	-3.23	1.43	-0.80	1.34
A. nguru	-2.23	2.16	-0.53	1.35
A. usambarica	-3.55	-0.61	2.43	0.04

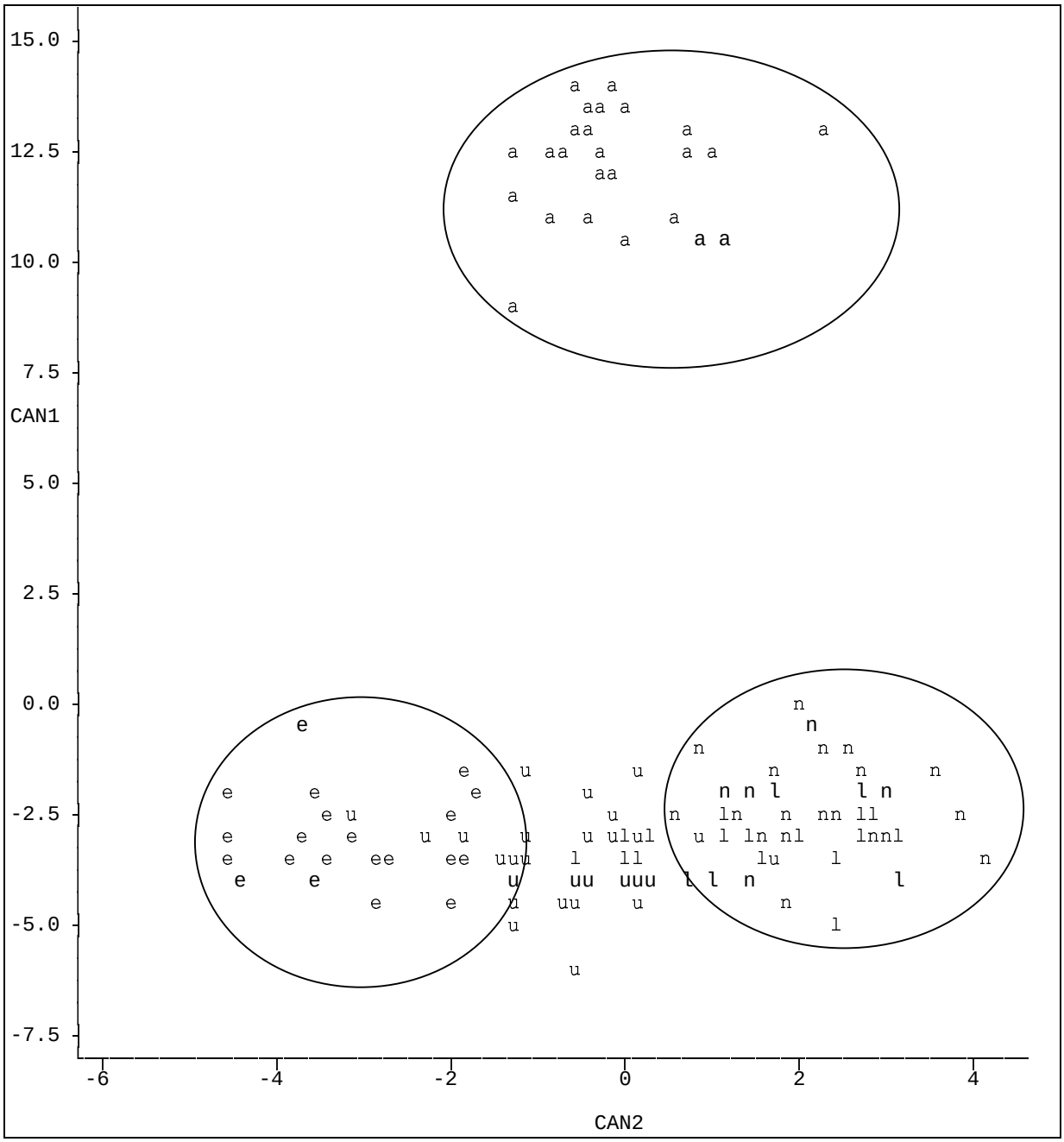


Fig. 21: Plot of canonical variates 1 and 2 of the discriminatory topology, illustrating the great distance between *P. arachniformis* (upper group) and the four *Afrophlaeoba* species (lower group); note: 21 objects are hidden, a = *P. arachniformis*, e = *A. euthynota*, l = *A. longicornis*, n = *A. nguru*, u = *A. usambarica*, circles mark the three species *P. arachniformis*, *A. euthynota* and *A. nguru*.

5.3.1.4 Contribution of Single Characters

The contribution of single characters to the complete analysis was calculated by a stepwise discriminant analysis. The stepwise analysis included 13 characters, which are given in table 25. The first character (TegW) belongs to the strongest characters of CAN1, while the second one (VertL) belongs to the strongest characters of CAN2 and CAN3 (table 26). Figure 22 shows a plot of those two most effective discriminating characters of the stepwise analysis against each other. In this

bivariate plot, *P. arachniformis* is easy to distinguish from the *Afrophlaeoba* species by its broader tegminae and *A. euthynota* by its shorter vertex. However, there are some outliers among the sample, including a *P. arachniformis* plotted close to *Afrophlaeoba* and an *A. euthynota* with a comparative long vertex, grouping it between the other *Afrophlaeoba* species. In a multivariate plot (figure 21) such outliers are partly corrected by other characters, although it has already been mentioned that even in the complete analysis some specimens were classified wrongly. The other three *Afrophlaeoba* species overlap largely in this plot, since the third and fourth variates are not presented.

Tab. 25: Included characters in the stepwise discriminant analysis in order of the steps in which they were included. The value of Wilks' Lambda illustrates the contribution of each value to the analysis – the value is decreasing with each character added.

Step	Character	Wilks' Lambda	Estimated F	df1	df2	Significance
1	TegW	0.121	244.8	4	135.0	0.0001
2	VertL	0.042	130.9	8	268.0	0.0001
3	FS6L	0.025	89.1	12	352.2	0.0001
4	VertW	0.017	71.2	16	403.9	0.0001
5	HeadW	0.010	66.7	20	435.4	0.0001
6	PostCar	0.006	61.6	24	454.7	0.0001
7	AntCar	0.005	56.9	28	466.5	0.0001
8	FS9W	0.004	50.7	32	473.6	0.0001
9	FS3L	0.004	46.1	36	477.7	0.0001
10	FS4L	0.003	44.3	40	479.6	0.0001
11	TegL	0.003	41.2	44	480.2	0.0001
12	HFemL	0.002	38.4	48	479.7	0.0001
13	Proz	0.002	36.7	52	478.5	0.0001

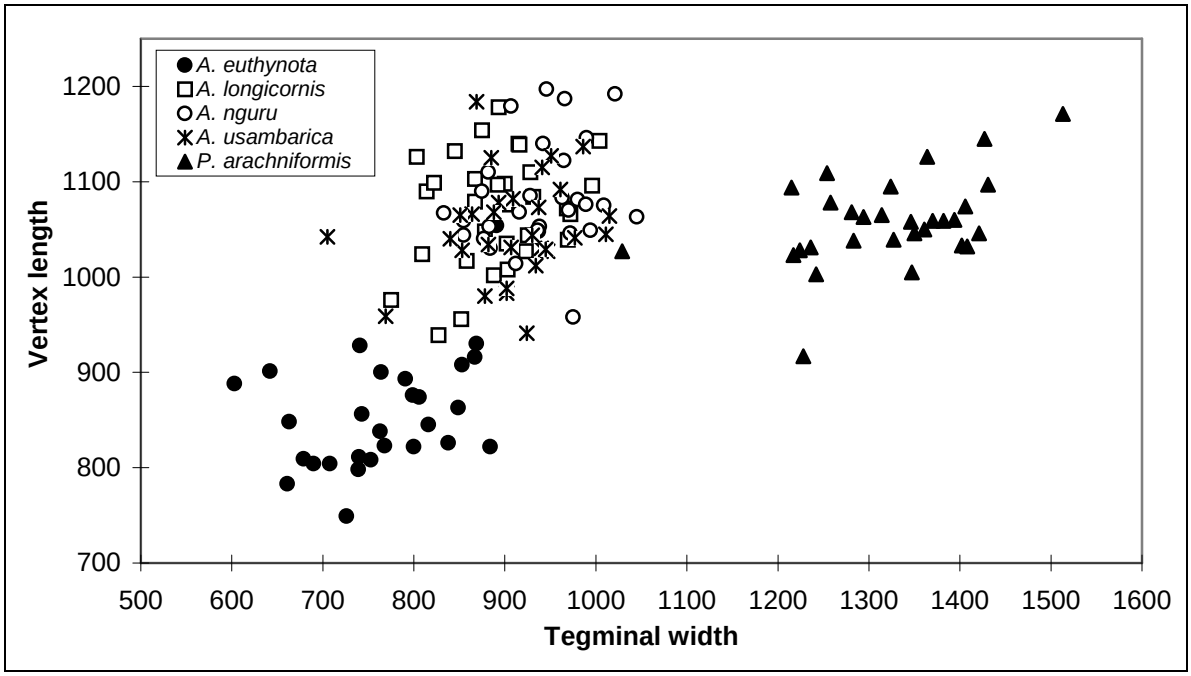


Fig. 22: Bivariate plot of the two most important variables of the stepwise discriminant analysis. Note the great distance between *P. arachniformis* and all *Afrophlaeoba* species, caused by the broad tegminae and the low vertex lengths in *A. euthynota*.

The description of the functions is represented by the standardized canonical coefficients (table 26). The characters with the highest amounts contribute most to the discriminant function. The three highest standardized canonical coefficients of the first variate are the vertex width (VertW: 1.21), the distance between the posterior lateral carinae of pronotum (PostCar: 0.76) and the tegminal width (TegW: 0.70). It is obvious that all those characters represent body widths, which characterize *P. arachniformis*. The two most effective variables of CAN1 are plotted against each other in figure 23. The higher posterior distance between the lateral pronotal carinae in *P. arachniformis* separates this group clearly from the *Afrophlaeoba* species. Among the *Afrophlaeoba* species *A. euthynota* is characterized by a narrower vertex and *A. usambarica* is characterized by a small posterior distance between the lateral carinae of pronotum in relation to its broader vertex.

Tab. 26: Standardized canonical discriminant coefficient for the canonical variates CAN1 to CAN4. The highest values are shaded.

	CAN1	CAN2	CAN3	CAN4
FlagL	0.260	-0.378	0.051	-0.894
FS1+2L	-0.190	0.003	0.106	0.635
FS3L	0.131	0.417	-0.510	-0.503
FS3W	-0.175	0.259	-0.344	0.036
FS4L	-0.291	-0.131	0.330	0.651
FS4W	-0.145	-0.274	0.712	-0.087
FS5L	-0.032	0.017	0.102	0.398
FS5W	0.239	0.043	-0.373	0.265
FS6L	0.259	0.113	-0.331	0.065
FS9L	-0.052	0.326	-0.287	0.073
FS9W	0.309	-0.323	0.255	-0.064
HeadL	0.149	0.097	-0.004	-0.316
HeadW	0.659	-0.250	0.397	-0.350
VertL	0.007	0.582	0.656	0.058
VertW	-1.205	0.206	-0.009	0.015
Intoc	-0.003	-0.026	0.385	0.160
AntCar	-0.643	0.125	-0.258	0.091
PostCar	0.764	-0.117	-0.309	0.761
Proz	-0.167	0.440	-0.163	-0.812
Metaz	-0.179	0.032	-0.067	0.005
TegL	-0.350	0.253	-0.087	0.162
TegW	0.698	0.007	0.329	-0.282
Cerc	0.138	0.145	-0.030	-0.175
HFemL	0.274	-0.618	-0.089	0.913
HFemW	0.210	0.242	0.151	-0.407

On CAN2 the length of hind femora (HFemL: 0.62), the length of the vertex (VertL: 0.58) and the length of the prozona (Proz: 0.44) account for most of the discriminatory power. The functions CAN2, CAN3 and CAN4 discriminate the four *Afrophlaeoba* species. However, the differences become more obvious if the discriminant analysis is calculated without *P. arachniformis*, since the great distance between *P. arachniformis* data and *Afrophlaeoba* data influences the visibility of the differences within *Afrophlaeoba*.

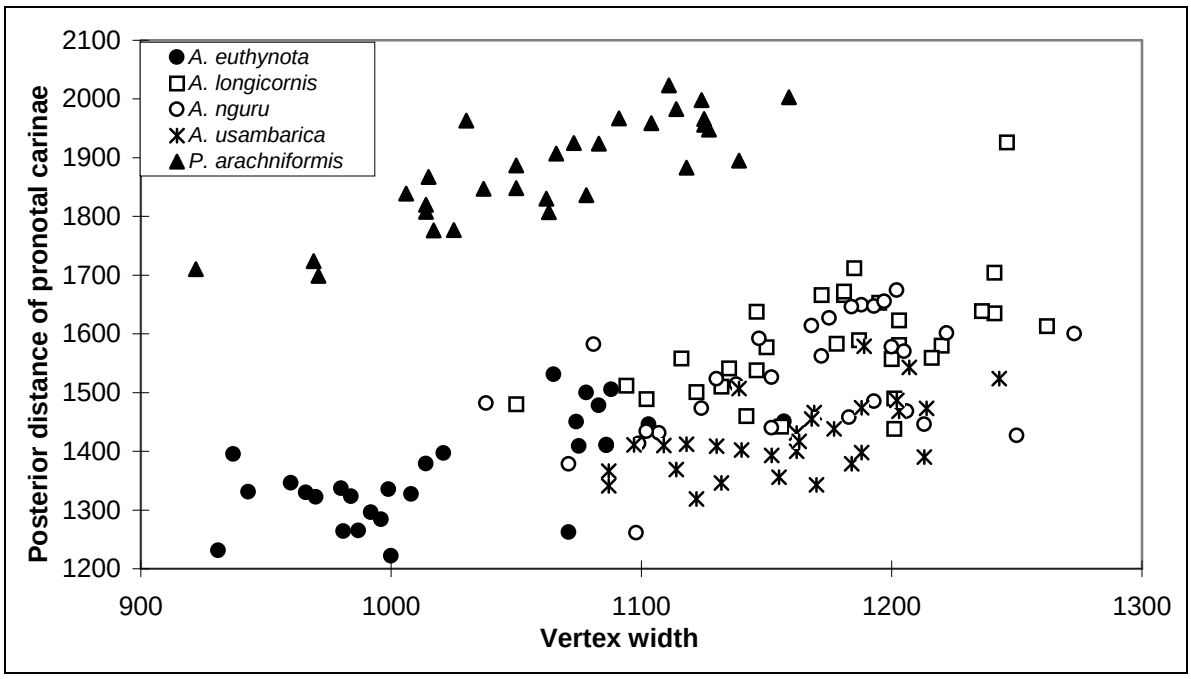


Fig. 23: Plot of the two most important variables of CAN1 against each other. Note the great distance of the posterior lateral carinae of pronotum in *P. arachniformis*, the narrow vertex in *A. euthynota* and the comparative small distance of posterior lateral carinae in *A. usambarica*.

5.3.2 Canonical Discriminant Analysis excluding *P. arachniformis*

5.3.2.1 Choice of the Characters

If only *Afrophlaeoba* species are compared, the number of correlated measures becomes higher than in the first analysis (91% of the 368 possibilities, $P < 0.05$). Less correlated characters include the flagellar widths (in particular of segments 4, 6 and 9). The univariate F-statistics revealed significant differences of all 27 characters ($P < 0.0001$). Again, two characters with high Wilks' Lambda had to be excluded due to the lower sample size of *A. euthynota*. Wilks' Lambda for each character is given in table 27, and the values of excluded characters are emphasized (FS6W and FS3W).

Tab. 27: Wilks' Lambda, F statistics, degrees of freedom and significance for the 27 characters measured (df1 = 3, df2 = 108, * = significant, $P < 0.0001$), grey shaded areas represent the two characters with the highest Wilks' Lambda, which were excluded from the analysis.

Character	FlagL	FS1+2L	FS2W	FS3L	FS3W	FS4L	FS4W	FS5L	FS5W	FS6L	FS6W	FS9L	FS9W	HeadL
Wilks' Lambda	0.377	0.427	0.784	0.432	0.787	0.557	0.714	0.412	0.704	0.387	0.802	0.418	0.703	0.468
F	59.59	48.38	9.91	47.26	9.75	28.69	14.43	51.36	15.14	56.98	8.89	50.05	15.19	40.92
Significance	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Tab. 27 (continued)

Character	HeadW	VertL	VertW	Intoc	AntCar	PostCar	Proz	Metaz	TegL	TegW	Cerc	HFemL	HFemW
Wilks' Lambda	0.544	0.290	0.401	0.580	0.505	0.519	0.399	0.594	0.549	0.505	0.629	0.415	0.465
F	30.23	88.10	53.68	26.12	35.29	33.38	54.19	24.66	29.63	35.32	21.27	50.68	41.42
Significance	*	*	*	*	*	*	*	*	*	*	*	*	*

5.3.2.2 Discriminating Power of the Analysis

All three functions show significant differences between the four species. Again Wilks' Lambda is quite low ($\Lambda = 0.027$; $\chi^2 = 349.3$; df = 75; $P < 0.0001$). For CAN2 to CAN3 it is 0.141 ($\chi^2 = 189.1$; df = 48; $P < 0.0001$) and for CAN3 0.453 ($\chi^2 = 76.5$; df = 23; $P < 0.0001$). Thus the high discriminating power of the discriminant functions can be confirmed. The values of Wilks' Lambda are even lower than those of CAN2 to CAN4 of the first analysis. The classification statistics assigned six specimens wrong. These included one *A. euthynota*, which was classified as *A. usambarica*, three *A. longicornis* as *A. nguru*, one *A. longicornis* as *A. usambarica* and one *A. nguru* as *A. longicornis*.

5.3.2.3 Interspecific Differences and Discriminant Structure

The discriminant analysis revealed significant differences between all species (F statistics; $P < 0.0001$). The greatest Mahalanobis distance occurred between *A. euthynota* and *A. nguru*, the smallest between *A. longicornis* and *A. nguru* (table 28). These relations correspond to the first analysis.

Tab. 28: Generalized Mahalanobis distances (D^2) between the four *Afrophlaeoba* species:

Species	*A. longicornis*	*A. nguru*	*A. usambarica*
A. euthynota	24.63	30.61	21.88
A. longicornis		10.04	16.02
A. nguru			19.85

The exclusion of *P. arachniformis* data leads to higher eigen values of those canonical functions, which discriminate between the *Afrophlaeoba* species (table 29). The first function discriminates the *A. euthynota* data from the data of *A. longicornis* and *A. nguru*. It explains 55.5% of the differences. This comparatively high value is mainly based on the smaller body size of *A. euthynota*, which is already visible in the figures 22 and 23. CAN2 explains 28.8% of the differences within *Afrophlaeoba* and CAN3 15.7%.

Tab. 29: Eigen values of the three canonical functions.

CAN	Eigen value	Difference	Proportion	Cumulative
1	4.26	1.99	0.555	0.555
2	2.21	1.10	0.288	0.843
3	1.21	-	0.157	1.000

The group centroids for the *Afrophlaeoba* species for the three canonical variates are given in table 30, illustrating again the discrimination of *A. euthynota* on CAN1, of *A. usambarica* on CAN2 and of *A. longicornis* and *A. nguru* on CAN3. Figure 24 shows a plot of the first two canonical variates with the locations of *A. euthynota*, *A. usambarica* and *A. nguru* marked. In figure 25 CAN2 and CAN3 are plotted against each other with *A. nguru*, *A. longicornis* and *A. usambarica* marked. This second plot is equivalent to a view from "below" (CAN2) on figure 24, if the canonical variate 3 is drawn in the third dimension. *A. longicornis* and *A. usambarica* are well separated on CAN2.

Tab. 30: group centroids for the five species on the four canonical variates; note the high distance between *P. arachniformis* and the *Afrophlaeoba* species on the CAN1.

Species	CAN1	CAN2	CAN3
A. euthynota	-3.29	-1.18	-0.15
A. longicornis	1.26	-0.39	1.63
A. nguru	2.17	-1.02	-1.33
A. usambarica	-0.38	2.42	-0.31

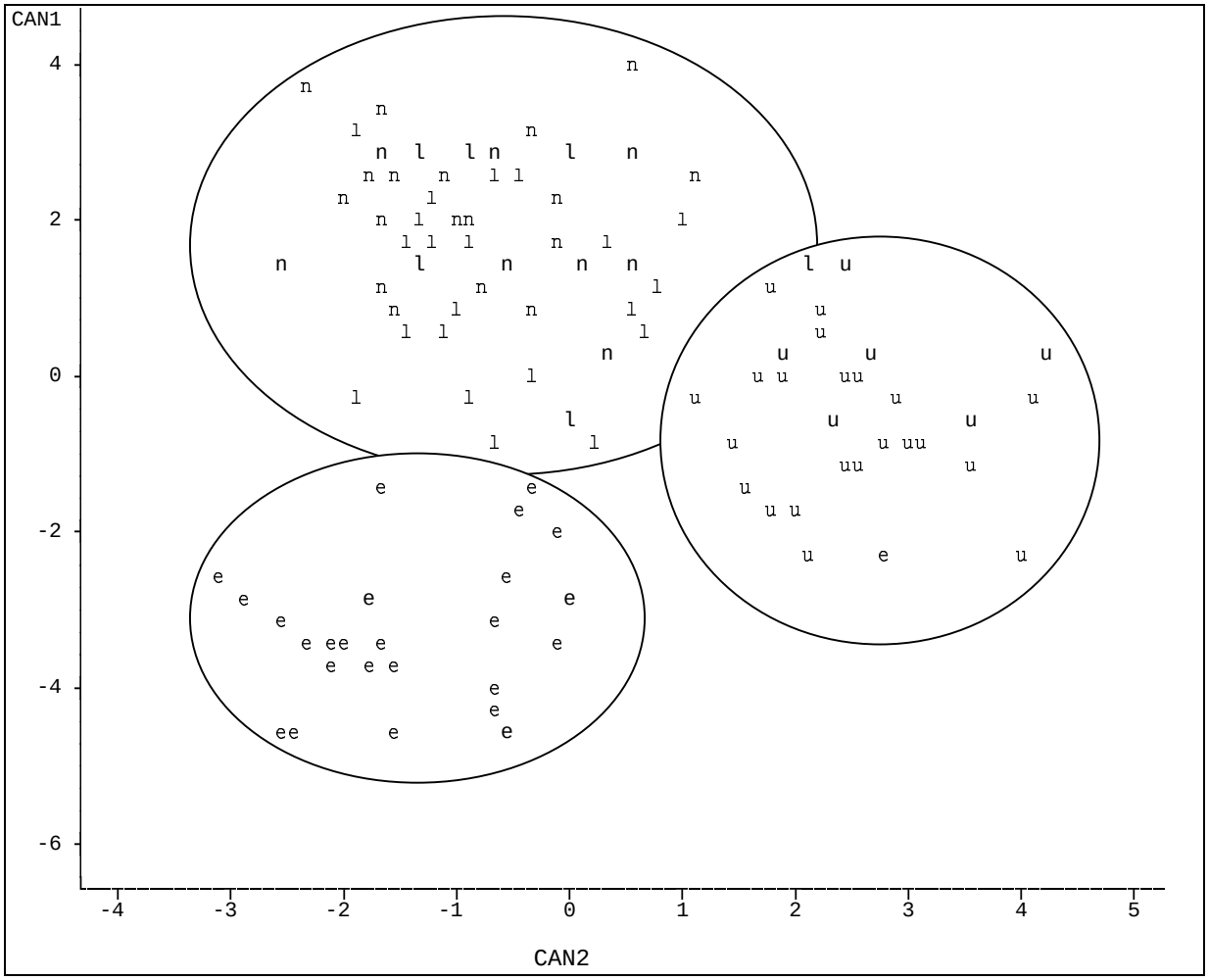


Fig. 24: Plot of canonical variates 1 and 2 of the discriminatory topology; it illustrates the separation of *A. euthynota* and *A. usambarica* on CAN2 and of *A. euthynota* from the *nguru-longicornis* group on CAN1; note: 5 objects are hidden, e = *A. euthynota*, l = *A. longicornis*, n = *A. nguru*, u = *A. usambarica.*

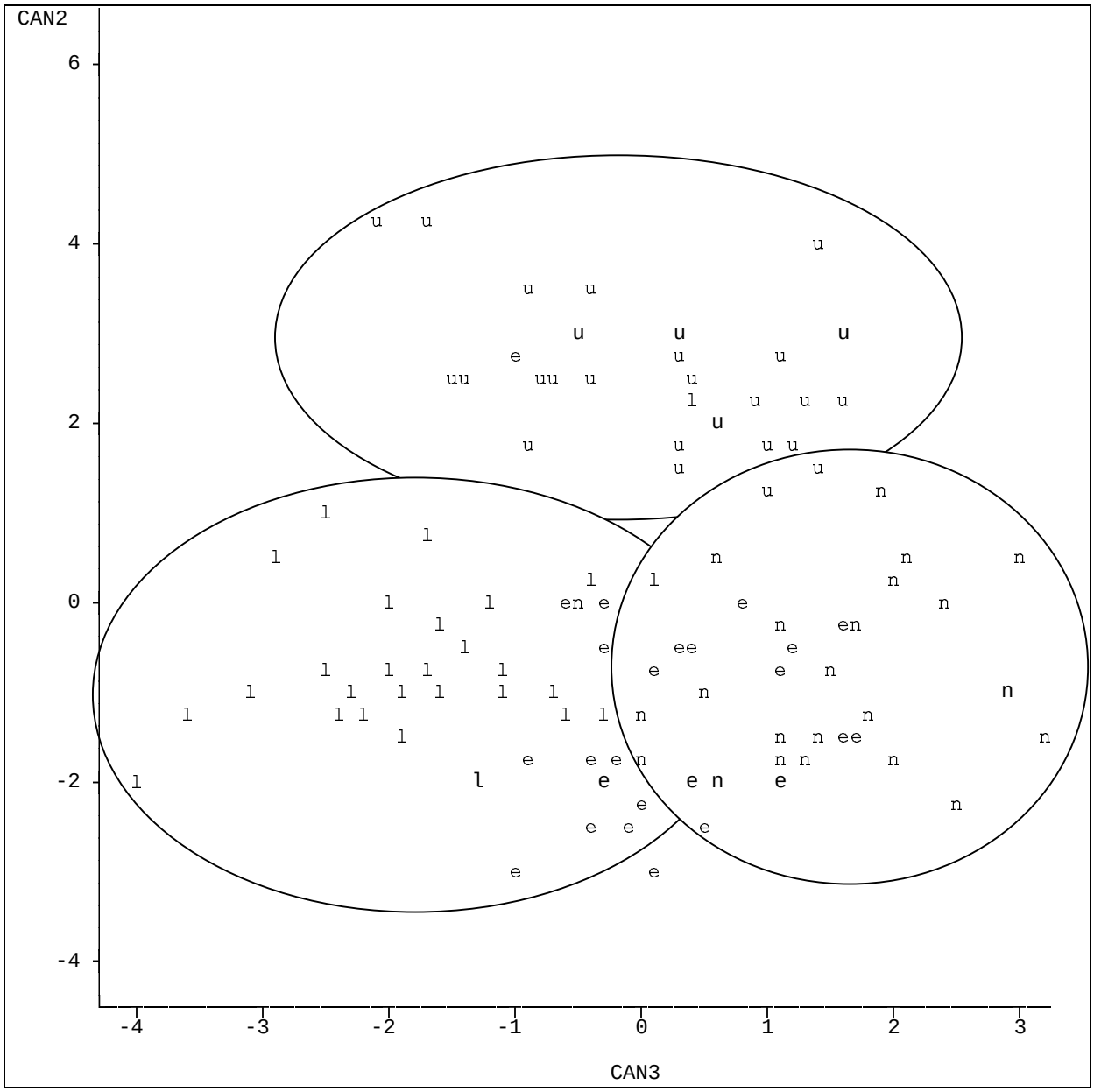


Fig. 25: Plot of canonical variates 2 and 3 of the discriminatory topology; it illustrates the separation of *A. longicornis* and *A. nguru* on CAN3 and *A. usambarica* on CAN2; note the overlap of *A. nguru* and *A. longicornis*; 9 objects are hidden; e = *A. euthynota*, l = *A. longicornis*, n = *A. nguru*, u = *A. usambarica*

5.3.2.4 Contribution of Single Characters

Ten characters were included in the stepwise analysis (table 31). These differ from the first stepwise analysis, illustrating the different power of characters for the discrimination of the *Afrophlaeoba* species. The most effective discriminating character (VertL) is the strongest of CAN1 and one of the strongest of CAN2, while the second one (FS3L) belongs to the most effective characters of CAN2 (table 32). A bivariate plot of those two variables (figure 26) shows that the most distinct group is represented by *A. euthynota*, due to the shorter vertex. *A. usambarica* is characterized by a comparatively short flagellar segment 3 in comparison to the other two *Afrophlaeoba* species. In all four species outliers are found.

Tab. 31: Included characters in the stepwise discriminant analysis in order to the steps in which they were included. The value of Wilks' Lambda shows the contribution of each value to the analysis – the value is decreasing with each character added .

Step	Character	Wilks' Lambda	estimated F	df1	df2	Significance
1	VertL	0.290	88.101	3	108.0	0.0001
2	FS3L	0.177	49.225	6	214.0	0.0001
3	FS1+2L	0.138	35.945	9	258.1	0.0001
4	FS9W	0.112	29.756	12	278.1	0.0001
5	TegW	0.097	25.427	15	287.5	0.0001
6	PostCar	0.084	22.715	18	291.8	0.0001
7	HeadW	0.075	20.487	21	293.4	0.0001
8	FS4L	0.067	18.875	24	293.5	0.0001
9	FS6L	0.058	17.852	27	292.7	0.0001
10	IntOc	0.054	16.608	30	291.3	0.0001

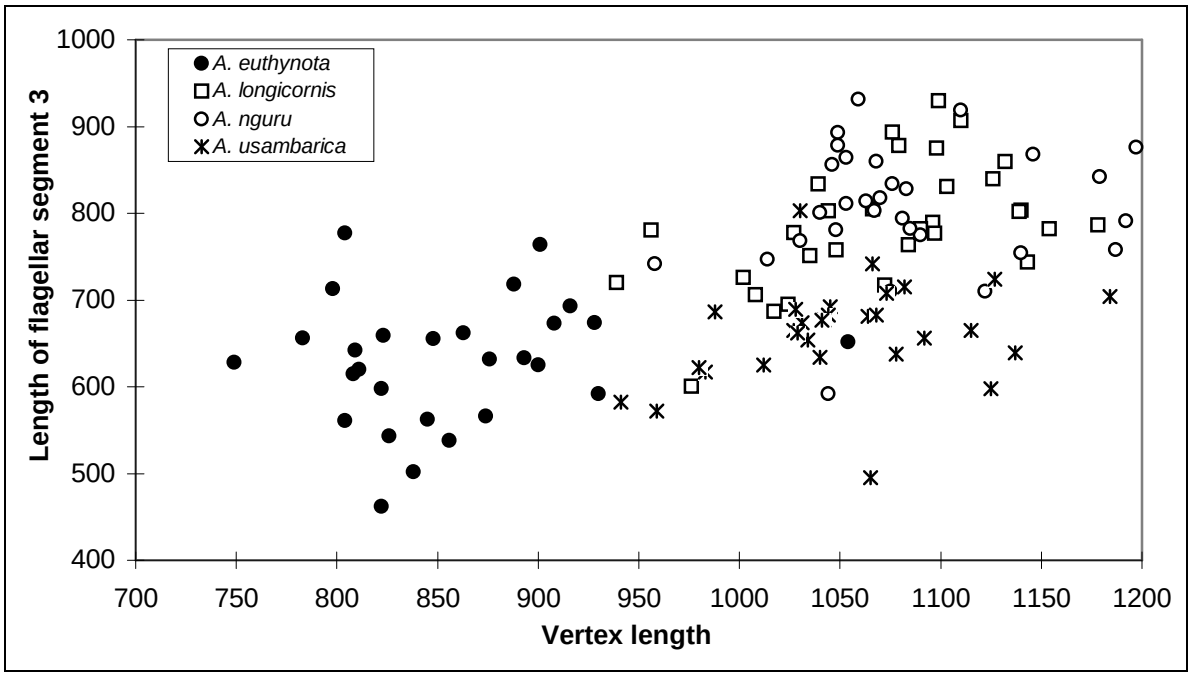


Fig. 26: Bivariate plot of the two most important variables of the stepwise discriminant analysis. While *A. euthynota* is characterized by a narrow vertex, *A. usambarica* has a shorter flagellar segment 3 in comparison to *A. nguru* and *A. longicornis*.

The standardized canonical coefficients for the second analysis are given in table 32. The three body dimensions that are particularly important for discriminating between the species in the first function are the vertex length (VertL: 0.67), the length of the hind femur (HFemL: 0.62) and the length of the prozona (Proz: 0.36). These characters are very similar to the most important characters of CAN2 of the first analysis. The two most effective discriminating characters of CAN1 are plotted against each other in figure 27, illustrating the high discriminating power of CAN1 for *A. euthynota*.

Tab. 32: Standardized canonical discriminant coefficient for the canonical variates CAN1 to CAN3. The three highest values are shaded for each variate.

	CAN1	CAN2	CAN3
FlagL	-0.032	0.231	-0.889
FS1+2L	0.236	0.339	0.564
FS3L	0.344	-0.629	-0.347
FS3W	0.311	-0.246	0.051
FS4L	-0.208	0.502	0.612
FS4W	-0.235	0.746	-0.114
FS5L	-0.103	0.237	0.516
FS5W	-0.080	-0.431	0.403
FS6L	-0.162	-0.812	-0.132
FS9L	0.183	-0.352	0.192
FS9W	-0.345	0.141	-0.041
HeadL	0.129	0.035	-0.098
HeadW	-0.034	0.060	-0.747
VertL	0.667	0.532	-0.278
VertW	-0.067	0.130	0.166
Intoc	0.083	0.414	0.020
AntCar	0.110	0.028	0.502
PostCar	-0.153	-0.395	0.399
Proz	0.359	-0.236	-0.496
Metaz	0.040	0.057	0.174
TegL	0.249	-0.032	0.192
TegW	0.275	0.166	-0.449
Cerc	0.229	-0.079	-0.138
HFemL	-0.618	-0.022	0.849
HFemW	0.290	0.044	-0.417

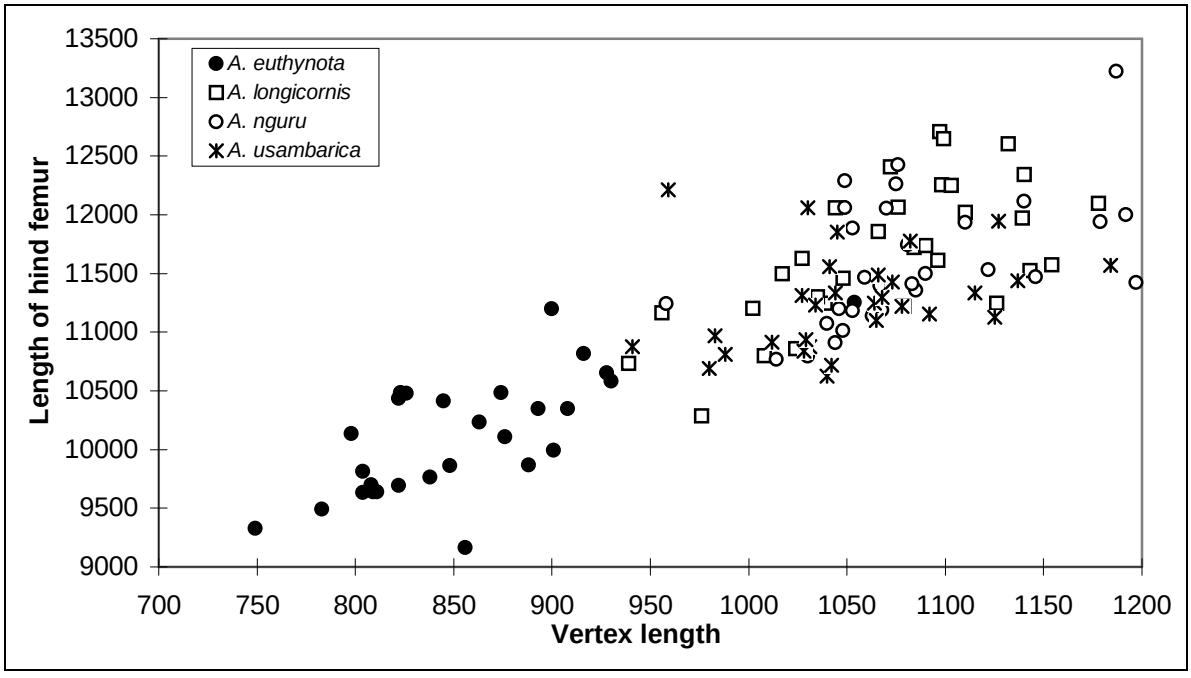


Fig. 27: Plot of the two most important variables of CAN1 against each other. Note the high discriminating power of these variables for the identification of *A. euthynota*.

CAN2 separates *A. usambarica* from the other species. The three characters with the highest standardized canonical coefficients are the width of flagellar segment 4 (FS4W: 0.75), the length of flagellar segment 3 (FS3L: 0.63) and the vertex length (VertL: 0.53). Again these three characters are identical to the three most effective characters of CAN3 of the first analysis. CAN2 explains 28.8% of the variation.

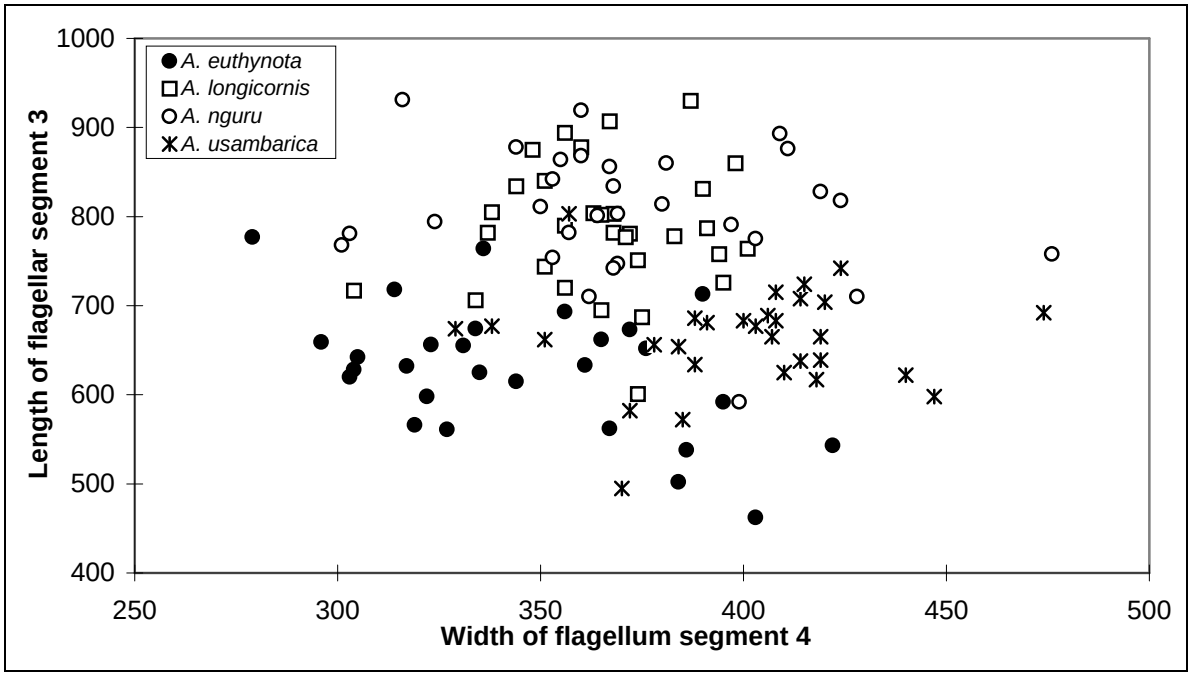


Fig. 28: Bivariate plot of the two most important variables of CAN2. In *A. usambarica* and *A. euthynota* the flagellar segment 3 is shorter, *A. usambarica* has a comparative broad flagellar segment 4.

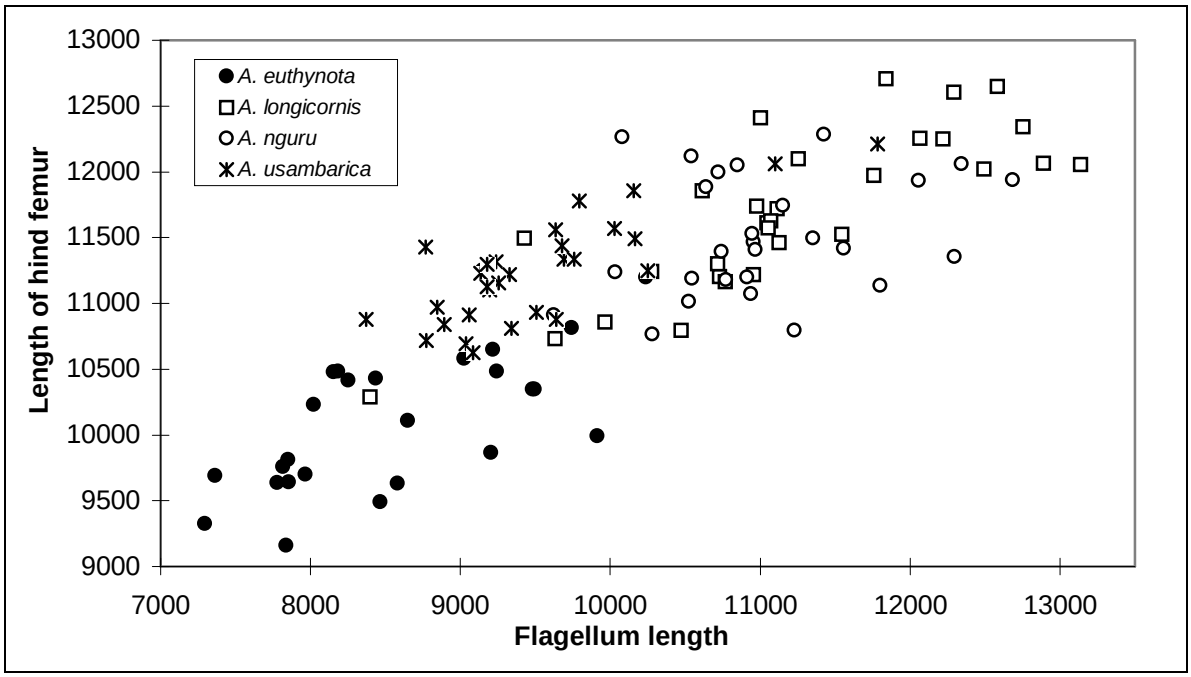


Fig. 29: Bivariate plot of the two most important variables of CAN3. The two length measures are correlated and sort the four *Afrophlaeoba* species according to their body length. It is visible that *A. euthynota* is the smallest species, followed by *A. usambarica*, while *A. nguru* and *A. longicornis* still overlap largely.

In the third canonical function, *A. nguru* and *A. longicornis* are separated (figure 25). These two species are still plotted close together and have the lowest distance. CAN3 has a proportional eigen value of only 15.7%. The function is mainly described by the length of the flagellum (FlagL: 0.89), the length of the hind femur (HFemL: 0.85) and the width of the head (HeadW: 0.75). Since the data sets are slightly overlapping even in the multivariate plot, it is not possible to distinguish them bivariately. A stepwise discriminant analysis just including *A. nguru* and *A. longicornis* revealed five characters (TegW, FS1+2L, FS3L, AntCar, HeadW) and a Wilks' Lambda of 0.448. However, even a plot of TegW with FG1+2L has a high overlap of the species (figure 30).

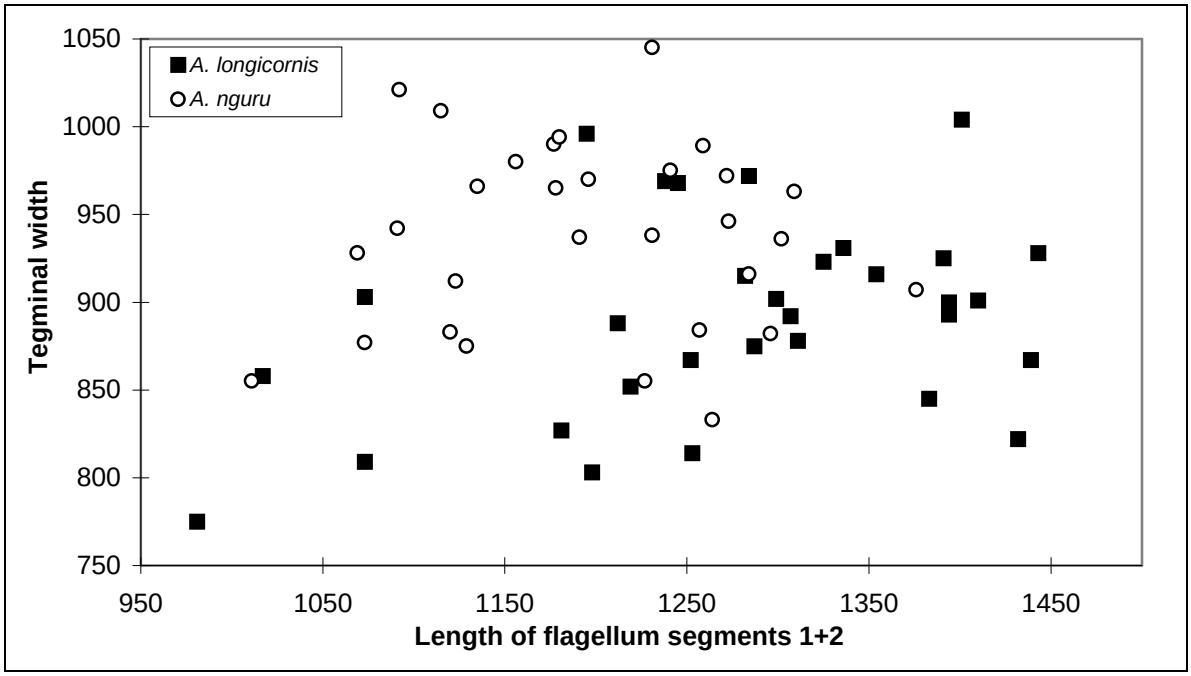


Fig. 30: Bivariate plot of the two most important variables of a stepwise canonical discriminant analysis just including the two species *A. nguru* and *A. longicornis*. The overlap of the two groups is still high, although the differences are significant.

5.3.3 Univariate Analysis

All characters were tested by means of pairwise univariate t-tests. The number of significant differences (table 33) ranges in most cases from 70% (*A. usambarica* and *A. longicornis*: 19 differences within a group of 27 characters) to 100% (*A. usambarica* and *A. euthynota*). One value is extremely low (*A. nguru* and *A. longicornis*: 37%). However, these values are influenced by the high number of antennal characters included (13 of 27 = 48%), weighting the flagellum extremely high compared to the head (18.5%), the pronotum (14.8%) or the femur (7.4%). Such weighting effects do not occur in the multivariate analysis. This also explains the comparatively low number of significant differences of *P. arachniformis* and the *Afrophlaeoba* species compared to *A. euthynota*, although it has very high distances in the multivariate analysis. In the following part only some important characters are presented.

Tab. 33: Pairwise number (lower left matrix) and proportion (upper right matrix) of univariate significant morphometric differences; the right column represents the mean percentage of all significant differences between one species and all other species; the lowermost line represents the total of all significant differences between one species and all other species; note the extremely low value between *A. nguru* and *A. longicornis*.

Species	*P. arachniformis*	*A. usambarica*	*A. euthynota*	*A. longicornis*	*A. nguru*	$\bar{x}$
P. arachniformis		0.74	0.93	0.78	0.85	0.82
A. usambarica	20		1.00	0.70	0.78	0.81
A. euthynota	25	27		0.96	0.96	0.96
A. longicornis	21	19	26		0.37	0.70
A. nguru	23	21	26	10		0.74
∑	89	87	104	76	89	

5.3.3.1 Length of the Flagellum

Nearly all species differ significantly in the flagellum length (t-test, df: 51-55, $P < 0.05$, figure 31), with one exception: *A. nguru* and *A. longicornis*. *P. arachniformis* has the longest flagellum ($\bar{x}$: 12.7 mm), *A. euthynota* has the shortest flagellum ($\bar{x}$: 8.6 mm). The length of the flagellum is probably correlated with the body length and may represent nothing more than the size of the different species. Jago (1983) used a relative length of the flagellum in his descriptions. Thus the flagellum length has been divided once with the length of the pronotal prozona and once with the length of the hind femur. In both cases all species proved to be significantly different with the exception of *A. usambarica* and *A. euthynota* (t-test, df: 51-57, $P < 0.05$). Figure 32 shows the relative flagellum length based on the length of the pronotal prozona.

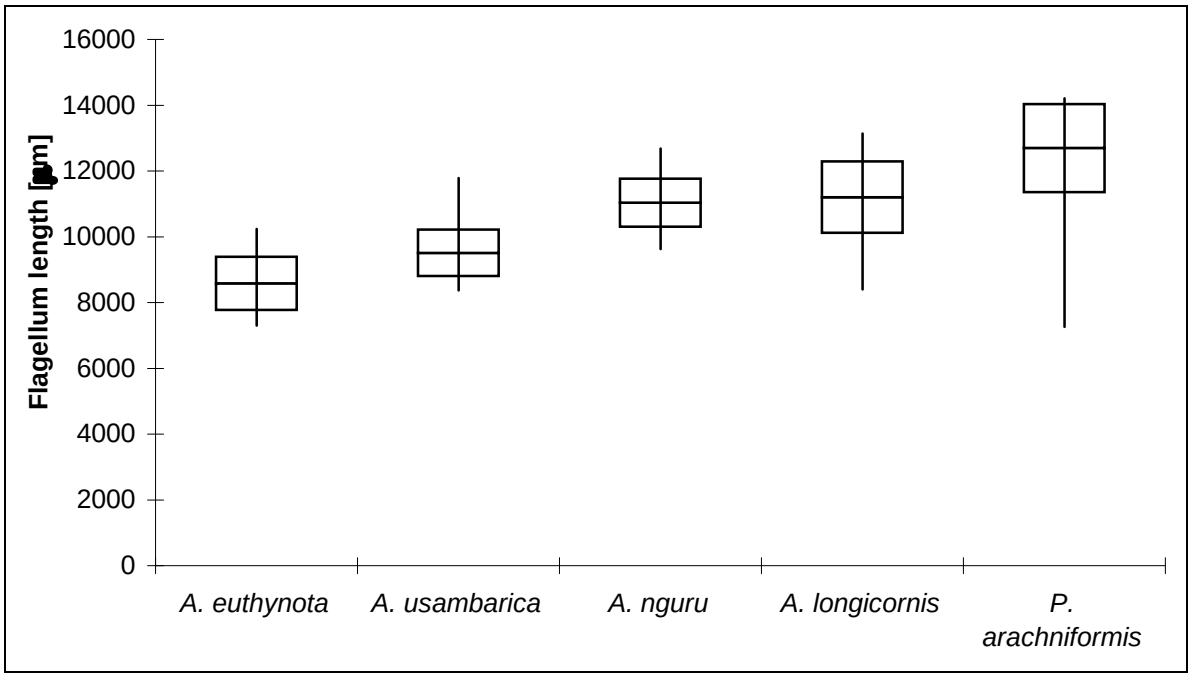


Fig. 31: Flagellum length (mean, standard deviation, range) of the five species; note the extremely long flagellum in *P. arachniformis* (the low minimum in this species is caused by a specimen with rudimentary antennae) and the similar flagellum length of *A. longicornis* and *A. nguru*.

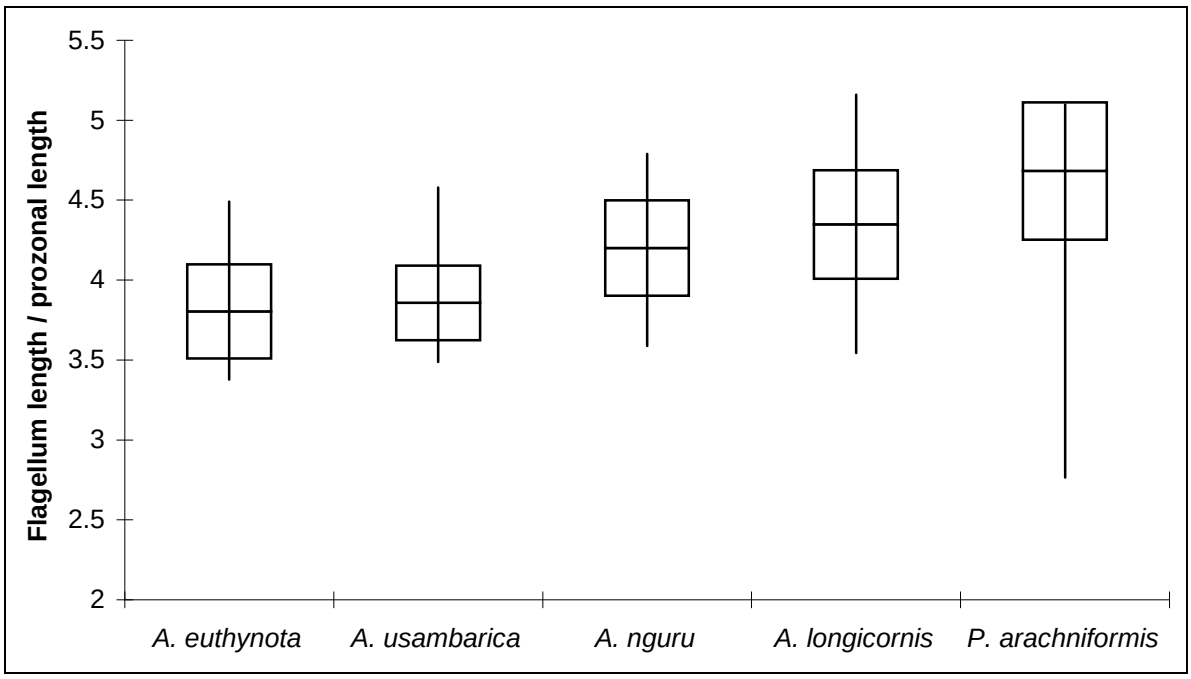


Fig. 32: Relative flagellum length (mean, standard deviation, range) of the five species; *A. euthynota* and *A. usambarica* is the only pair of species, which does not differ significantly in this regard.

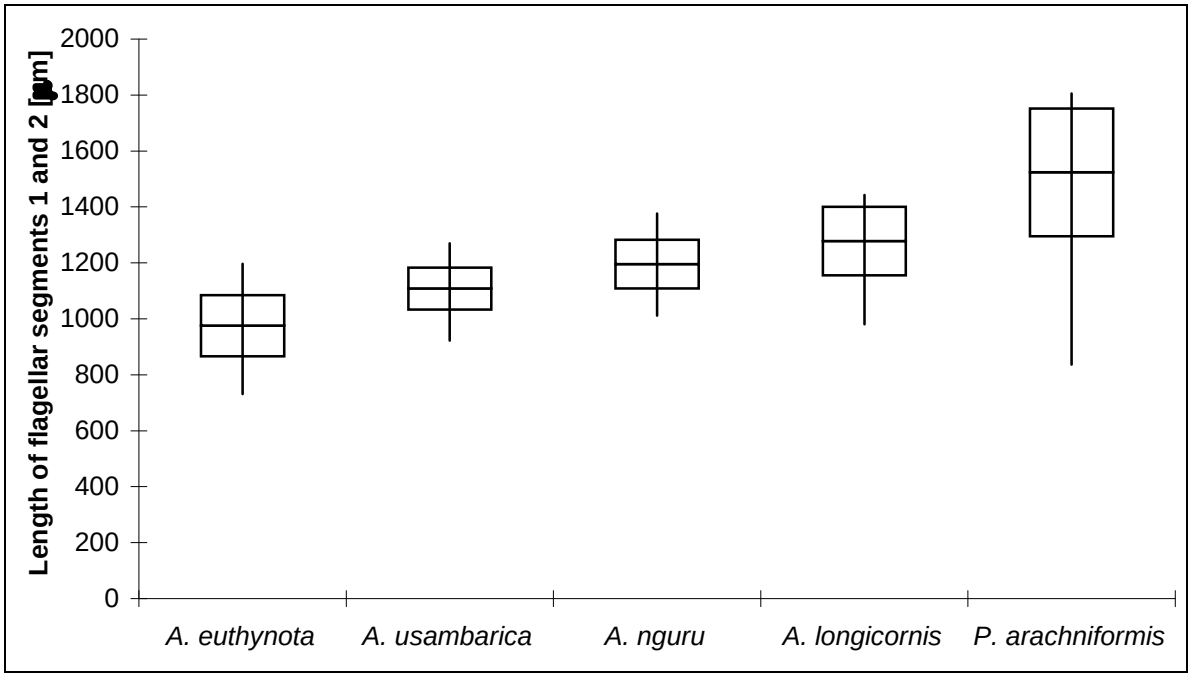


Fig. 33: Length of the first two (usually fused) flagellar segments (mean, standard deviation, range); the species are arranged according to their body length. Again the low minimum in *P. arachniformis* is caused by the specimen with vestigial antennae.

5.3.3.2 Flagellar Segments 1+2

In many specimens of all species the flagellar segments 1 and 2 are fused. In some *P. arachniformis* specimens, a suture was visible in the first segment, indicating that this may consist originally of two segments again. However, the homology of the measured segments is out of question, since the shape of the following segments is rather typical. The length of the first two flagellar segments differs significantly among all species (t-test, df: 56-59, $P < 0.05$, figure 33). It correlates with other length measures, such as the flagellum length ($R = 0.90$), the length of other flagellar segments (all $R > 0.8$) or the length of the hind femora ($R = 0.87$). In the widths of flagellar segments differences are less pronounced than in lengths measures (figure 34). This is also true for the flagellar segment 2, which does not differ significantly in this regard among four of the species. Only *A. euthynota* differs significantly from all other species in this character (t-test, df: 56-59, $P < 0.05$). To illustrate the proportions of the basal flagellar segments, the ratio of the length of segment 1+2 and the distal width of segment 2 has been calculated. All species differ significantly in this regard, with the exception of *A. usambarica* and *A. nguru* (t-test, df: 56-59, $P < 0.05$, figure 35).

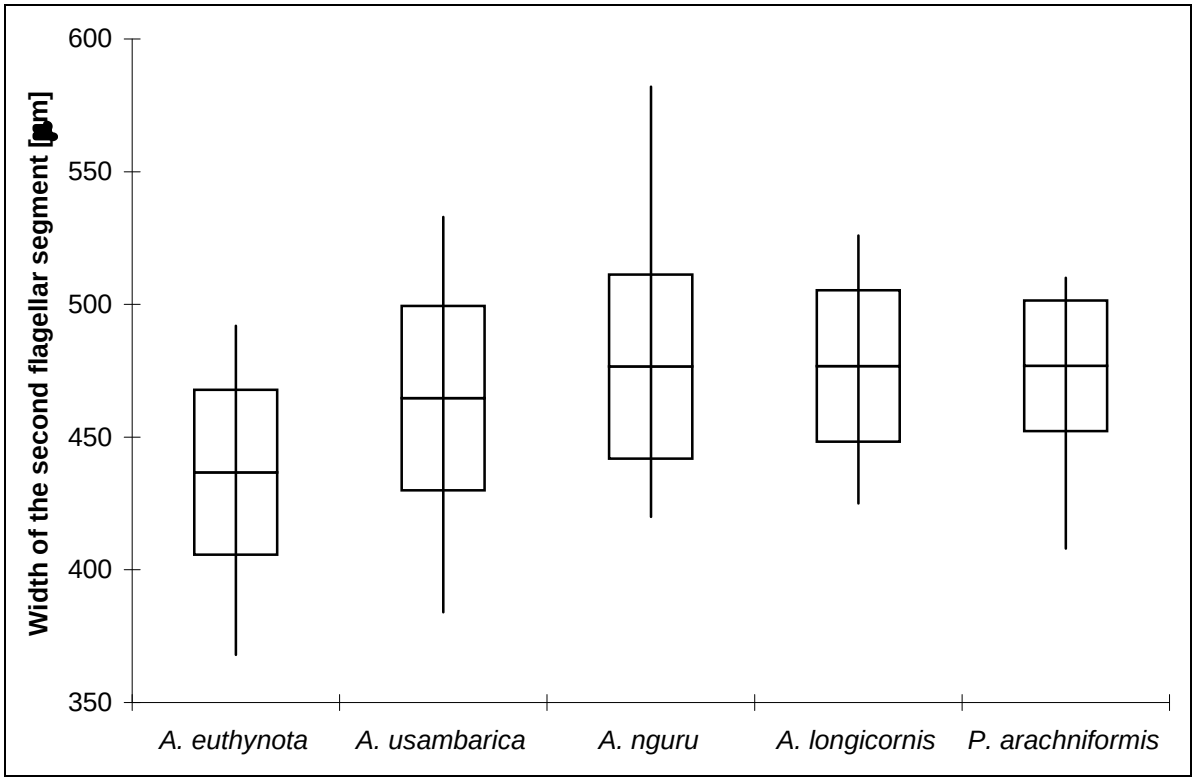


Fig. 34: Width of the second flagellar segment (mean, standard deviation, range) of the five species; the species are arranged according to their body length; note the significantly lower values in *A. euthynota*.

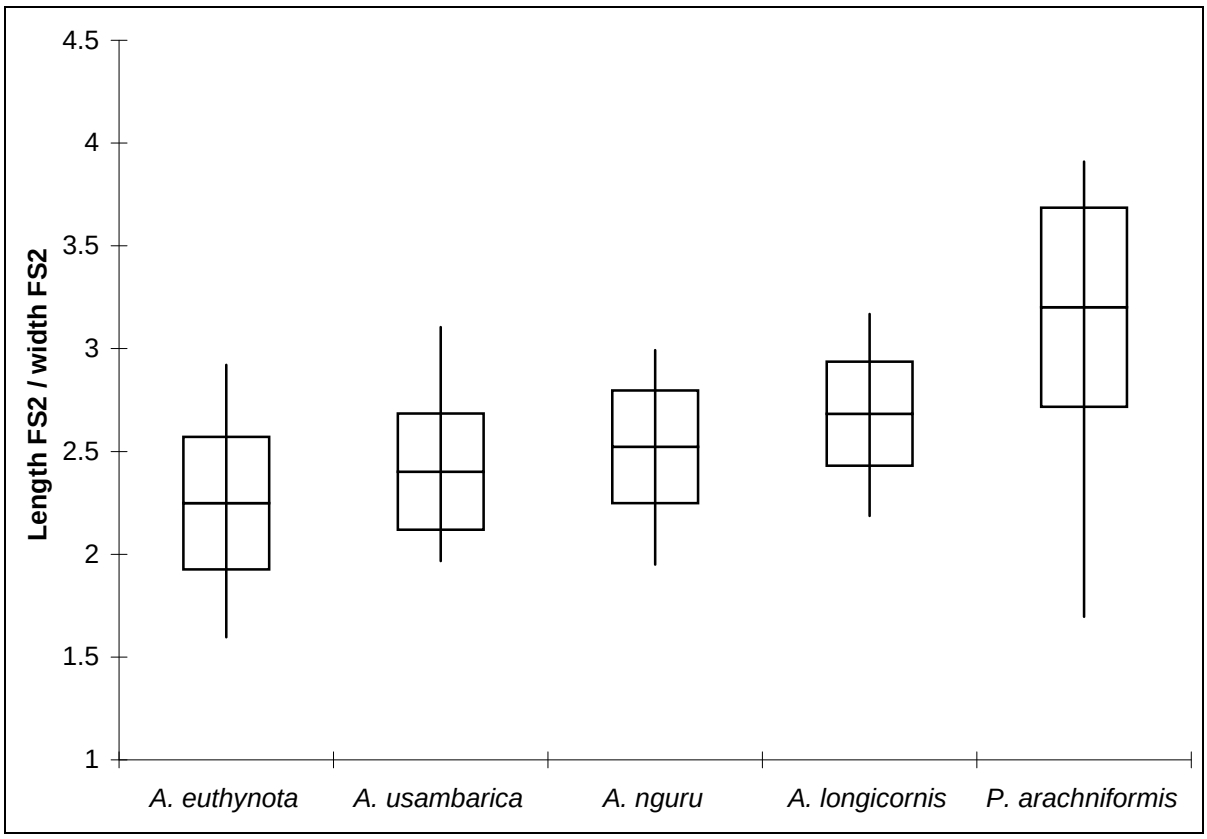


Fig. 35: Proportions of the first two (usually fused) flagellar segments, based on the length and distal width of the five species (mean, standard deviation, range); the species are arranged according to their body length; *A. nguru* and *A. usambarica* do not differ significantly in this regard.

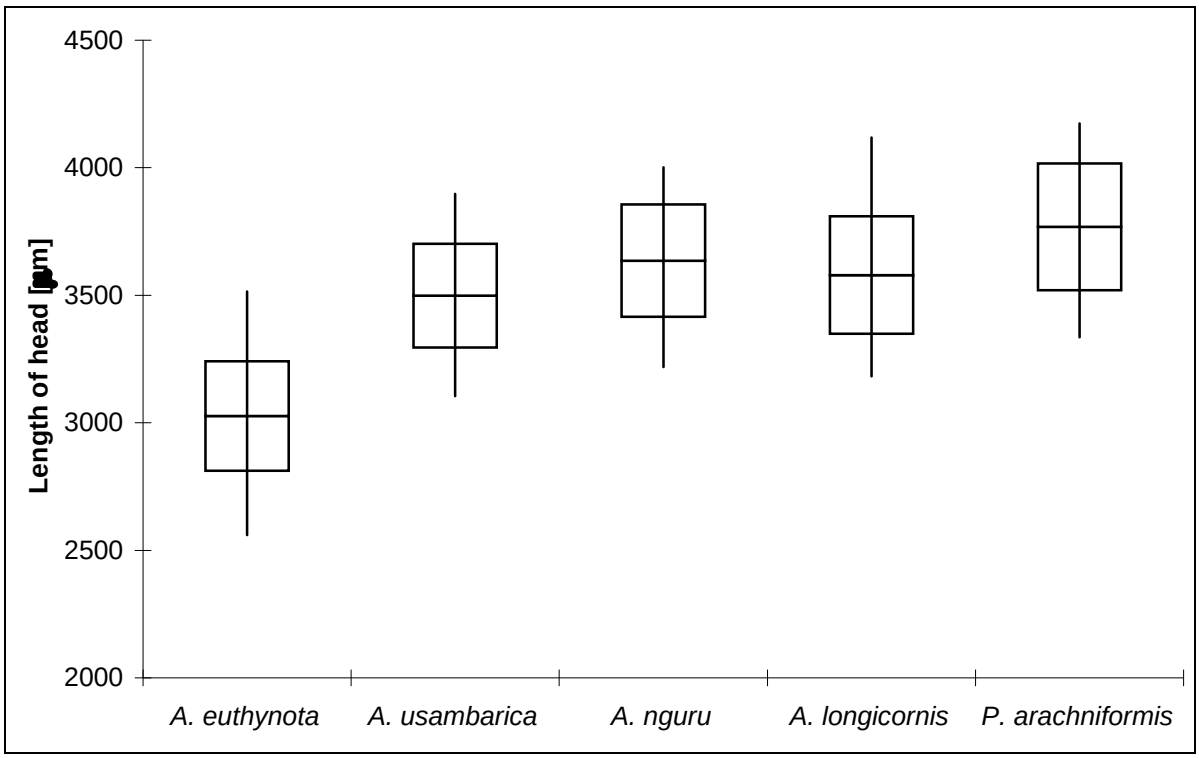


Fig. 36: Length of head (mean, standard deviation, range) of the five species; the species are arranged according to their body length; in this case *A. nguru* differs not significantly from *A. usambarica* and *A. longicornis*

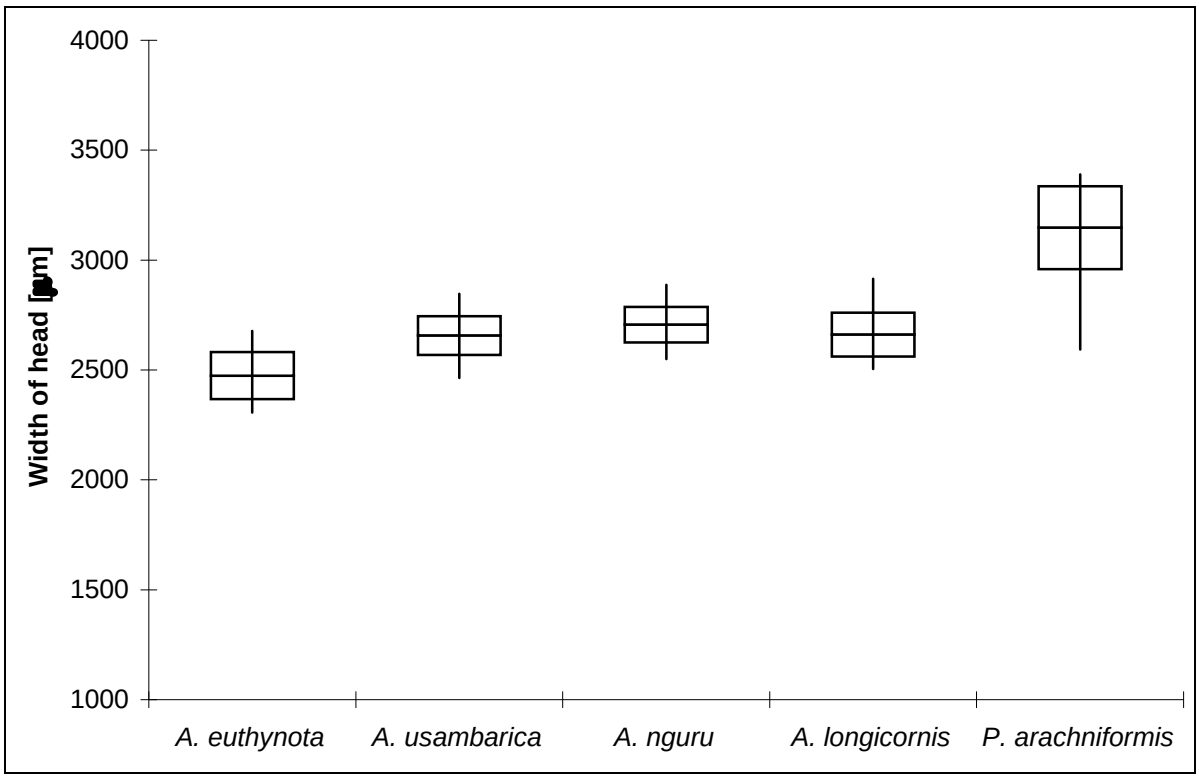


Fig. 37: Width of head (mean, standard deviation, range) of the five species; the species are arranged according to their body length; in this case *A. usambarica* and *A. longicornis* differ not significantly from each other; note the extremely broad head of *P. arachniformis*.

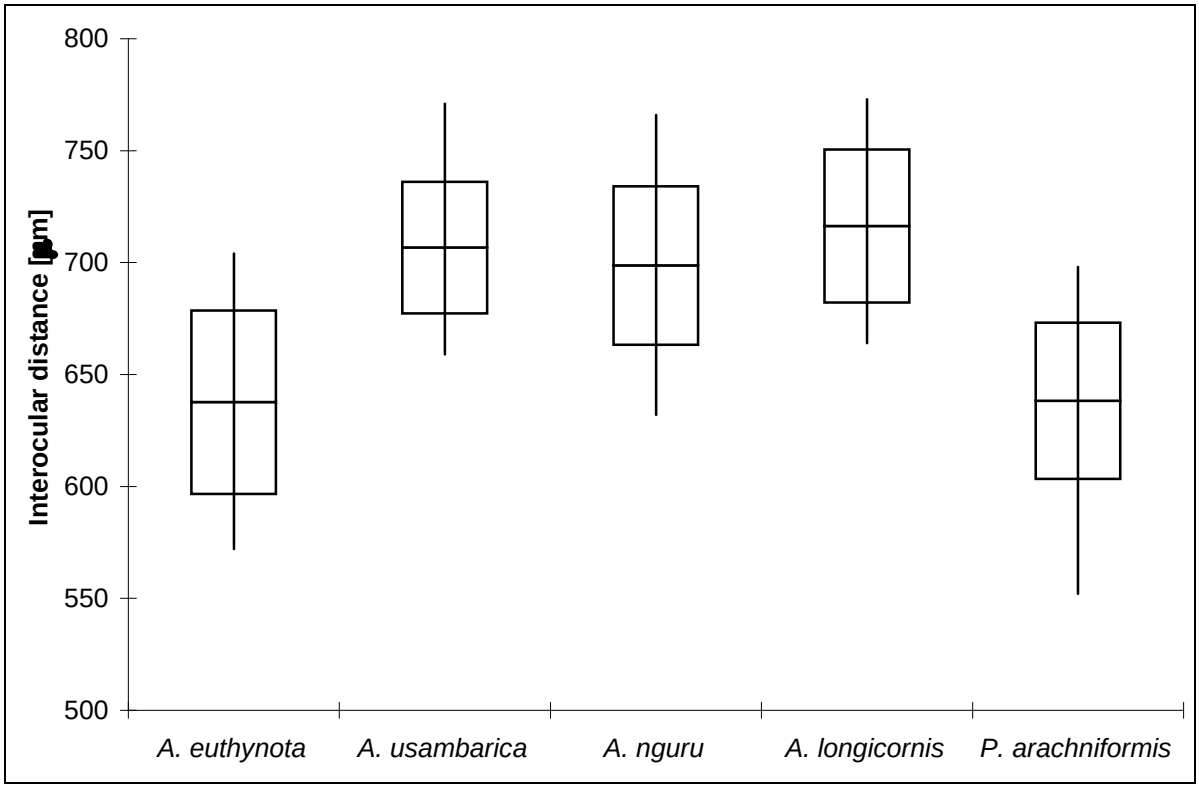


Fig. 38: Interocular distance (mean, standard deviation, range) of the five species; the species are arranged according to their body length; *P. arachniformis* has a small interocular distance compared to its body size, caused by its large protruding eyes.

5.3.3.3 Head

The length of the head differs between nearly all species (t-test, df: 56-59, $P < 0.05$, figure 36). Exceptions are the groups *A. usambarica* / *A. nguru* and *A. nguru* / *A. longicornis. A. euthynota* has the shortest head ($\bar{x}$ = 3026 µm) and *P. arachniformis* has the longest head ($\bar{x}$ = 3768 µm). The head width also differs significantly between most of the species (t-test, df: 56-59, $P < 0.05$, figure 37). In this case only *A. usambarica* and *A. longicornis* do not differ. If the species are arranged according to their body size, it becomes obvious that *P. arachniformis* has an extremely broad head. This is caused mainly by the large, protruding eyes. Figure 38 illustrates the interocular distance of the five species examined. It is obvious that the interocular distance in *P. arachniformis* is lower than in most of the *Afrophlaeoba* species, despite its broader head. In *Afrophlaeoba* the interocular distance seems to increase in relation to the body size. No significant differences were found between the species pairs *A. usambarica* / *A. longicornis*, *A. usambarica* / *A. nguru*, and *A. euthynota* / *P. arachniformis*. All other species pairs differed significantly in this character (t-test, df: 56-59, $P < 0.05$).

5.3.3.4 Vertex

The vertex length differs in the case of six of the ten species pairs (table 34, figure 39). The only species, which is differing from all other species is *A. euthynota*. It has a very short vertex ($\bar{x}$ = 856 µm) compared to the other species ($\bar{x}$ = 1051-1081 µm).

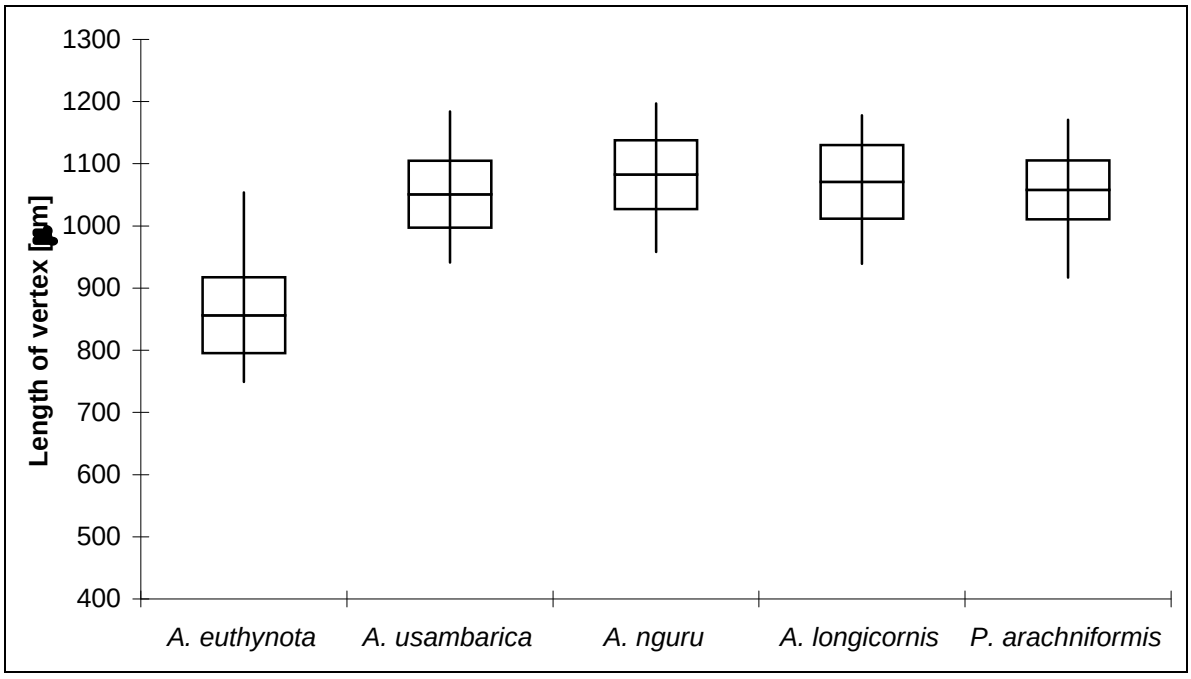


Fig. 39: Length of vertex (mean, standard deviation, range) of the five species; the species are arranged according to their body length; note the extremely short vertex in *A. euthynota*.

Tab. 34: Significant differences (P < 0.05) and species pairs which do not differ significantly (n.s.) in the vertex length. Note that *A. euthynota* is the only species, which differs significantly from all other species.

Vertex Length	*A. usambarica*	*A. euthynota*	*A. longicornis*	*A. nguru*
P. arachniformis	n.s.	P < 0.05	n.s.	P < 0.05
A. usambarica		P < 0.05	n.s.	P < 0.05
A. euthynota			P < 0.05	P < 0.05
A. longicornis				n.s.

The ratio vertex width / vertex length is negatively correlated with the body length (figure 40). In *P. arachniformis* it is in mean 1.01, while in *A. euthynota* it is in mean 1.20. All species differ significantly in this character, with the exception of *A. longicornis* and *A. usambarica* (t-test, df: 56-59, P < 0.05).

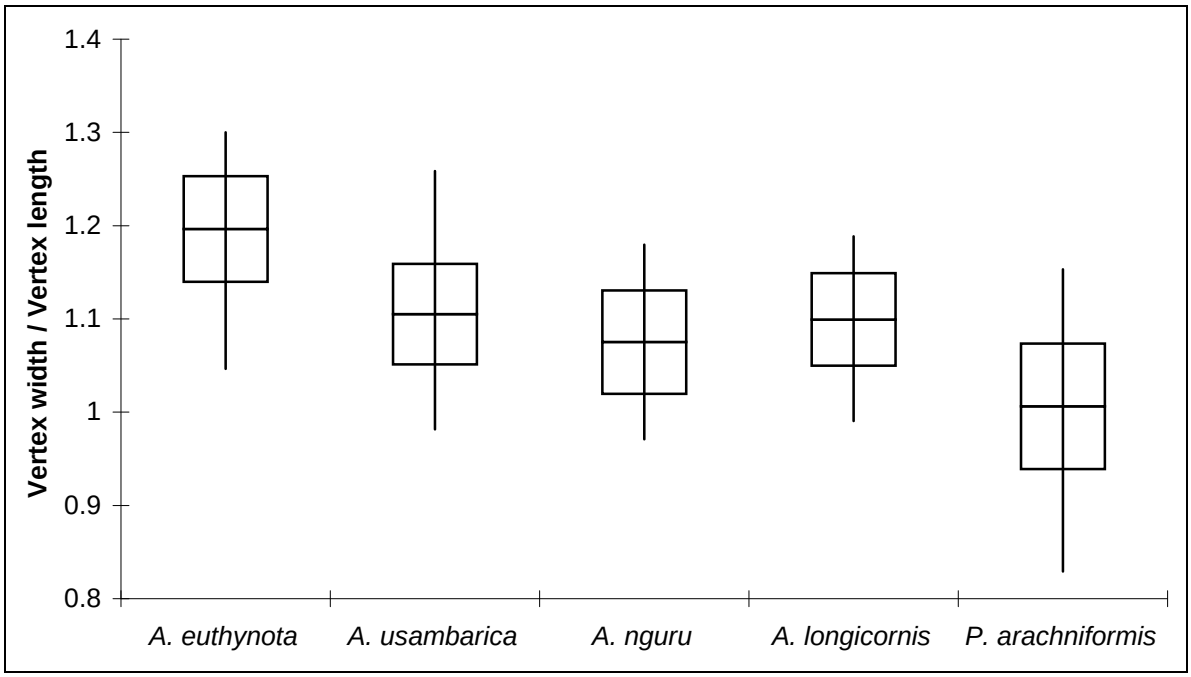


Fig. 40: Ratio of the vertex width to the vertex length (mean, standard deviation, range) of the five species; the species are arranged according to their body length; the ratio decreases with body length. It is extremely high in *A. euthynota* and extremely low in *P. arachniformis*.

5.3.3.5 Pronotum

The ratio of the posterior distance between the lateral carinae of the pronotum and the anterior distance varies in mean from 1.07 (*A. usambarica*) to 1.11 (*A. longicornis*) for *Afrophlaeoba*, but reaches 1.42 in *P. arachniformis* (figure 41), caused by the diverging lateral carinae in this species. *A. euthynota*, *A. longicornis* and *A. nguru* do not differ significantly in this regard, while *A. usambarica* differs from the other *Afrophlaeoba* species (t-test, df: 56-59, P < 0.05). The average ratio of the prozonal length to that of the metazona ranges from 3.06 to 3.11 in *Afrophlaeoba*, while it is 2.82 in *P. arachniformis* (figure 42). No significant difference was found within *Afrophlaeoba*, while *P. arachniformis* differs significantly from all other species (t-test, df: 56-59, P < 0.05).

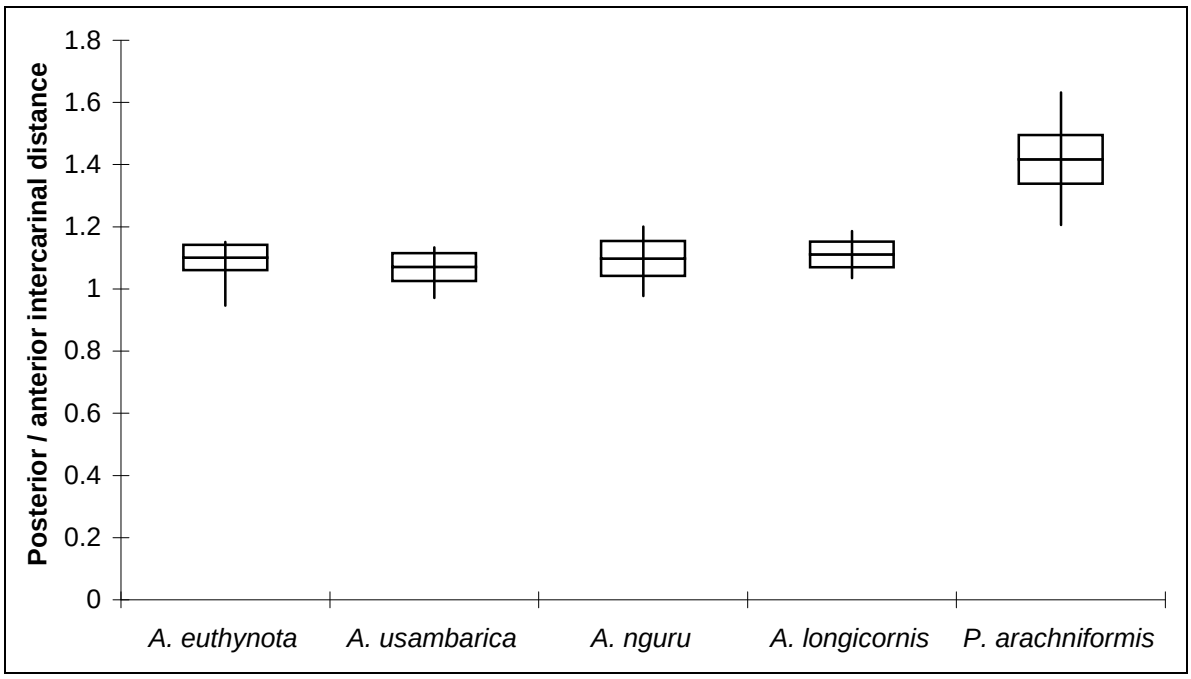


Fig. 41: Ratio of posterior intercarinal distance to the anterior intercarinal distance (mean, standard deviation, range); the species are arranged according to their body length; note the relatively large intercarinal distance of *P. arachniformis.*

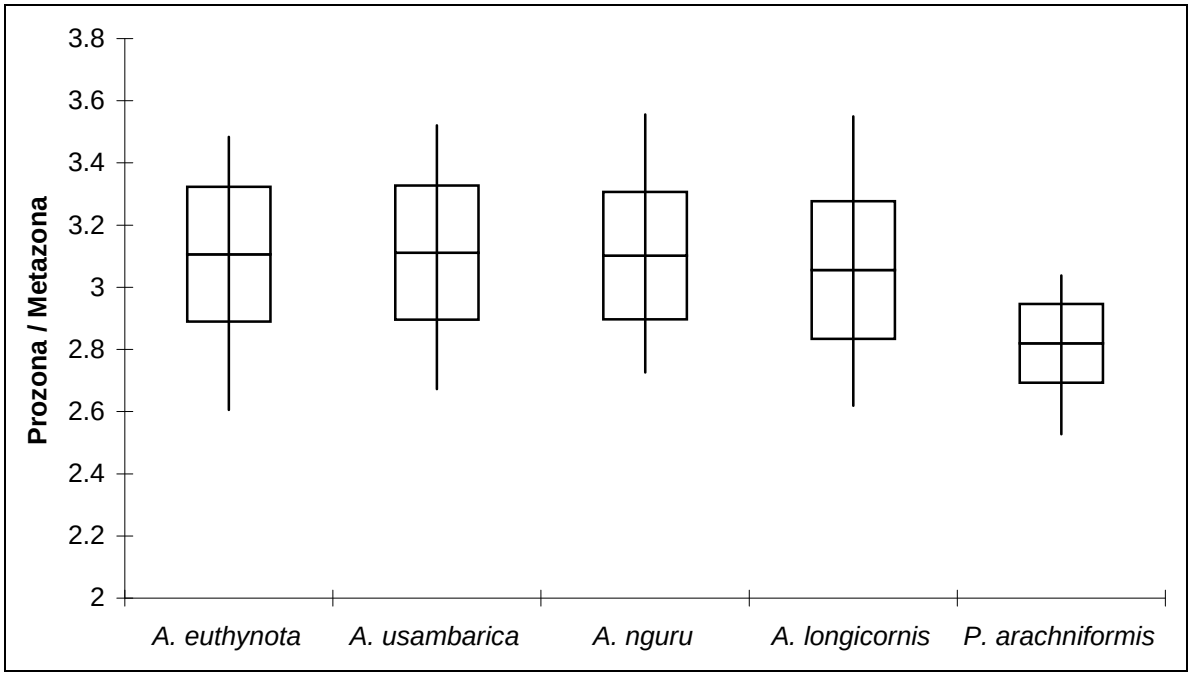


Fig. 42: Ratio of prozonal length to length of metazona (mean, standard deviation, range); the species are arranged according to their body length; note the comparatively short prozona in *P. arachniformis.*

5.3.3.6 Tegminae

The average ratio of the tegmen length to its width ranges from 3.34 to 3.53 in *Afrophlaeoba*, while it is 2.29 in *P. arachniformis* (figure 43). No significant difference was found between *A. nguru*, *A. euthynota* and *A. usambarica* and between *A. longicornis* and *A. nguru*. *P. arachniformis* differs significantly from all other species (t-test, df: 56-59, $P < 0.05$) and there is only little overlapping.

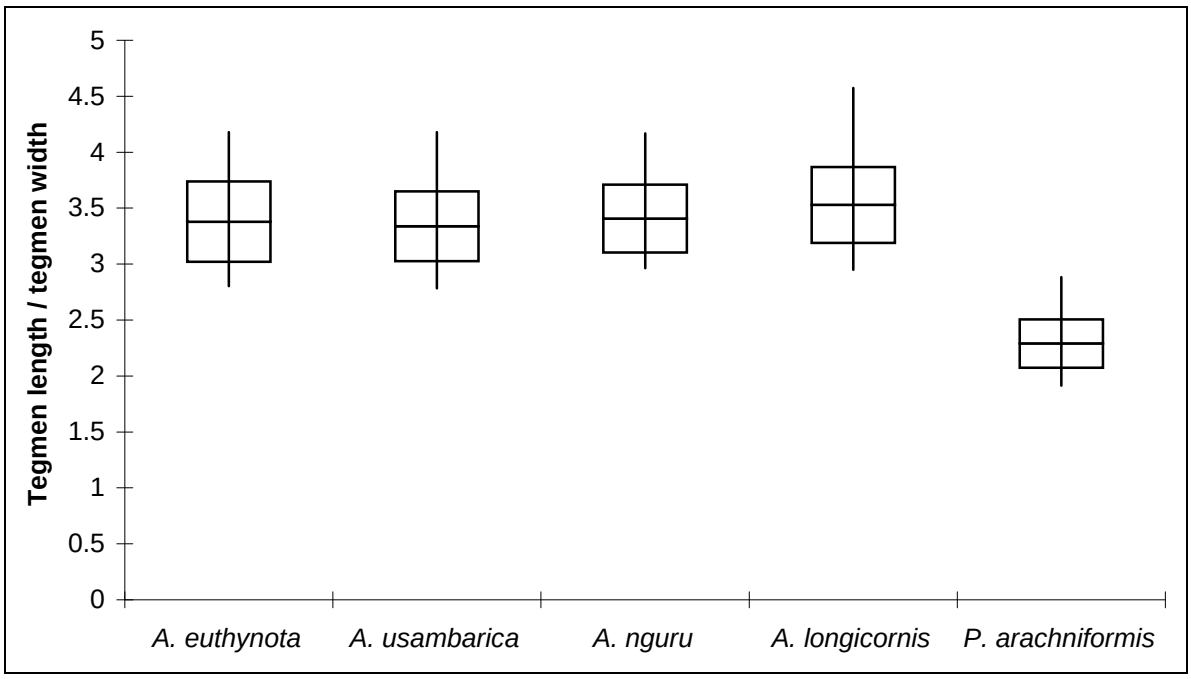


Fig. 43: Ratio of tegmen length to tegmen width (mean, standard deviation, range); the species are arranged according to their body length; note the broad tegminae in *P. arachniformis*.

5.3.3.7 Hind Femur

The proportions of the hind femora were calculated by dividing the length by the width. The only intrageneric differences found were between *A. longicornis* and *A. euthynota* and between *A. longicornis* and *A. nguru* (t-test, df: 56-59, $P < 0.05$, figure 44). *A. longicornis* has a more slender hind femora ($\bar{x}$: 5.2, range: 4.6-5.7) than those two species ($\bar{x}$: 5.1, range: 4.6-5.6), but the overlap is high. The hind femora of *P. arachniformis* are stockier.

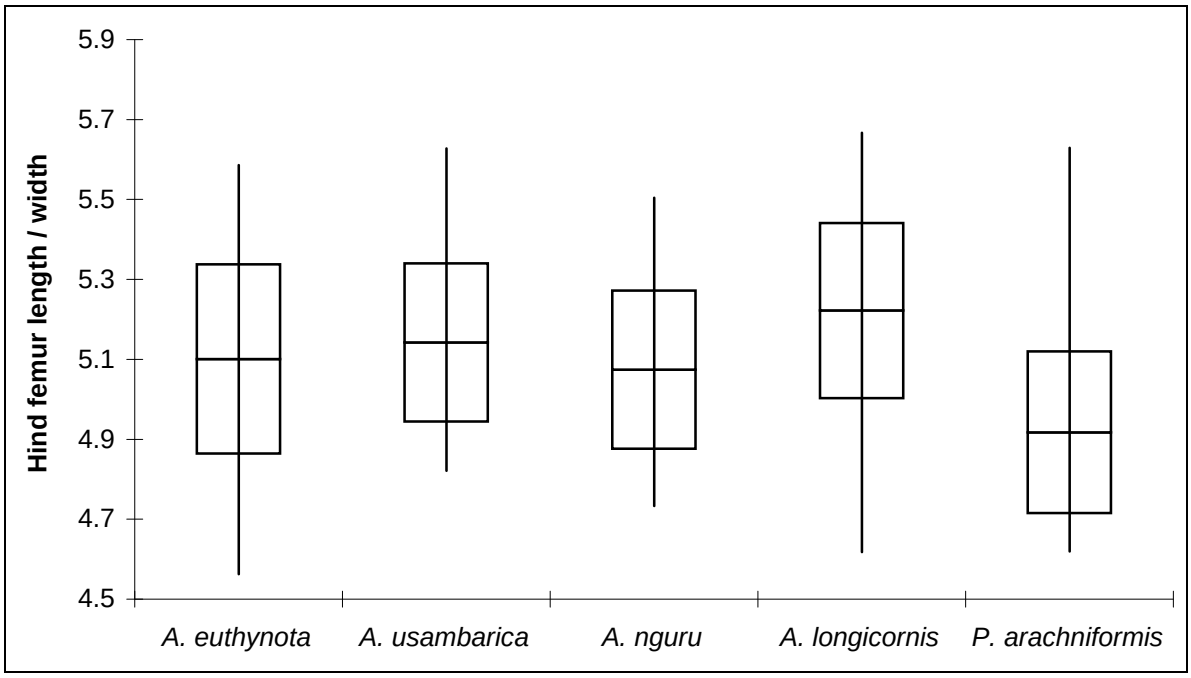


Fig. 44: Ratio of hind femur length to hind femur width (mean, standard deviation, range); the species are arranged according to their body length; note the broad femora in *P. arachniformis*.

5.4 Results: Qualitative Characters

5.4.1 Antennae

Antennal characters proved to be highly variable. In all species there is a trend of the basal flagellar segments to fuse (figure 45). In *Afrophlaeoba* this usually includes the first two flagellar segments, while in *P. arachniformis* these are normally separated. The suture of the first flagellar segment in *P. arachniformis* may indicate that this originally consisted of segments. In *Afrophlaeoba* this suture is not visible. The homology of the flagellar segments can be followed from the shape of the following segments. Since it remains unknown, whether the suture in the first flagellar segment of *P. arachniformis* represents the apomorphous or plesiomorphous state, the numeration followed the state of *Afrophlaeoba*. Additionally, some more segments were fused in some specimens of all species. These included fusions of the segments 1-3 and 4-5 or 1-4 and 5-6. Fusion events usually generate contractions of the participating segments (figure 45). Generally the first six to seven flagellar segments are flattened, but in some specimens all segments are flattened. The variation of the antennal characters is high and no typical interspecific differences were found.

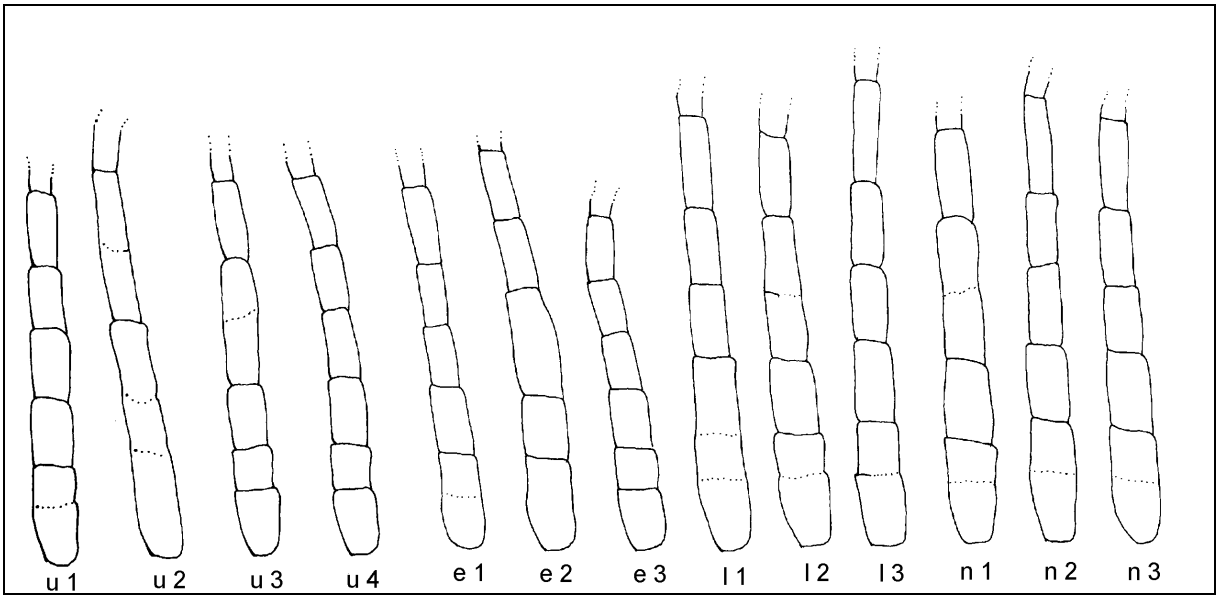


Fig. 45: Basal flagellar segments of some specimens of Afrophlaeoba (u 1-u 4 = A. usambarica, e 1-e 3 = A. euthynota, l 1-l 3 = A. longicornis, n 1-n 3 = A. nguru); dotted lines represent visible sutures between fused segments.

5.4.2 Frontal Ridge

The frontal ridge and the fronto-lateral angle of the frons are marked by clear carinulae in all *Afrophlaeoba* species and *P. arachniformis*. The carinulae of the frontal ridge are continuous down to the fronto-clypeal suture in most specimens. In some specimens they are flattened ventrally. The frons are strongly sulcate in all species. No interspecific differences were found.

5.4.3 Fastigium Verticis

The shape of the vertex is highly variable in all species. The strong median carinula extends across the occiput, but it fades at the rear of the head. The fastigium verticis is usually concave, sometimes flat, but never convex. The sides of the rear half are lightly divergent to the front. Anteriorly the vertex can be obtuse- or acutangular. In some *A. euthynota* specimens it is rounded due to the broader shape of the vertex (see morphometrics). A part of the aerodynamic organ (a hairy field) described by Weis-Fogh (1956) is present close to the eyes in all species. No interspecific differences were found in the qualitative characters of the vertex.

5.4.4 Head and Compound Eyes

No differences were found in the shape of the eyes, the mouthparts or other parts of the head between the *Afrophlaeoba* species. *P. arachniformis* differs from the *Afrophlaeoba* species in having large, protruding eyes (see also morphometrics).

Tab. 35: Pronotum: Character states with number of specimens in each *Afrophlaeoba* species.

Character	Character state	*A. longicornis*	*A. nguru*	*A. usambarica*	*A. euthynota*
Lateral carinae at front	divergent	4	4	2	0
	parallel	16	16	18	18
	convergent	0	0	0	2
Lateral carinae at rear	divergent	5	2	8	4
	parallel	15	18	12	16
	convergent	0	0	0	2
Shape of median carina	broad	8	12	6	10
	slender	12	8	14	10
	high	12	8	12	8
	low	8	12	8	12
Shape of lateral carinae	broad	5	12	12	10
	slender	15	8	8	10
	high	15	8	10	12
	low	5	12	10	8
Anterior sulcus	crosses all carinae	20	20	20	20
Posterior sulcus	visible	7	2	10	6
	not visible	13	18	10	14
Pronotal shape	coarse	20	20	20	20
Pronotal disc	two frontal dots	18	20	19	20
	without those	2	0	1	0
Lateral lobe: frontal margin	concave	0	1	0	0
	straight	1	1	0	0
	convex	19	18	20	20
Lateral lobe: lower margin	with concavity	20	20	20	20
	without concavity	0	0	0	0
Lateral lobe: rear margin	concave	20	20	20	20
	not concave	0	0	0	0

5.4.5 Pronotum

The lateral carinae of the pronotum are usually nearly parallel-sided in all *Afrophlaeoba* species (table 35). In some specimens of *A. usambarica*, *A. longicornis* and *A. nguru* the pronotal disc is widened at rear or front. Only in some *A. euthynota* specimens they are convergent forwards or backwards. The shape of the lateral and median carinae is variable from flat and broad to high and slender in all species. The posterior sulcus (separating the prozona from the metazona) always crosses all three carinae, while the anterior sulcus is either not or only faintly visible. The pronotal disc is always coarse and usually has some dark dots, of which two parallel dots can be found near the front. The lateral lobe of the pronotum is nearly always similarly shaped with a straight or slightly convex frontal margin (in one specimen a concavity was found ventrally), a concavity at the anterior lower margin (the rear half is straight, but sloping downwards) and a concave rear margin.

5.4.6 Tegmina

The tegminae of all *Afrophlaeoba* species are rather similarly shaped. They are usually at least slightly expanded distally and apically rounded. The anterior lower margin is straight or slightly concave, the anterior upper margin straight to convex. The tegminae are usually reaching or slightly exceeding the rear edge of the first abdominal tergite. The number of veins varies from four to six, but in most specimens five veins are visible. The variation of these characters is correlated with the tegminal length. Four veins were found comparatively often in *A. euthynota*, the species with the shortest tegminae, while *A. longicornis* more often had six visible veins and an obtuse-angulate apex (table 36).

Tab 36: Examined tegminal characters. Character states with number of specimens in each *Afrophlaeoba* species.

Character	Character state	*A. longicornis*	*A. nguru*	*A. usambarica*	*A. euthynota*
Tegmina	expanded distally	17	20	16	18
	not expanded	3	0	4	2
Apex of Tegmina	rounded	15	15	19	19
	obtuse-angulate	5	5	1	1
Lower margin	concave	4	10	9	2
	straight	16	10	11	17
	convex	0	0	0	1
Upper margin	concave	0	0	0	0
	straight	9	7	4	8
	convex	11	13	16	12
Apex reaching:	¾ way tergite 1	0	1	0	3
	rear edge of tergite 1	19	16	20	17
	half way tergite 2	1	3	0	0
Number of veins	4	4	2	5	7
	5	11	16	13	11
	6	5	2	2	2

5.4.7 Abdomen

No clear differences were found in abdominal characters. The abdominal tergites of all specimens are sharp carinate dorsally and lightly pitted. Tergite ten is always divided and obtusely angularly emarginate. The subgenital plate is always rounded and bluntly pointed. The cerci are conical and hairy. Only the relative length of the cerci and their shape is variable. In *A. longicornis* and *A. nguru* the tips of the cerci are nearly always exceeding the apex of the supra-anal plate, while in the smaller species, *A. usambarica* and *A. euthynota*, only 50% of the specimens have such long cerci (table 37). The tip of the cerci usually point slightly ventrally, but in some cases they are straight.

Tab. 37: Examined abdominal characters, which proved to be variable. Character states with number of specimens in each *Afrophlaeoba* species.

Character	Character state	*A. longicornis*	*A. nguru*	*A. usambarica*	*A. euthynota*
Cerci reaching	apex of supra-anal plate	18	17	10	10
	exceeding it	2	3	10	10
Tip of cerci	pointing ventrally	17	19	18	18
	straight	3	1	2	2

Tab. 38: Examined characters of legs, which proved to be variable. Character states with number of specimens in each *Afrophlaeoba* species.

Character	Character state	*A. longicornis*	*A. nguru*	*A. usambarica*	*A. euthynota*
lower outer lobe	acutangulate	13	12	16	8
of hind knee	rounded	7	8	4	12
Number of outer dorsal spines of right post-tibia	10+2	1	1	0	2
	11+2	2	3	2	4
	12+2	13	6	11	9
	13+2	0	3	1	1
	14+2	0	0	1	0
Number of inner dorsal spines of right post-tibia	10+3	1	0	0	0
	11+3	6	0	0	1
	12+3	9	12	11	14
	13+3	0	1	3	1
	14+3	0	0	1	0
Number of outer dorsal spines of left post-tibia	10+2	1	1	0	2
	11+2	7	6	5	7
	12+2	8	8	9	9
	13+2	2	3	4	1
	14+2	0	1	0	0
	15+2	1	0	0	0
Number of inner dorsal spines of right post-tibia	10+3	0	1	0	2
	11+3	2	2	5	7
	12+3	16	14	9	9
	13+3	1	1	4	1
	14+3	0	1	0	0

5.4.8 Legs

Some characters of the legs are variable, others proved to be very uniform. The lower outer lobe of the hind knee varies in all species from acutangulate to rounded, while the lower inner lobe is always rounded. The upper pair of lobes is always equal in length. Both, fore and mid-femora are always bicarianate above. All specimens have a longitudinal carinula on the outer side of the mid-femur and the inner side of the fore-femur. The highest variation was found in the number of tibial spines (table 38). The differing sample sizes of posttibial characters are caused by missing single hind legs in some specimens.

5.4.9 Epiphallus

The epiphalli of *Afrophlaeoba* are characterized by broad lophi merging into the broad posterior lateral projections. The lophal interspace proved to be variable, depending on the dissection technique. If the epiphallus is extracted with a pin, the epiphallic bridge may bend or even break. This subsequently changes distances like the lophal interspace or the lateral projections. If the epiphallus remained attached to the ectophallus, the lophal interspace was always narrow, while it became widened when the epiphallus was removed. This also leads to stronger lateral projections. The distance between the ancorae was always broad.

The intraspecific variation in the shape of the lophi was higher than the interspecific variability. The hind margin was slightly concave or straight, the rear upper margin was always convex with a small concavity often occurring at the front. The dimension of this concavity was variable and not specific. In some cases the concavity produced a small elevation at the frontal edge. The frontal margin of the lophi was always steep but low. At the rear the lophi are curved outwards. Figure 46 illustrates some examples of the similar lophal shape of the four species.

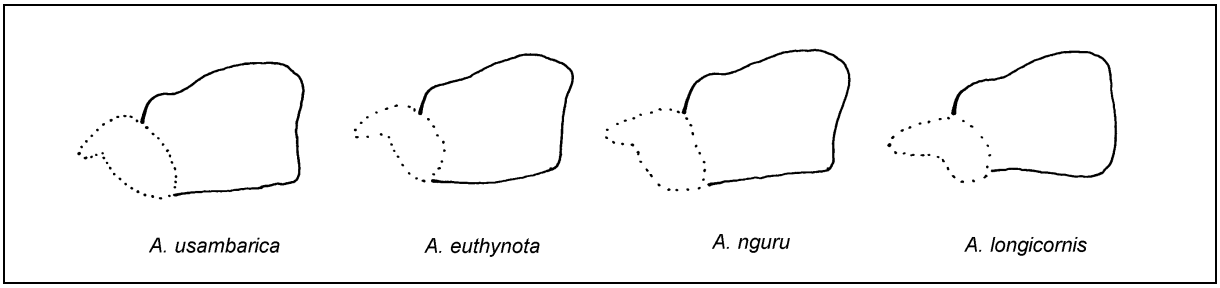


Fig. 46: Lophi shape of four *Afrophlaeoba* specimens, illustrating the high similarity. In each species some variability was found, including the dimension of the concavity at the frontal upper margin and the angle of the hind margin.

5.5 Phenetic Relationships and Correlation with the Genetic Distances

Since no discrete dissimilarities were found within the genus *Afrophlaeoba*, a phylogenetic analysis based on discrete characters was not possible. Instead a phenetic method was applied, using the roots of the pairwise Mahalanobis distances as proposed by Blackith & Reyment (1971). The phenogram inferred from neighbor joining and rooted with the outgroup *P. arachniformis* is given in figure 47. The structure does not differ from the trees inferred with molecular data. The great distance between *P. arachniformis* and the *Afrophlaeoba* species is clearly visible. The two groups *euthynota-usambarica* and *nguru-longicornis* are branched together despite the unusual body dimensions of *A. euthynota*. This is probably caused by the correction of the rate heterogeneity with the neighbor joining method. If the UPGMA method was chosen, *A. euthynota* branched basally to the other *Afrophlaeoba* species. Like in the genetic analysis the lowest distance was found between *A. longicornis* and *A. nguru*. The distance between *A. usambarica* and *A. euthynota* is higher than the distance between *A. usambarica* and *A. longicornis* or *A. nguru*. A strong correlation between morphological and genetic distances ($R^2 = 0.9837$) was found (figure 48), which was mainly caused by the high distances between *Afrophlaeoba* and *P. arachniformis*. Within *Afrophlaeoba* the correlation is lower ($R^2 = 0.3664$).

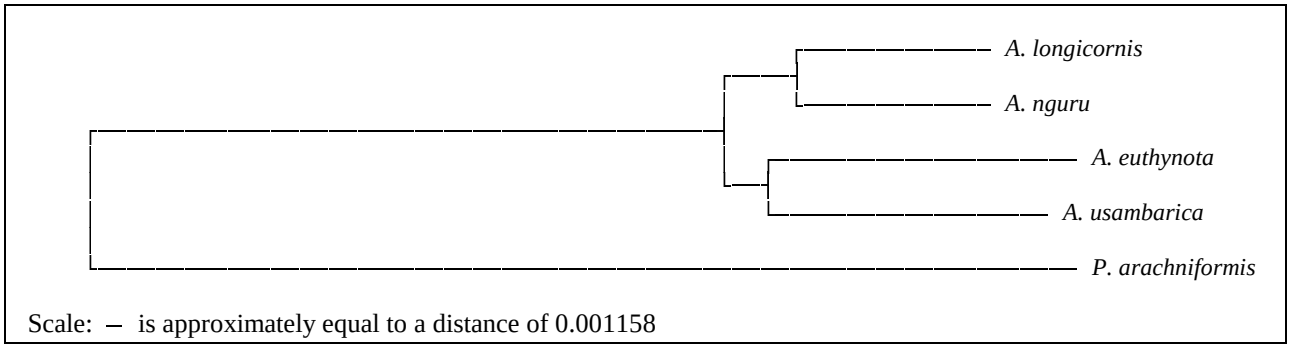


Fig. 47: Phenogram for the morphometric data inferred from the pairwise distances of the discriminant analysis.

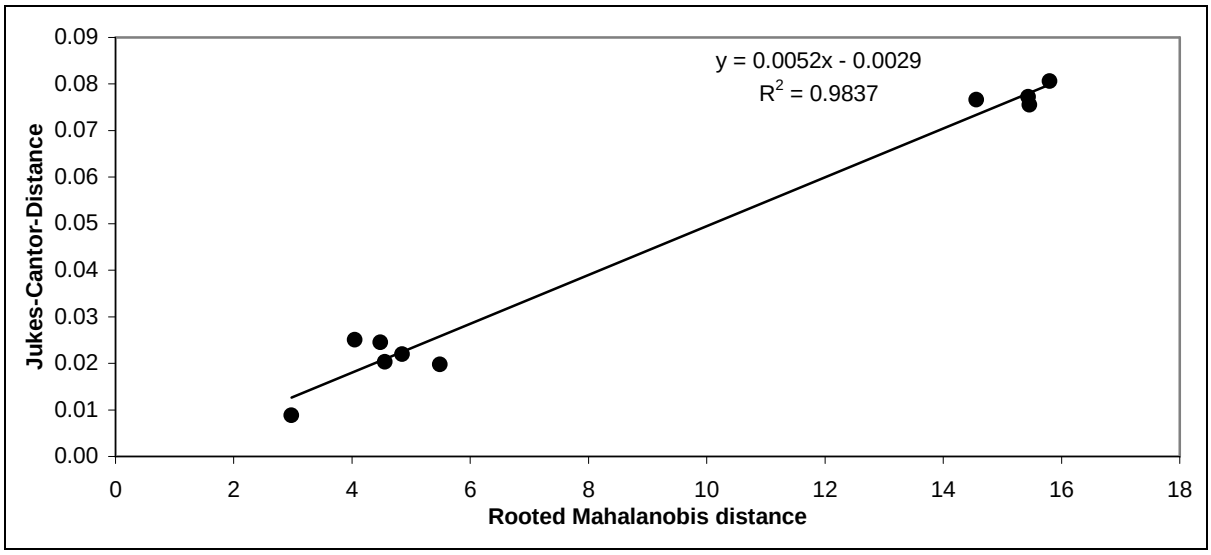


Fig. 48: Ratio of hind femur length to hind femur width (mean, standard deviation, range); the species are arranged according to their body length; note the broad femora in *P. arachniformis*.

5.6 Discussion

5.6.1 Evaluation of the Identification Keys

Jago (1983) presents keys to all species of the genus *Afrophlaeoba* in his revision of the Phlaeobini. According to these keys the main character distinguishing *Afrophlaeoba* from *Parodontomelus* is the shape of the lateral carinae, which are almost parallel in *Afrophlaeoba* but divergent in *Parodontomelus*. The high taxonomic value of this character can be confirmed from the measurements. The posterior distance of the lateral carinae is one of the most important characters discriminating *P. arachniformis* from *Afrophlaeoba*. The ratio of the posterior distance between the lateral carinae and the anterior distance is much higher in *Parodontomelus* than in *Afrophlaeoba*. It clearly separates the two genera and is even a good character for distinguishing them in the field. The other generic characters of *Parodontomelus* given by Jago (1983) are also of high quality (Hochkirch 1999b).

In the key to the males of *Afrophlaeoba* (Jago 1983), the first species keyed out is *A. nguru*, which has relatively short antennae and stocky hind femora (length to depth ratio 4.9). The measurements presented above suggest that these characters are not reliable for identifying *A. nguru*. The flagellum of the species is not differing significantly from *A. longicornis*, which is the species with the longest flagellum within *Afrophlaeoba*. The ratio of the flagellum length and the length of the pronotal prozona illustrates that *A. usambarica* and *A. euthynota* and not *A. nguru* have the shortest relative antennal lengths. In *A. nguru* the relative antennal length is not always “equal to distance from tip of vertex to half way along second abdominal tergite” (Jago 1983). In some cases it reaches the rear edge of the abdominal tergite two, which should be typical for the other species. The comparatively stocky hind femora of *A. nguru* can be confirmed. The length to depth ratio has the smallest value (5.07) within the genus *Afrophlaeoba*, but in this regard it only differs significantly from *A. longicornis* (5.22). However, the overlap is very high and the character is, therefore, not suitable for the identification of the species. The two species *A. longicornis* and *A. nguru* are very similar in morphometric features. The distance within the discriminant function between the species pair is the smallest and the overlap higher than among other species of *Afrophlaeoba*. This can also be concluded from the classification statistics, in which four specimens of *A. nguru* were assigned to *A. longicornis*. This means that even by measuring 25 body dimensions a small uncertainty in identification remains.

The second species keyed out by Jago (1983) is *A. euthynota*, which has a smaller body size. The precise citation is “small insects, overall length from tip of vertex to apices of folded hind femora 17 mm.” The body size is truly a very typical characteristic of *A. euthynota*. In all measures of lengths *A. euthynota* is the smallest species within the genus. This is illustrated by the high distances between *A. euthynota* and all other *Afrophlaeoba* species and by the high importance of length measures in the

discriminant function, separating *A. euthynota*. The overall length was not measured for this analysis, since it is prone to measurement errors (e. g. the angle, in which the hind femora are folded). In the case of the specimens with the longest femora and pronota the overall length was measured with an ocular micrometer. In these cases the length varied from 17.5-20.5 mm including the stretched hind legs, while the smallest *A. usambarica* specimens were always ≥ 20.7 mm long. The multivariate analysis reveals that the best measure for distinguishing *A. euthynota* from other species is the length of the vertex, which usually ranges from 749 µm to 930 µm. The smallest vertex length measured in *A. longicornis* was 939 µm, in *A. usambarica* 941 µm and in *A. nguru* 958 µm. However, one specimen of *A. euthynota* (e125) had an extremely long vertex of 1054 µm, which causes a wrong classification. This specimen also has the longest prozona and the longest hind femur. On the second canonical variate (CAN2) of the discriminant analysis excluding *P. arachniformis* (figure 24), the specimen was plotted in the *A. usambarica* group. Several causes for such an outlier can be imagined. Since the specimen was plotted on CAN1 in the range of *A. euthynota*, mislabelling is rather unlikely, although it cannot be completely excluded. Mistakes in measurement can be neglected, since all doubtful measures were proved. Probably the specimen just represents a "real" outlier, which can be found in all populations.

In the last step of his key, Jago (1983) distinguishes *A. usambarica* and *A. longicornis*. For *A. usambarica* he mentions that the pronotal disc is slightly widened at the extreme front and rear. In *A. longicornis* he describes that the pronotal prozona is parallel-sided or slightly convergent to the front. The measurements of the pronotal disc and the examination of qualitative characters do not confirm these observations. All species have very similarly shaped pronotal discs with more or less parallel lateral carinae. Sometimes the lateral carinae are slightly diverging to the front, but only in two specimens of *A. euthynota* they are slightly convergent.

It is obvious that the identification key to the males of *Afrophlaeoba* given by Jago (1983) is not suitable. The overlap of measures is very high in most of the morphometric characters, and there are no distinct differences in the qualitative characters. The only species which is easy to distinguish from the external morphometrics, is *A. euthynota*, which is the smallest species within the genus. *A. longicornis* and *A. nguru* are very similar and even on CAN3 their plots overlap slightly, although they differ significantly. According to Blackith & Reyment (1971) difficulties in using keys for the identification frequently arise from the fact that they are built on monothetic principles. They state that "it has to be admitted that sometimes dichotomous keys, especially those dealing with rare material of which the constructor may have seen few specimens, or have been compelled to rely on earlier descriptions which themselves have been of poor quality, are partly incorrect." This proved to be true also for *Afrophlaeoba*. Based upon the findings from the multivariate analysis, it is not possible to present a new key to the species, using only a few morphological characters. While *A. euthynota* may be distinguished quite easily, measurements of many characters are needed to

discriminate the other species. Apparently, at present the use of genetic analyses represents a more powerful tool for identification than morphology, although it has to be admitted that the genetic intraspecific variability of the species remains virtually unknown. For practical reasons, the location remains the most suitable field character for identification. Although this may not be sufficient from a taxonomical point of view, the clear discrimination of the four species in multivariate space demonstrates that such a simplification is quite reliable.

5.6.2 Evaluation of the Descriptions

Due to the deficient quality of the keys the question arises, whether the descriptions are more suitable for the identification. Three of the four *Afrophlaeoba* species known were described by Jago (1983), and *A. usambarica* (RAMME, 1929) was redescribed in the same paper. While the redescription of *A. usambarica* is rather long, the description of the other three species are comparatively short and often based upon comparisons of measurements with one of the other species, mostly *A. usambarica*. The characters used in the description will be discussed in the following part. The order, in which the species are discussed and all statements in quotation marks refer to Jago (1983).

A. euthynota JAGO, 1983

According to Jago (1983) this species "resembles a miniature of *A. nguru*." Concluded from the morphometric distances, it is plotted nearer to *A. usambarica* than to *A. nguru*. *A. euthynota* is smaller than the other species in all characters, including the antennae. This confirms Jago's (1983) observations. The relative length of the flagellum is significantly smaller in *A. euthynota* than in *A. nguru* and *A. longicornis*. However, it does not differ from *A. usambarica* in this regard. The statement that the antennae are only "weakly widened basally" is true on an absolute scale, but not with regard to its length. *A. euthynota* is the only species, which differs significantly from the other species by narrower flagellar segments 2 and 3. The length to width ratio of the flagellar segment 1+2, however, is significantly smaller ($\bar{x}$: 2.25) than in the other species (2.4-2.7), which means that the relative width is higher. Since the width of segment 2 is not correlated with length measures, it is also less broad in the specimen e125, which is extremely large in all measures of lengths and plotted with *A. usambarica* on CAN2. This supports the hypothesis that the specimen is a real outlier and not mislabelled.

The vertex of *A. euthynota* is shorter and broader in proportion (width to length ratio 1.2) than in the other *Afrophlaeoba* species (1.1). It is not narrower, as stated by Jago (1983). The proportions and the shape of the vertex are highly variable in all species, but the character proved to be the strongest for discriminating *A. euthynota*. Although *A. euthynota* is the only species in which convergent pronotal carinae exist, most specimens examined had parallel carinae, which characteristic for all other *Afrophlaeoba* species. The carinae are not typically "slightly widened at front" and rear, as described by Jago (1983). The ratio of the length of the pronotal prozona to the length of the metazona is not

significantly differing from any of the other *Afrophlaeoba* species. It is in mean 3.11 and not "less than three times." The emargination of the rear margin of the pronotal metazona is highly variable in all species and no specific shape was found. As it is true for all measures of lengths, *A. euthynota* has the shortest hind femora. However, unlike statements by Jago, they are not more slender than in *A. nguru*, but they differ from *A. longicornis*, which in mean has more slender femora than any other *Afrophlaeoba* species. The overlap in this character is very high and only the outgroup *P. arachniformis* is obviously differing from the other species. The ratio of the upper inner area of the hind femur to its upper outer area has been ignored, as it is too sensitive to minimal changes of the angle, in which the femur is viewed and to the area, in which the measurement is taken.

Different from Jago's (1983) observations, the proportion of the tegmina of *A. euthynota* is not significantly differing from *A. usambarica*, but rather from that of *A. longicornis*. It is 2.80 to 4.18 longer than wide ($\bar{x}$: 3.37). Tegminal characters seem to be highly variable, but somehow correlated with the body length. Epiphallic differences between the species were not found. The three characters mentioned by Jago (1983), the lophal interspace, the posterior lateral projections and the distance between the ancorae, proved to be sensitive to dissection techniques. The epiphallic bridge may bend or even break, when the epiphallus is pulled out with a pin. This process will subsequently change the lophal interspace and the lateral projections or the distance between the ancorae. The low values of these characters can already be concluded from comparing the drawings of the epiphallus of *A. usambarica* by Jago (1983) and Popov (in press). The lophal interspace is much broader in Popov's drawings than in Jago's drawings and resembles the state of *A. longicornis* presented by Jago (1983). In the sister genus *Parodontomelus* other epiphallic characters proved to be more useful, in particular the shape of the lophi (Hochkirch 1999b). However, even these proved to be variable in *Afrophlaeoba* and no interspecific differences were found. Table 39 compares some measurements of Jago's (1983) description with own measurements. The table illustrates that Jago (1983) missed the high intraspecific variability, because he examined only few specimens.

In conclusion, it is obvious that many of Jago's (1983) observations cannot be confirmed and most characters have very high overlaps. The statement that *A. euthynota* differs from all other species by the smaller size and proportionally shorter antennae can be confirmed. The only suitable character for identifying *A. euthynota*, however, is the vertex length, in which only one outlier was found, grouping with *A. usambarica.*

Tab. 39: Comparison of Jago's (1983) and own measurements of *A. euthynota*

	Jago (1983); n = 7	Own measurements; n = 28
Head width	2.07-2.48 ($\bar{x}$: 2.25)	2.31-2.68 ($\bar{x}$: 2.47)
Tegminal length	2.00-2.72 ($\bar{x}$: 2.27)	1.90-3.17 ($\bar{x}$: 2.57)
Tegminal width	0.67-0.81 ($\bar{x}$: 0.74)	0.60-0.89 ($\bar{x}$: 0.77)
Pronotal length	2.55-3.07 ($\bar{x}$: 2.77)	2.77-3.31 ($\bar{x}$: 2.98)
Hind femur length	9.52-10.90 ($\bar{x}$: 10.01)	9.16-11.25 ($\bar{x}$: 10.13)
Hind femur depth	1.81-2.13 ($\bar{x}$: 1.94)	1.79-2.30 ($\bar{x}$: 1.99)

***A. longicornis* JAGO, 1983**

According to Jago (1983) this species should be "closely similar to *A. usambarica*." The discriminant analysis showed that the species that is most similar to *A. longicornis* is *A. nguru* and not *A. usambarica*. Of course, all species are very similar in the outer appearance and without a morphometric analysis they are difficult to distinguish. Jago's (1983) description of the species is based on the differences to *A. usambarica*. Amazingly, *A. usambarica* and *A. longicornis* are the only species, which do not differ significantly from each other in the proportion of the vertex, in contrast to Jago's (1983) observations. The lateral pronotal carinae of *A. longicornis* are usually parallel or slightly widened at the front and not "parallel or slightly convergent forwards." No interspecific differences were found in the proportion of the prozonal length to the length of the metazona, but the mean of 3.06 is consistent with Jago's (1983) description. The taxonomic value of the shape of the outer genicular lobes seems to be very low, as they are highly variable in all species. The hind femora proved to be significantly more slender as in *A. euthynota* and *A. nguru*, but not as in *A. usambarica*, as stated by Jago (1983). The only observation, which proved to be true is that of a longer and more slender shape of the tegmina, compared to *A. usambarica*. However, although the tegmen are significantly longer ($\bar{x}$: 3136 µm) than in *A. usambarica* (3018 µm) and *A. euthynota* (2572 µm), the overlap with *A. usambarica* is still great. They are also more slender ($\bar{x}$: 3.53 times longer than broad) than in *A. usambarica* (3.34), but again with a high overlap (range 2.95-4.57 for *A. longicornis* and 2.78-4.18 for *A. usambarica*). Jago does not describe the epiphallus, but a figure is included, which is characterized by a broad lophal interspace. As stated above, this character is prone to errors. It can be seen from table 40 that the examination of longer series of specimens increased the variability of Jago's (1983) measurements in most cases.

In conclusion, *A. longicornis* cannot be easily distinguished from either *A. usambarica* or *A. nguru*. The lowest overlap between *A. usambarica* and *A. longicornis* was found in the posterior distance of the lateral carinae of the pronotum, which is usually higher in *A. longicornis*. In a multivariate plot, however, *A. usambarica* and *A. longicornis* are represented by distinct groups. In the classification statistics only one *A. longicornis* was assigned to *A. usambarica*, but three were assigned to *A. nguru*, indicating the close morphometric relationships between the two species.

Tab. 40: Comparison of Jago's (1983) and own measurements of *A. longicornis*

	Jago (1983); n = 4	Own measurements; n = 31
Head width	2.60-2.75	2.51-2.92 ($\bar{x}$: 2.66)
Tegminal length	2.90-3.40	2.73-3.76 ($\bar{x}$: 3.14)
Tegminal width	0.84-1.10	0.76-1.00 ($\bar{x}$: 0.89)
Pronotal length	3.51-3.66	3.14-3.74 ($\bar{x}$: 3.42)
Hind femur length	11.49-12.28	10.29-12.71 ($\bar{x}$: 11.68)
Hind femur depth	2.11-2.41	2.05-2.45 ($\bar{x}$: 2.24)

***A. nguru* JAGO, 1983**

A. nguru is a larger species than *A. euthynota* and *A. usambarica* and rather similar in length compared to *A. longicornis*. The species is not "smaller… than either *usambarica* or *longicornis*" (Jago 1983). The pronotal prozona is even significantly longer than in *A. longicornis*. Like in *A. longicornis*, Jago (1983) described the species on the basis of differences to *A. usambarica*. As stated above, the fastigium verticis is highly variable and its shape not suitable for distinguishing the species. A fading median dorsal carinula of the vertex, described by Jago (1983), can be found in all species to some degree. This was already mentioned by Ramme (1929) in his description of *A. usambarica*. According to Jago (1983) the pronotal prozona should be "twice in length" compared to the length of the metazona. Considering the examination of the types it can be concluded that this is obviously wrong. In the specimens studied the ratio varied from 2.7 to 3.6 with a mean of 3.1. The lateral carinae are parallel or slightly widened at the rear and the front, but never "converging forwards." Jago's drawing of the epiphallus is characterized by weak posterior lateral projections and a narrow lophal interspace, characters that proved not to be stable. Comparisons of measurements are given in table 41. The four specimens measured by Jago (1983) proved to be smaller than those collected in 1998 with regard to nearly all characters.

The species is insufficiently described. It can be concluded from the discriminant analysis that this species significantly differs from all other species, but not one single character separates this species from *A. longicornis*. The strongest variable separating those two species is the width of the tegmen, but the overlap is very great. Even if 25 characters are included in a multivariate analysis, the two species cannot be separated completely. Three specimens of *A. longicornis* were classified as *A. nguru* in the classification statistics.

Tab. 41: Comparison of Jago's (1983) and own measurements of *A. nguru*

	Jago (1983); n = 4	Own measurements; n = 30
Head width	2.48-2.61	2.55-2.89 ($\bar{x}$: 2.71)
Tegminal length	2.32-3.04	2.86-3.77 ($\bar{x}$: 3.19)
Tegminal width	0.77-0.97	0.83-1.05 ($\bar{x}$: 0.94)
Pronotal length	3.08-3.46	3.25-3.86 ($\bar{x}$: 3.50)
Hind femur length	10.06-10.78	10.77-13.22 ($\bar{x}$: 11.60)
Hind femur depth	2.15-2.28	2.12-2.66 ($\bar{x}$: 2.29)

***A. usambarica* (RAMME, 1929)**

A. usambarica is the best described species of the genus, since Jago (1983) redescribed the species as a reference for his other descriptions. Most of his and Ramme's (1929) observations proved to be true for *A. usambarica*, but also for the whole genus. In the following part only differences to Jago's (1983) and Ramme's (1929) observations are discussed. Jago (1983) does not mention the tendency of flagellar segments to fuse. An examination of the holotypes and the paratypes at the Natural History

Museum (London) showed that no fusions occurred in these specimens (this does not include the possible fusion of the first two flagellar segments which is visible in *P. arachniformis*). All flagellar segments measured in this study were longer than wide. Segment 4 is not "as long as wide." The first six to seven segments are flattened, sometimes even the complete flagellum. The tendency of the flagellar segments to fuse and the high variation of their lengths suggest that these characters are not suitable for the identification of any species within the genus. The vertex is not always "acutangular anteriorly", sometimes it is obtuseangular (in all species) or even rounded (in *A. euthynota*). Ramme (1929) describes the vertex of *A. usambarica* as anteriorly rounded. Different from Ramme's (1929) observations, the vertex is also not always slightly longer than wide, but the range of the width / length ratio varies from 0.98 to 1.23.

The lateral carinae of the pronotum are not always "lightly and equally divergent anteriorly and posteriorly", as proposed by Jago (1983). Usually they are parallel or lightly divergent. The emargination of the rear edge of the metazona is variable and not always "broadly obtusely angularly emarginated." The lower margin of the tegmina is not convex, as described by Jago (1983), but rather straight or concave. The tegmen are in mean 3.34 times longer than wide (not "about four times"), ranging from 2.78 to 4.18 in *A. usambarica*. The number of visible tegminal veins varies from four to six, but five veins are visible in most specimens, as described by Jago (1983). The cerci tips do not always "level with apex of supra-anal plate." In 50% of the specimens they are shorter. The shape of the lower outer lobes of the hind knee is variable and not always "acutangulate." The number of posttibial spines is variable, but most specimens had 12 outer spines and 12 inner spines. Jago's (1983) drawing of the epiphallus shows strong lateral projections and a medium lophal interspace, but these characters cannot be confirmed. The measurements given below (table 42) are quite similar to those of Jago (1983). This is probably due to the longer series of specimens examined by him in this species.

Again it is difficult to find any characters separating *A. usambarica* clearly from *A. nguru* and *A. longicornis*. However, the discriminant analysis showed, that it is easier to distinguish than the latter species pair. The antennae are shorter than in these species (also in relation to the body length). The third flagellar segment is very short, and nearly all segments are comparatively broad. The fourth, the sixth and the ninth segment proved to be significantly broader than in *A. longicornis* and *A. nguru*.

Tab. 42: Comparison of Jago's (1983), Ramme's (1929) and own measurements of *A. usambarica*

	Jago (1983); n = 7	Ramme (1929); n = 1	Own measurements; n = 30
Head width	2.55-2.80 ($\bar{x}$: 2.67)	-	2.46-2.84 ($\bar{x}$: 2.66)
Tegminal length	2.86-3.37 ($\bar{x}$: 3.07)	3.3	2.67-3.51 ($\bar{x}$: 3.02)
Tegminal width	0.90-1.07 ($\bar{x}$: 0.99)	-	0.71-1.02 ($\bar{x}$: 0.91)
Pronotal length	3.15-3.57 ($\bar{x}$: 3.33)	3.4	3.01-3.60 ($\bar{x}$: 3.27)
Hind femur length	11.24-11.90 ($\bar{x}$: 11.57)	11.8	10.62-12.21 ($\bar{x}$: 11.26)
Hind femur depth	2.14-2.46 ($\bar{x}$: 2.31)	-	2.00-2.46 ($\bar{x}$: 2.19)

5.6.3 Morphological Discontinuity

Only one species, *A. euthynota*, is distinguishable by using one single character (the short vertex). In the other species no single character by itself can be used to discriminate between the species, but most of them can be clearly distinguished from each other in a multivariate context. There is a substantial morphological discontinuity in all body dimensions between the four species of *Afrophlaeoba*. Thus, the discriminant analysis provides morphometric characters for separating the morphologically very homogenous group. Only a few specimens fall into each other's data cloud, especially the two species *A. nguru* and *A. longicornis*, which have the smallest morphometric and genetic distances. Hence, each of the species is distinct in morphometric space.

According to Slaney & Weinstein (1996) morphological modifications are likely to occur in populations, which have been isolated, experienced stronger selective pressures, or have a greater fixation rate of genetic mutations within a population. Thus a separation of the populations seems likely. This can also be inferred from the close restriction of the genus *Afrophlaeoba* to the Eastern Arc (Jago 1983) and the general fragmentation of forests. Of course, this picture might be influenced by the low availability of distribution records. However, grasshoppers have been collected in substantial parts of Tanzania, but nowhere *Afrophlaeoba* has been found in dry woodland or grassland, so far.

The close morphometric relationship between *A. nguru* and *A. longicornis* supports the hypothesis that these two species have been separated rather recently. The low Mahalanobis distance is consistent with the low genetic distance. A final solution of the question, whether a habitat connection between the two species still exists or not could only be found by intense collection efforts in the area between the two sites. It cannot be excluded that the unusual body dimensions of *A. euthynota* are influenced by environmental factors, since the habitat from which the species was obtained was located at nearly treeless sites at higher altitudes with a high radiation. The high amount of parasitic mites (see chapter 7) may indicate the high degree of disturbance of the habitat.

5.6.4 Taxonomic Rank

For practical reasons all four species have been described as morphospecies. Hence, one would expect that qualitative morphological differences clearly distinguish them. Ramme (1929) already realised that many characters of grasshoppers are variable within species and sometimes even within populations, which leads to wrong descriptions, if only a few specimens are examined. This was probably the case in the paper of Jago (1983). He examined only four specimens of *A. longicornis* and *A. nguru* and seven specimens of *A. euthynota* and *A. usambarica*. Since Jago dissected only few specimens, he missed the intraspecific variability of the genitalia. From this point of view the missing qualitative differences between the species suggest that they may be conspecific. The male genitalia are similar enough to consider them to be subspecies of the same taxon. At least *A. longicornis* and

A. nguru are genetically extremely closely related and they are morphologically difficult to identify. Since genetic drift will lead to clear morphological differences even between closely located populations (Gries et al. 1973), the present taxonomic rank of at least these two species must be severely doubted. On the other hand, in some taxa no morphometric differences occur, although they can be clearly distinguished by qualitative characters (Burckhardt & Basset 2000). Species of the grasshopper genus *Chrotogonus* SERVILLE, 1838 can only be multivariately identified or by a dissection of the genitalia (Blackith & Kevan 1967). This demonstrates that morphological characters alone are not suitable to provide an answer to the questions of the species status (Mayr 1942). However, although or because isolation may not be given, it is reasonable to suggest a fairly recent divergence of these two taxa. A final conclusion regarding the species status is only possible by testing their potential to interbreed, which is experimentally difficult and not meaningful for the research objectives of this study. The problem will be discussed further, after the communicative behaviour has been dealt with.

5.6.4 Phenetic Relationships

The neighbor-joining phenogram depicts phenetic relationships based on the morphometric distances. This may be interpreted as a phylogenetic tree, if morphometric characters do not evolve in abnormally different rates (Blackith & Reyment 1971). Omland (1997) suggested that morphological and genetic distances are usually highly correlated. This also seems to be the case in the studied taxa. A factor, which is often thought to cause differences between a phenetic tree based on morphometrics and the “real phylogeny” is the adaptation to local ecological conditions (Sites & Willig 1994). This might be reflected in the small body dimensions of *A. euthynota*, which was collected from the highest locations with the highest degree of disturbance (see chapter 7). However, the neighbor joining analysis corrects the rate heterogeneity and, therefore, the species was branched with *A. usambarica*. In an UPGMA phenogram *A. euthynota* would branch basally to the other *Afrophlaeoba* species, since this analysis does not correct different evolutionary rates. The structural similarity of the neighbor joining phenogram and the trees inferred from mtDNA data is quite a good argument for the soundness of each method. Unfortunately, there is no statistic method available to test the confidence of a phenetic tree based on distances alone. It merely represents the shortest way to connect the species in morphometric space. Due to the missing discrete differences, this typological technique to connect species was the only method available, to find any hierarchical structure in the data.

6 Communication Behaviour

6.1 Introduction

The communicative behaviour of grasshoppers has been studied intensely due to the conspicuous songs of European grasshoppers. Aristotle mentioned the song production of grasshoppers by rubbing with the hind legs already 330 BC (Thompson 1910). Some authors described the songs of several European Orthoptera already in the middle of the 19th century (Von Siebold 1842) and Yersin (1854) illustrated them by musical notation and realised the high value for identification. The first identification key for Orthoptera entirely based on their songs was provided by Faber (1928). He also established the species status for the two closely related species *Chorthippus parallelus* (ZETTERSTEDT, 1821) and *Chorthippus montanus* (CHARPENTIER, 1825), based upon their sound production (Faber 1929). The extensive descriptions of the behaviour of European grasshoppers pioneered by Faber (1953) and Jacobs (1953) were followed by detailed studies of the songs from the ethological (von Helversen 1979) and taxonomical point of view (Ragge & Reynolds 1998). In Africa taxonomical studies based on songs are rare and mainly focus on Tettigoniidae and Gryllidae (Bailey 1975, Pitkin 1977, Otte & Cade 1983, Rentz 1988). Only few descriptions of songs of Acrididae are available (Green 1995). This is partly caused by the fact that sound production in the communicative behaviour of Acridoidea is absent in many "primitive" groups, while it evolved independently in some subfamilies, such as Gomphocerinae or Oedipodinae, which dominate in Europe. The "silent majority" of grasshoppers communicate by visual means, usually by signals of the hind legs or antennae (Otte 1970, Riede 1987). It is reasonable to suggest that sound production is derived from such visual signalling (Bailey 1991). Some typical visual signals include "knee-waving", "femur-shaking" and antennal movements (Riede 1987). The special value of songs and other kinds of communicative behaviour in taxonomy stems from the fact that they form a mate recognition system and are thus likely to be particularly reliable (Ragge & Reynolds 1998). In the sibling species *Chorthippus brunneus* (THUNBERG, 1815), *Ch. mollis* (CHARPENTIER, 1825) and *Ch. biguttulus* (LINNAEUS, 1758) the songs seem to provide the only barrier for interbreeding (Ragge & Reynolds 1998). According to Ragge & Reynolds (1998), "songs are particularly useful in … deciding on the status of allopatric populations showing small morphological differences. Their use in assessing relationships between species, and therefore as a tool in phylogenetics, is more limited."

The intention of the analyses of the communicative behaviour in this study was to examine interspecific differences and evaluate the importance as barriers. For this purpose 52 video sequences were analysed and the movements of antennae, hind legs and palpi were studied for the *Afrophlaeoba* species and *P. arachniformis*. The analysis included the amplitude of the antennal movement in all species and of the hind femora in *P. arachniformis*. The frequency of the movements was analysed as well, including the number of strokes and the duration of a display.

6.2 Methods

6.2.1. Video Records

The visual displays were recorded with a High 8 video camera (Sony video Hi8, CCD-TR3100E) in the field. The number of records per species differs substantially due to the rareness of displays occurring. In some cases many hours or days of observations preceded a successful observation of a display. A total of 51 records were analysed. Most displays were recorded for *A. euthynota*, but only 26 of them were analysed. From the other species a substantially smaller sample was obtained, including eleven sequences of *A. usambarica*, four of *A. nguru*, four of *A. longicornis* and six of *P. arachniformis*. All field records were obtained during the second field trip to Tanzania (table 43). The temperature was usually noted during the ecological records obtained from the same specimen. In some cases, however, the ecological records had already been finished (*P. arachniformis*: 19. & 20.01.1998, *A. nguru*: 03.02.1998) and the temperature was then estimated from the temperature measured at the same site and time one day before. With the exception of *A. euthynota*, all species were recorded at temperatures between 24°C and 26°C. The higher temperatures of the *A. euthynota* records (c. 34°C) may have influenced the frequency and the duration of the display, as was documented in Gomphocerinae songs (von Helversen 1972). The angle, from which the video sequences were recorded, depended on the circumstances of the location. In most cases it was not possible to obtain records from the straight dorsal or the lateral view. This influenced the angles measured for antennal or femoral movements, which have to be interpreted as relative angles and not as absolute measures.

Tab. 43: Date, localities and altitude of the video records.

Species	Dates	Locality	Altitude	Temperature
A. euthynota	09.12.1997	Uluguru Mts. near Morogoro	c. 1200 m	c. 34°C
A. usambarica	06.01.1998	East Usambara Mts. near Amani	c. 900 m	c. 24°C
P. arachniformis	17., 19. & 20.01.1998	East Usambara Mts. near Amani	c. 750 m	c. 25-26°C
A. nguru	03.02.1998	Nguru Mts. near Mhonda	c. 700 m	c. 25-26°C
A. longicornis	11.02.1998	Rubeho Mts. near Kilosa	c. 550 m	c. 26°C

6.2.2 Videography

In videography, the image is analysed sequentially frame-by-frame. A full video frame is interleaved, i. e. composed of 50/s frames (20 ms per interleave). Provided that the movements exceed the duration of a half-frame scan, all movements will be captured on the video. The frame-by-frame analysis was made with a video player (Panasonic NV-HS 1000) and a monitor screen. The measurements were performed by attaching a transparent film to the monitor and drawing one line along the body axis and another along the antennae and the upper edge (respectively the inner edge) of the femora to the point where those lines meet the body axis (figure 49). The angle between the body axis and the femoral and antennal positions was measured with a protractor (antennae:

front = 0°, rear = 180°, hind legs: rear = 0°, front = 180°). The movements of the hind legs in *Afrophlaeoba* proved to consist mainly of minor angles (up to 3°), which are difficult to measure. Hence, the main and the minor strokes of hind leg movement were noted, to obtain at least the frequency of the hind leg. Moreover, these hind leg movements were very fast and even the direction of the movement was difficult to disentangle. This might also be caused by the three-dimensional character of those movements. Seen from above, a horizontal movement could be observed, while a vertical movement was visible from the lateral view. Main hind leg strokes were noted with a "1", while minor movements were noted with "0.5." The video material had a resolution of 0.02 seconds (25 Hz). Thus, 50 pictures had to be analysed for a video sequence of one second. The movements were noted in a table, with each cell representing a new picture. In addition, the day, the time, the temperature (if available), the orientation and the location of the insect and the view were noted. Special features, such as the presence of another male or female or missing legs were noted as well. The descriptions of visual displays in literature are too rare so that no universal terminology has been proposed yet. Although the movements resemble those of sound production, a different terminology is required due to the different quality. In this study a display refers to an "echeme" in the sense of Ragge & Reynolds (1998) and a stroke refers to a "syllable".

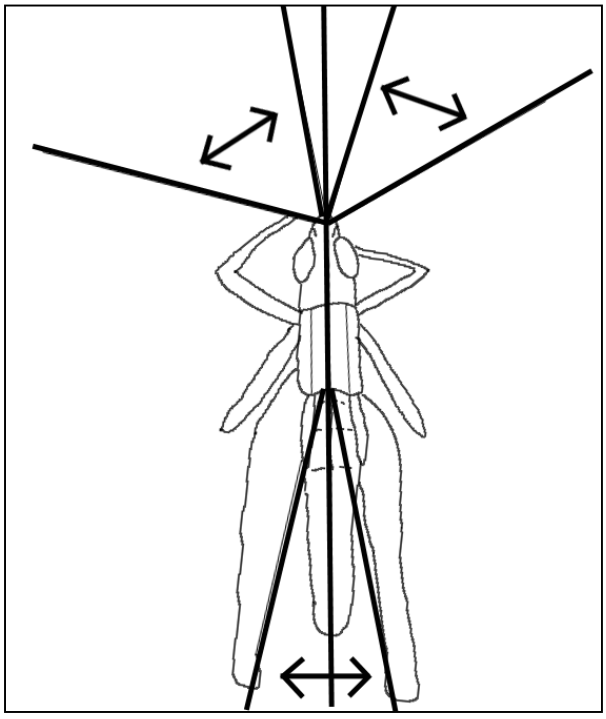


Fig. 49: Illustration of the main movements of *Afrophlaeoba*, redrawn from a transparent film. The arrows indicate the main directions of antennal and femoral movements. Both, the hind femora and the hind legs also move in a vertical direction, which is not illustrated. The angle of movements is measured in relation to the body axis, represented by the central line.

6.2.3 Statistical Analysis

From the video sequences the following data could be obtained:

- duration of a display
- amplitude of each antennal stroke (in *P. arachniformis* also of the hind legs)
- total amplitude of the antennae within a display
- frequency of the antennal movements and average per display
- number of antennal movements per display
- frequency of the hind leg movements and average
- number of hind leg movements
- frequency and number of palpi movements in *P. arachniformis*

These measures were analysed univariately and multivariately. The number of strokes per display proved to be rather variable between the species and was also influenced by the quality of the video records. In some cases minor strokes preceding the analysed part were possibly overlooked or the display started before recording. Therefore, some sequences had to be excluded from the multivariate analysis. For the same reason the homology of single movements is difficult to assess. It was decided to homologise the strokes from behind to avoid errors based on incomplete recordings. From such incomplete displays the existing data, such as the frequency or the amplitudes of the last strokes were included in the averages for the main descriptions and univariate analyses. Student's t-tests were performed on normal distributed data. The highest P accepted was 0.05. Characters, which were present in most sequences (especially the amplitude and the distance of the last strokes) were included in a stepwise discriminant analysis. This method has already been presented in the morphological part (chapter 5).

6.3 Results

The visual display of *Afrophlaeoba* includes rapid synchronous movements of the hind legs and the antennae. These vibratory bursts are not continuous, but rather expressed in short rhythmical strokes with typical intervals. While the hind legs perform only minor vibrations ("femur shaking" sensu Otte 1970), the antennae cover larger amplitudes in most of the species. The hind legs are slightly spread from the abdomen and thus a sound production is unlikely to occur. For each species a description of one typical display is presented. A first chart illustrates the spatial pattern of the movement, while the second chart illustrates the difference to the situation of the previous frame, which allows a clearer identification of the rhythm of the main strokes. In addition, the average amplitude of each stroke and the average frequency of the strokes is presented for each species. In a second part the main descriptors of the sequences of each species are compared univariately. The third part represents a discriminant analysis of some parameters, which have been chosen according to their availability.

6.3.1 Descriptions of the Visual Displays

A. euthynota

Most video sequences of displays were obtained from this species, and 26 of them were analysed. In comparison to the other species the temperature was much higher (c. 34°C), which might have influenced the frequency and the duration of the display. A female was observed pursuing a male after a display. A typical display (record nr. 4) is presented in the figures 50 and 51. Since the vibrations of the hind femora are synchronous, only one femur is presented. The hind femur movement is given in different categories (0.5 for a minor movement, 1 for a major movement). The number of hind femur strokes was eight and the frequency decreased in the last three intervals (5 Hz, 4.6 Hz, 5.6 Hz, 4.6 Hz, 4.6 Hz, 3.6 Hz, 2.6 Hz). A similar rhythm could be observed in the antennal movements, but there was one more stroke. The right antenna was not visible very well and thus it showed smaller amplitudes. The angle of the left antenna to the bodyline was rising with each stroke, representing a movement to the rear. With the last stroke a rapid forward movement of the left antenna could be observed. Each stroke was characterized by a major movement in one direction and a following smaller movement back. The amplitude of the antennal movement increased with each stroke (Fig. 50: 1°, 2°, 3°, 8°, 8°, 14°, 19°, 32°, 58°). The complete amplitude of the left antenna was 82° with a highest velocity of 2,200° per sec. The rhythmical patterns of the antenna and the femur were highly synchronized. The length of the display was 2.48 seconds.

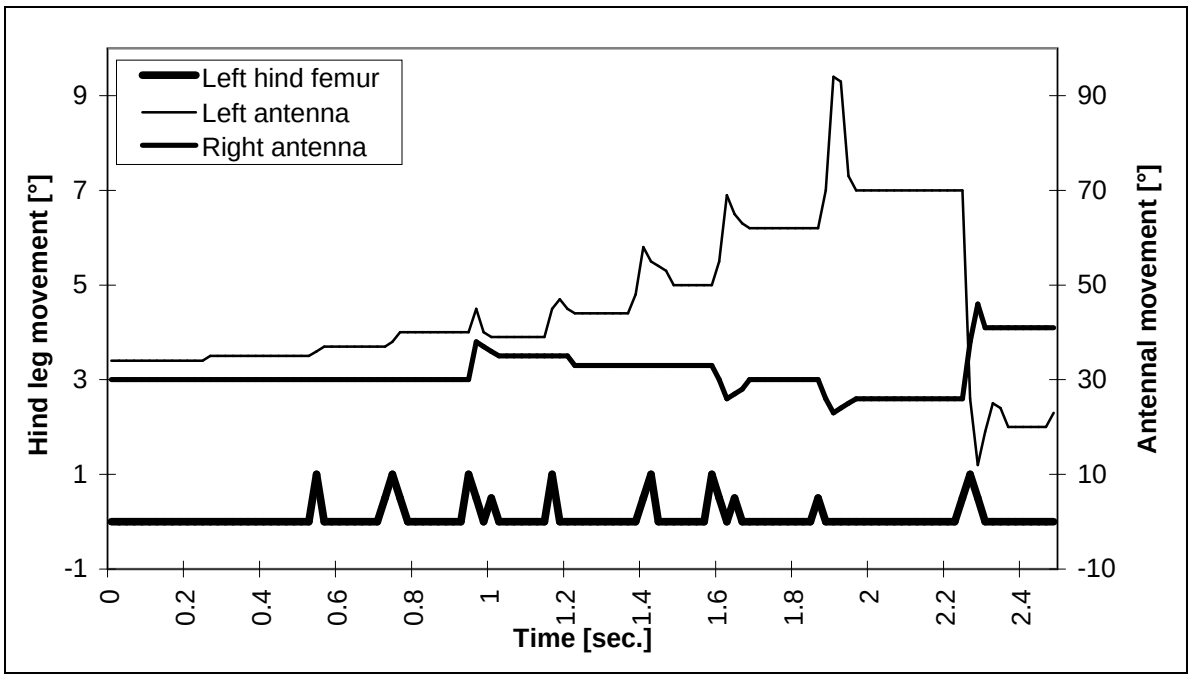


Fig. 50: Characteristic, continuous sequence of the male visual display in *A. euthynota* (record 4, 09.12.1997, 10:19, c. 34°C, dorsal view, location: leaf of a forb)

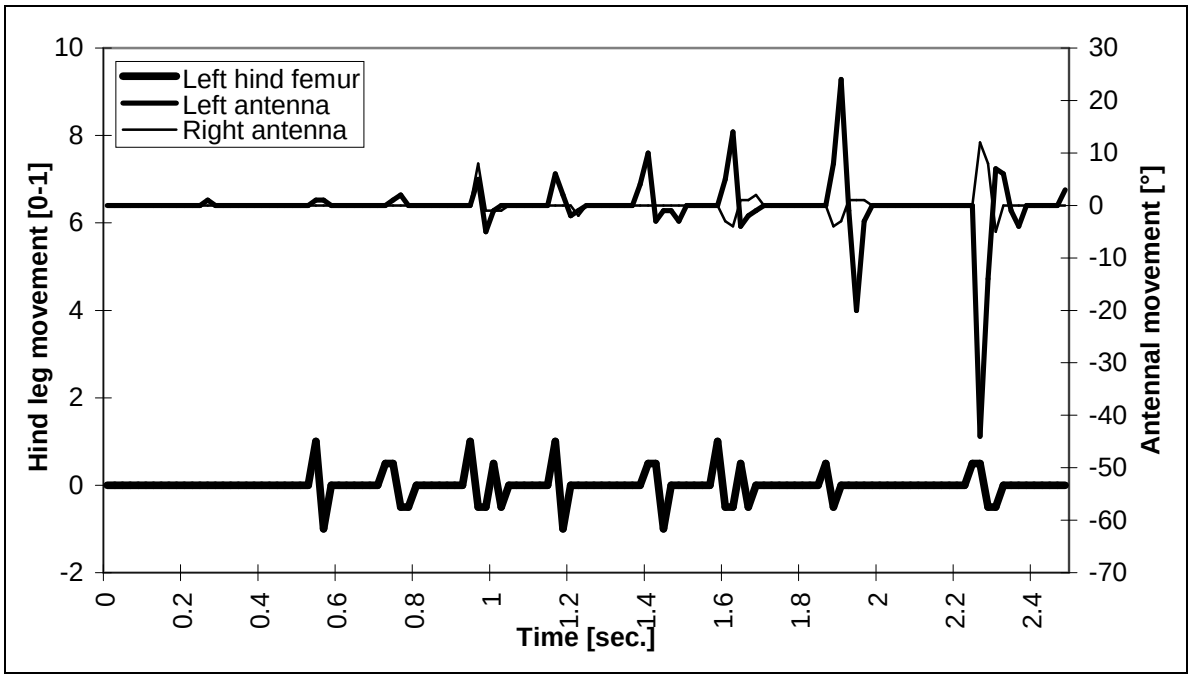


Fig. 51: Relative movements of hind legs and antennae in relation to the preceding position of *A. euthynota* (record 4, 09.12.1997, 10:19, c. 34°C, dorsal view, location: leaf of a forb)

Figure 52 illustrates the development of the average frequency with each interval of the complete *A. euthynota* data set. The number of strokes per display ranged from five to eleven, with an average of 7.83 (s.d.: 1.40). It is visible that the frequency of the first strokes was around 5 Hz on average, while it decreased in the last strokes to 3-4 Hz. This decreasing rhythm of the frequency seems to be rather typical. The total average of the frequency was 4.4 Hz. The mean amplitude of the antennal movement increased with each stroke, as illustrated in figure 53. The direction of the antennal

movement was variable between the sequences, but often an increasing angle was observed due to the low resting position of the antennae (c. 30°). The mean total amplitude was 41.9°, but the variation was very high (s.d.: 20.1°). The average of the highest velocity per display was 1,270° per second. A display lasted 1.73 seconds (s.d.: 0.29) on average. In summary the visual display of *A. euthynota* was characterized by antennal movements of medium amplitude and short duration with a comparatively high frequency, which decreased during the display.

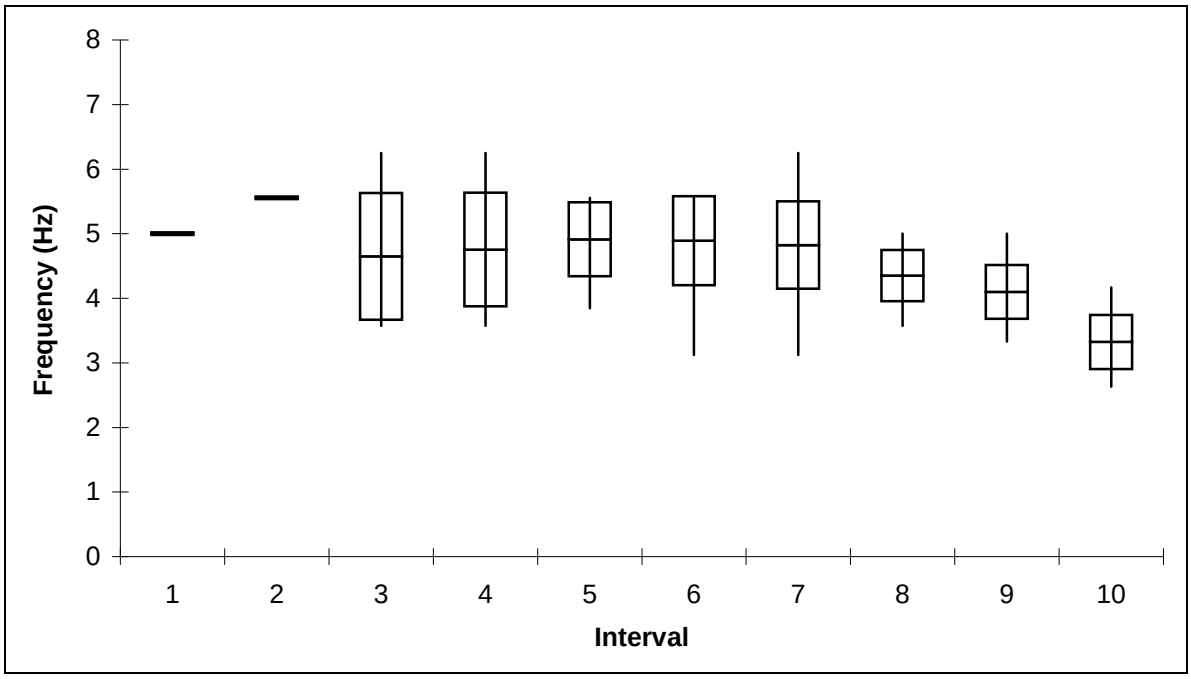


Fig. 52: Development of the frequency of the strokes of *A. euthynota* with each interval (mean, standard deviation, range). The frequency is typically decreasing from 5 to 3-4 Hz.

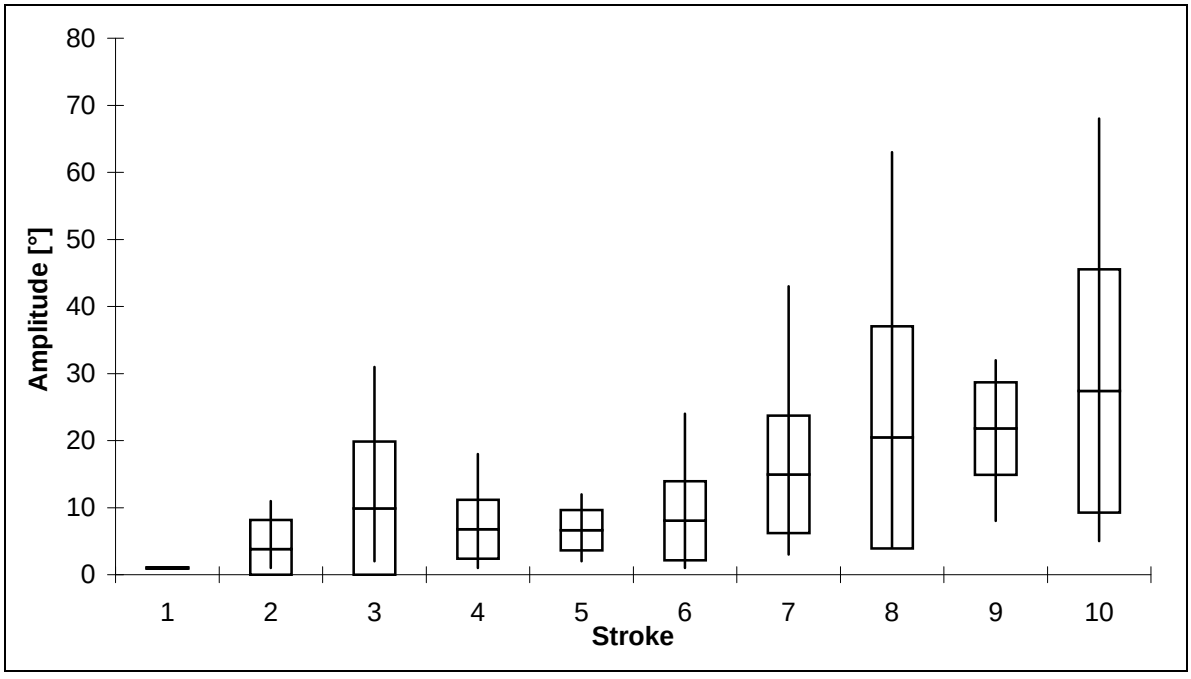


Fig. 53: Development of the amplitude of the antennal movement with each stroke in *A. euthynota* (mean, standard deviation, range). The amplitude increases with each stroke.

A. usambarica

From this species eleven video sequences of visual displays were obtained and analysed. The temperature was lower than in the displays of the other species, but only in *A. euthynota* the temperature was substantially higher. Some of the sequences were recorded from two males located close to each other and alternating in displaying, which was termed “vibratory dialogue” by Riede (1987). The figures 54 and 55 illustrate a typical display of *A. usambarica* (record nr. 37). As described in *A. euthynota*, only one hind femur is presented. The hind femur movement is given in different categories (0.5 for a minor movement, 1 for a major movement). The number of the rhythmically synchronous strokes of the hind femur and the antennae was nine. The frequency increased with the first four strokes and decreased with the last three intervals (2 Hz, 2.8 Hz, 3.6 Hz, 3.6 Hz, 2.9 Hz, 2.9 Hz, 2.4 Hz, 2.2 Hz). The antennal movement did not always follow the same direction, but the same rhythmical pattern. In this special case the direction of the left antennal movement alternated, but this pattern was variable between the records. The antennal resting position was approximately 30°. It varied from –7° to 59° and thus covered a total amplitude of 66°. As in *A. euthynota* a backward movement of the antennae could be observed after each stroke, which sometimes lasted until the next stroke occurred. The amplitude of the antennal movement increased with each stroke, but decreased in the last stroke (Fig. 54: 1°, 2°, 5°, 9°, 15°, 36°, 38°, 45°, 37°). The pattern of an initially increasing and afterwards decreasing amplitude was rather typical for the species, while the direction of the antennal movement was variable. The length of the display was 3.1 seconds, covering a total antennal amplitude of 66° with a highest velocity of 1250° per sec.

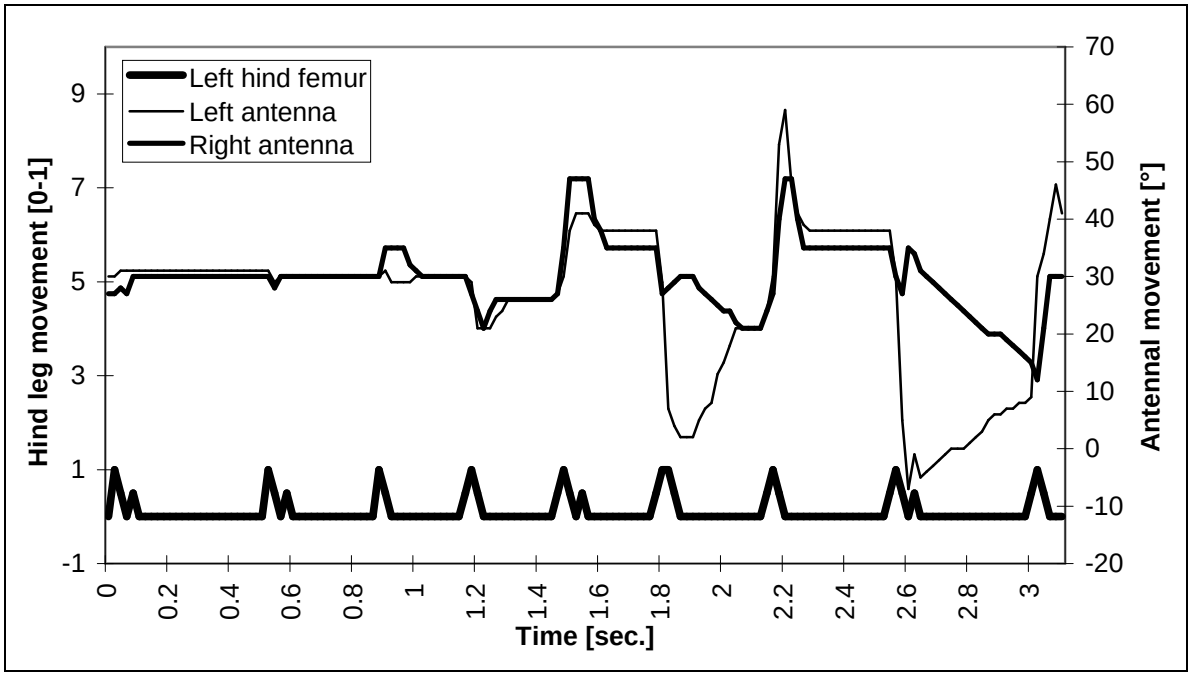


Fig. 54: Characteristic, continuous sequence of the male visual display in *A. usambarica* (record 37, 06.01.1998, 16:10, c. 24°C, dorsal view (slightly from the right), location: leaf of a grass)

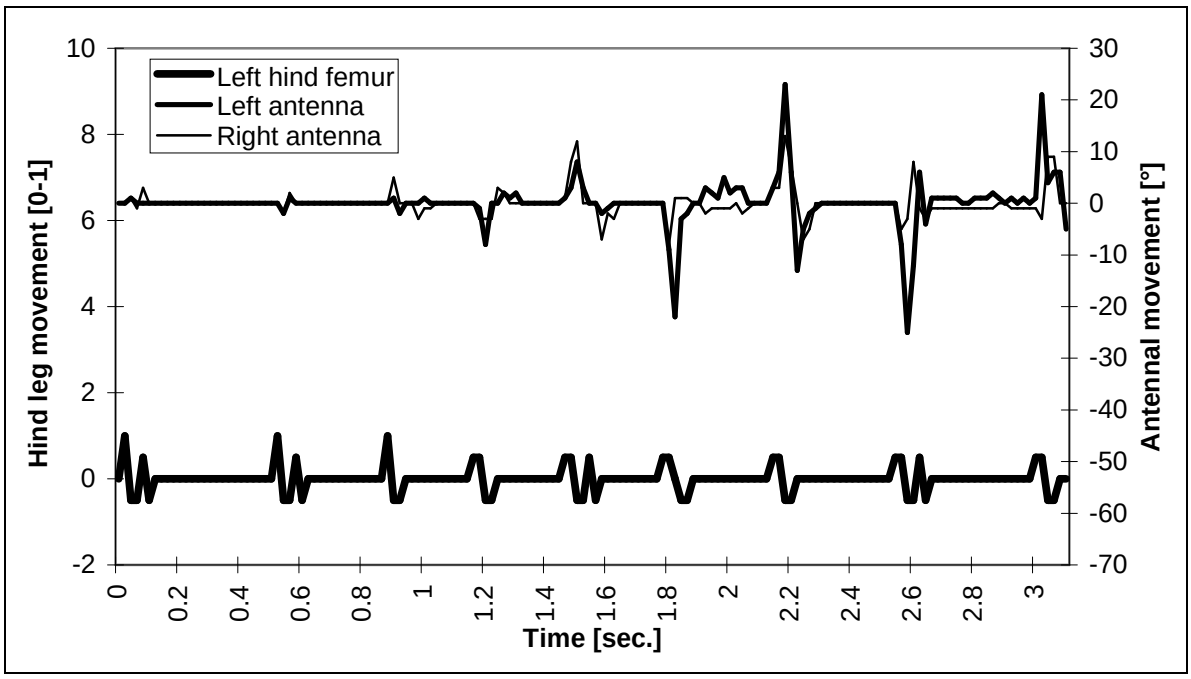


Fig. 55: Relative movements of hind legs and antennae in relation to the preceding position of *A. usambarica* (record 37, 06.01.1998, 16:10, c. 24°C, dorsal view (slightly from the right), location: leaf of a grass)

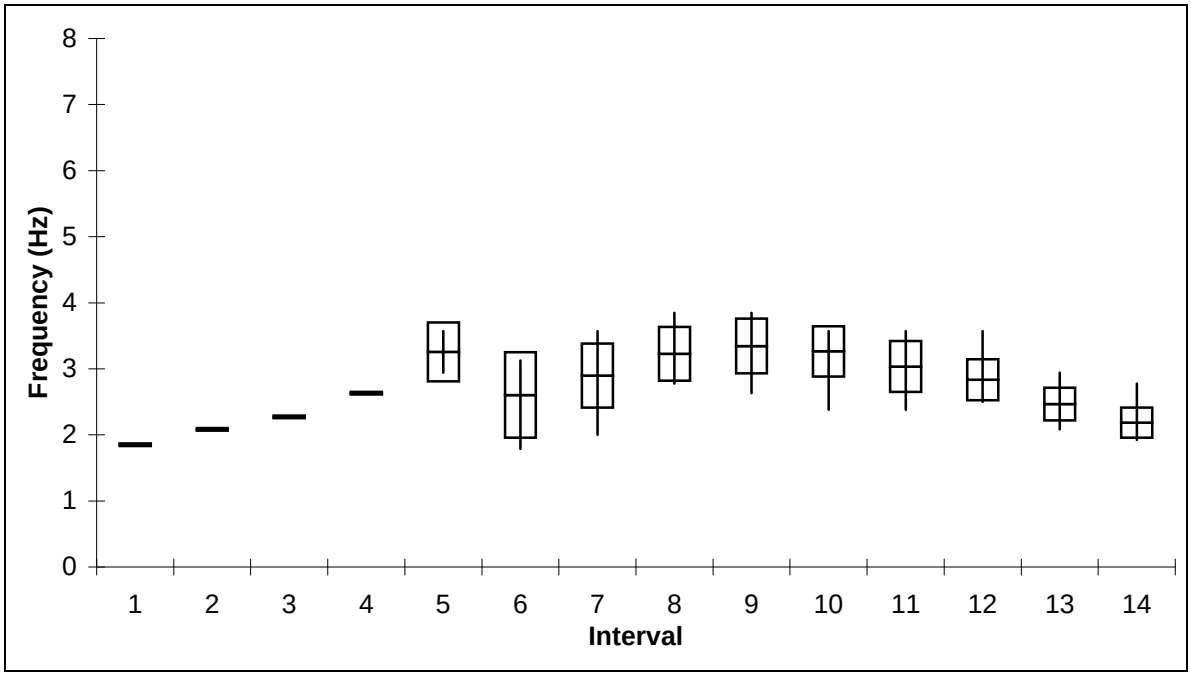


Fig. 56: Development of the frequency of the strokes of *A. usambarica* with each interval (mean, standard deviation, range). While the frequency is increasing until the ninth interval, it is decreasing afterwards.

The development of the average frequencies for the complete data set of *A. usambarica* are given in figure 56. The number of observed strokes per display ranged from seven to fifteen, with an average of 9.6 (s.d.: 2.11). Fifteen strokes were observed only once, while the other displays included seven to ten strokes. Hence, the first four frequencies in the chart are given without the range and standard deviation. The display with only seven strokes (record 38) followed the cleaning of the hind leg without transition. Generally, the frequency increased from 2 Hz in the first part of the display to 3-

4 Hz in the central part and decreased afterwards to an average of 2.2 Hz between the last two strokes. In only one display this pattern was less pronounced (the above mentioned reduced record 38). The total average of the frequency was 2.85 Hz. While the amplitude of the first eleven strokes was comparatively low (usually below 20° on average), it was higher in the three penultimate strokes (around 30°) and decreasing again to 20° in the last stroke (figure 57). The mean total amplitude was 45.8°, but showed a very high variation (s.d.: 33.3°). The average of the highest velocity per display was 1,390° per second. A display lasted 3.22 seconds (s.d.: 0.76) on average. In summary the visual display of *A. usambarica* is characterized by antennal movements of medium amplitude and of medium duration with a comparative low frequency.

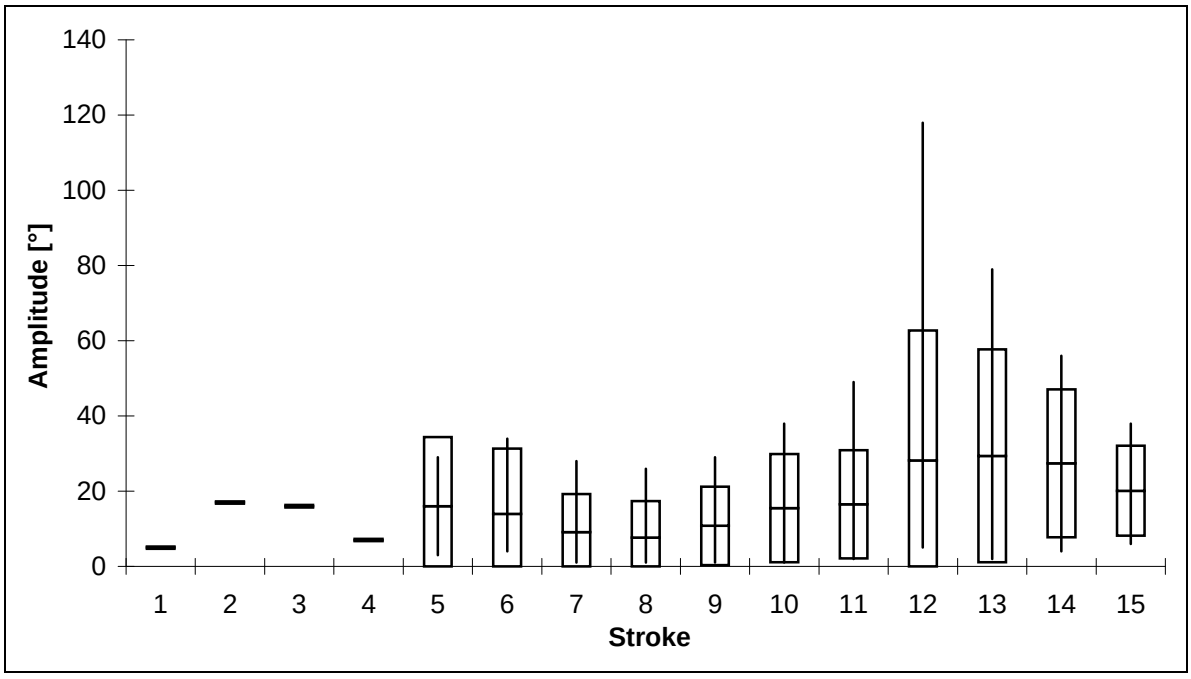


Fig. 57: Development of the amplitude of the antennal movement with each stroke in *A. usambarica* (mean, standard deviation, range). While the most of the first eleven strokes and the last stroke have average amplitudes up to 20°, the penultimate three strokes have higher amplitudes.

A. nguru

Only four displays of one specimen of this species were recorded and analysed. This specimen had only one hind leg. The temperature was approximately 25-26°C. It cannot be excluded that the missing hind leg influenced the display. Since the hind leg movements of all other *Afrophlaeoba* species were synchronous, it was treated like those species. One record was incomplete. The figures 58 and 59 illustrate a typical display of *A. nguru* (record nr. 48). Due to the high quality of the record, the angle of the hind femur was included (right lateral aspect). The number of main femoral strokes was 14, that of the antennal strokes 13. A longer gap (0.56 sec.) was observed between the fourth and the fifth stroke. Since the length of the gap is approximately twice the usual interval, it can be assumed that one stroke was lost. When this gap was omitted, the frequency was increasing up to the 11th interval and decreasing slightly afterwards (2.6 Hz, 3.1 Hz, 3.6 Hz, 1.8 Hz, 3.3 Hz, 3.9 Hz,

3.3 Hz, 3.6 Hz, 4.2 Hz, 3.6 Hz, 4.2 Hz, 2.6 Hz, 3.1 Hz). Due to the synchronous movements of the antennae and the hind legs the antennae followed the same rhythm. The amplitude of the antennae was more or less increasing continuously, but especially within the last four strokes (Fig. 58: 1°, 9°, 5°, 13°, 7°, 34°, 27°, 18°, 22°, 103°, 125°, 79°, 108°). The total amplitude of the antennal movement was 201° (ranging from –41° to 160°) with a highest velocity of 3,900° per sec. The backward movement of the antennae was visible as well. The length of the display was 4.4 seconds.

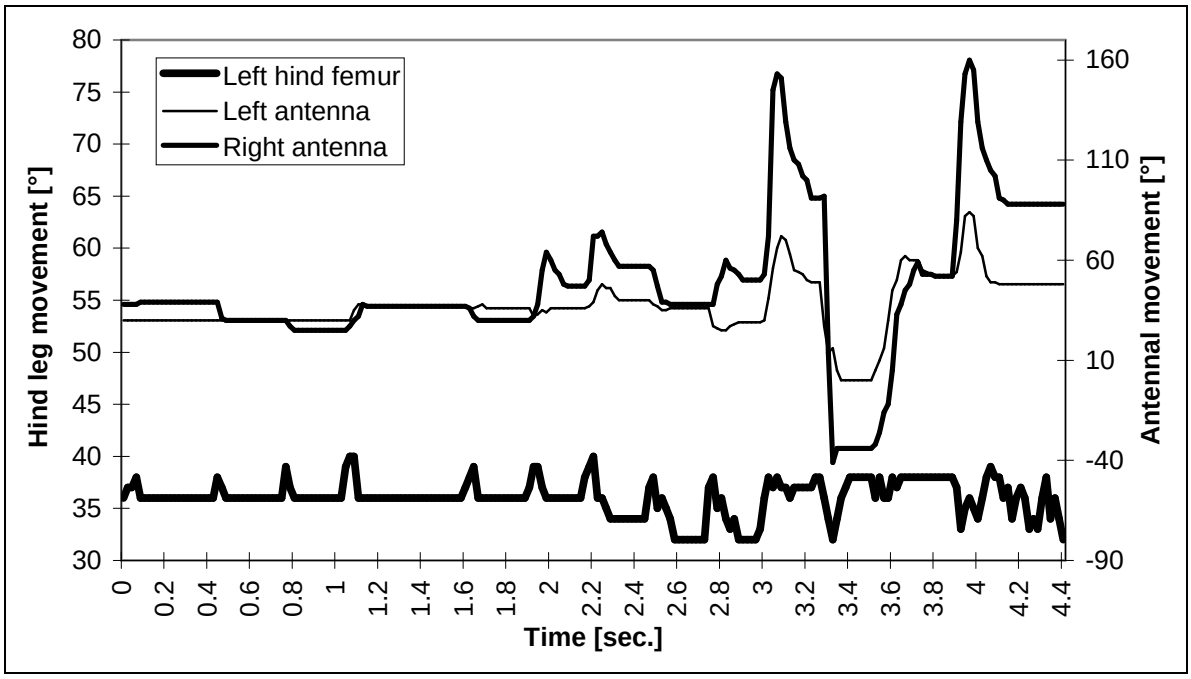


Fig. 58: Characteristic, continuous sequence of the male visual display in *A. nguru* (record 48, 03.02.1998, 11:06, c. 25-26°C, lateral view (from the right), location: stem of a grass)

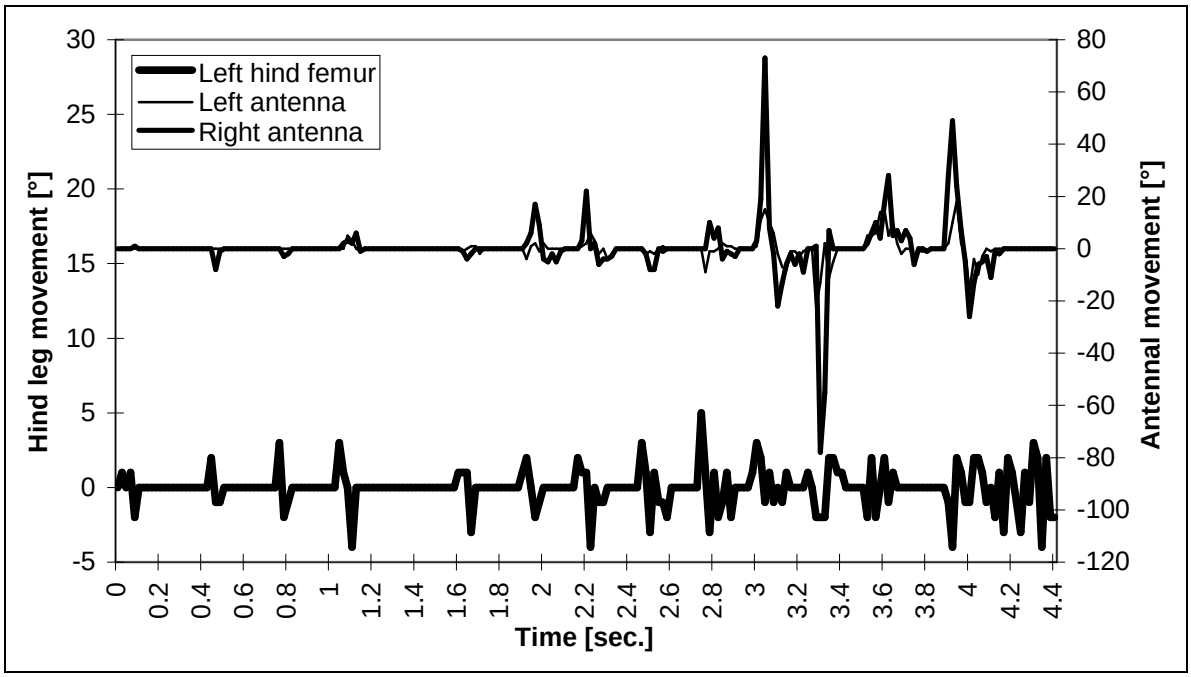


Fig. 59: Relative movements of hind legs and antennae in relation to the preceding position of *A. nguru* (record 48, 03.02.1998, 11:06, c. 25-26°C, lateral view (from the right), location: stem of a grass)

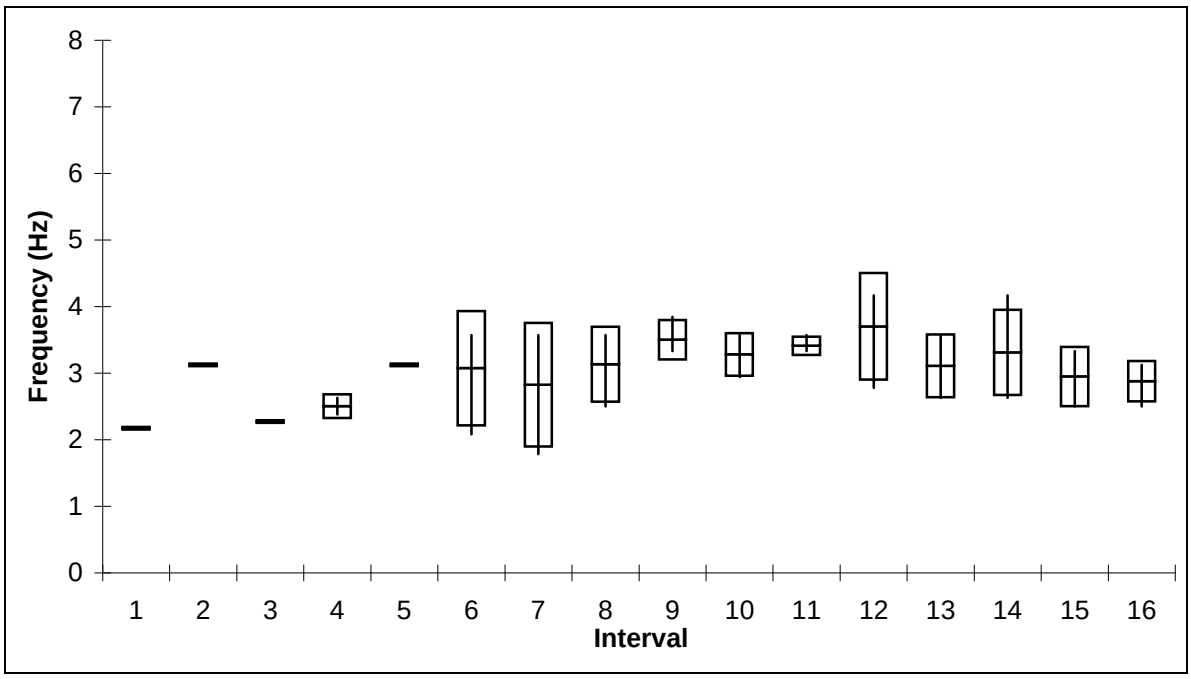


Fig. 60: Development of the average frequency of the strokes of *A. nguru* (mean, standard deviation, range) with each interval. The frequency increases up to the 12th interval and decreases slightly afterwards.

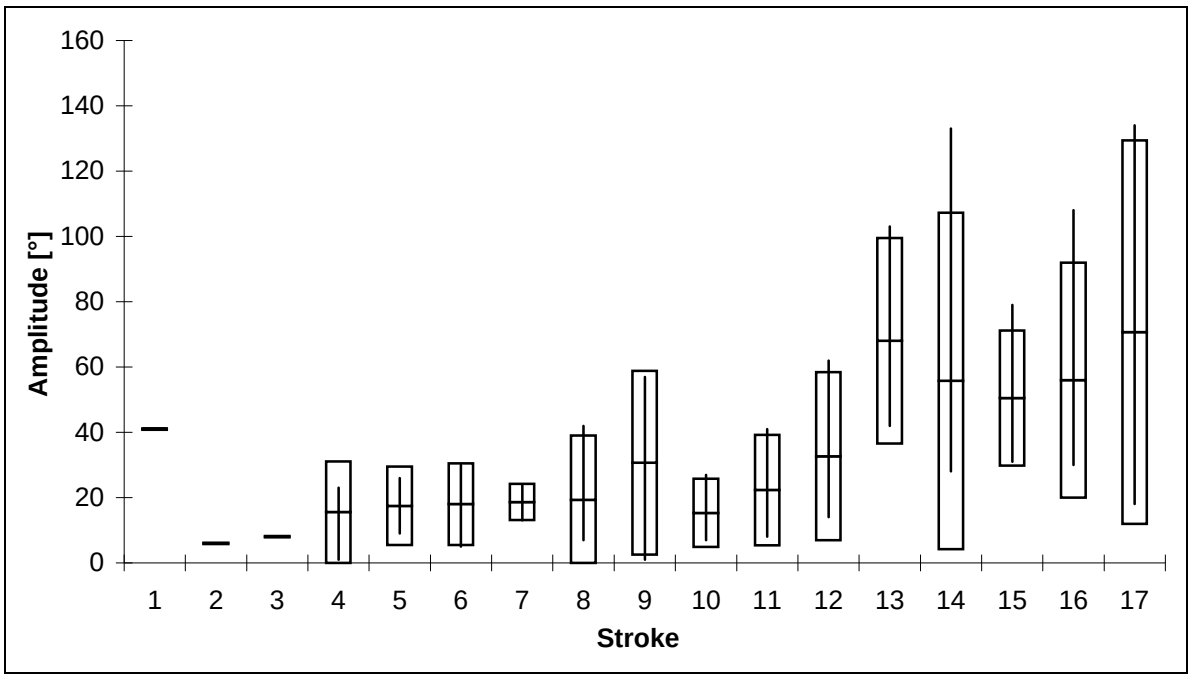


Fig. 61: Development of the amplitude of the antennal movement with each stroke in *A. nguru* (mean, standard deviation, range). The amplitude is much higher than in the other species and more ore less continuously increasing to the last stroke.

Figure 60 illustrates the development of the average frequency for the records of *A. nguru*. It should be taken in consideration that only four records of one specimen were available, of which one record was incomplete. The number of observed strokes ranged from 12 to 17 in the complete displays (the incomplete record included four strokes), with an average of 14.3 (s.d.: 2.52). Thus the observed displays included more strokes than in *A. euthynota* or *A. usambarica*, similar to *A. longicornis*. The

frequency increased slightly from 2-3 Hz to 3-4 Hz with the first twelve intervals. Afterwards it decreased to an average of 2.8 Hz between the last two strokes. The total average of the frequency was 3.1 Hz. The amplitude of the antennal movement was increasing continuously. The first eight strokes had amplitudes lower than 20° on average, the next four strokes varied from 20-40° and the next three covered amplitudes higher than 40°. The last stroke was usually the highest, with an average amplitude of 70° (figure 61). The mean total amplitude was 102.6°, but showed a very high variation (s.d.: 48.9°). The average of the highest velocity per display was 3,275° per second, but a maximum of 5,100° per second occurred (102° amplitude between two frames). A display lasted 4.69 seconds (s.d.: 0.74) on average. In summary, the recorded displays of *A. nguru* were characterized by a high duration, a comparatively low frequency and extremely high amplitudes of the antennal movements.

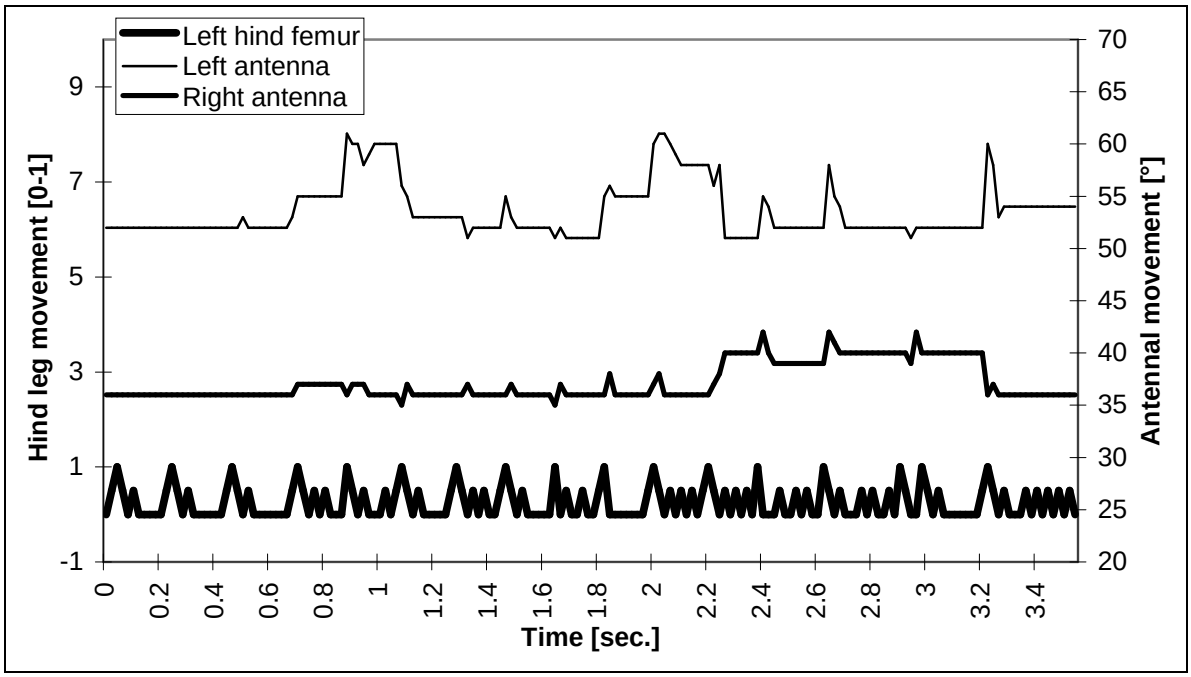


Fig. 62: Characteristic, continuous sequence of the male visual display in *A. longicornis* (record 51, 11.02.1998, 15:46, 26°C, dorsal view (slightly from the right), location: leaf litter)

A. longicornis

In *A. longicornis* four displays were analysed, which were recorded at a temperature of 26°C. Two specimens were involved, which were located close to each other and alternating in display. A typical display of *A. longicornis* (record nr. 51) is given in the figures 62 and 63. The hind femur movement is given in different categories (0.5 for a minor movement, 1 for a major movement). The number of main antennal strokes was 14, while the hind femora showed many smaller movements, which made it difficult to identify the major strokes. Since most of the femoral strokes were synchronous to the antennal strokes, 16 major strokes were identified. Thus the femoral movement started two strokes earlier than the antennal movement. The frequency of the first strokes varied from 3.9 Hz to 7.2 Hz,

while it decreased in the last two intervals (3.3 and 3.6 Hz). The full sequence was 5 Hz, 3.9 Hz, 5.6 Hz, 5 Hz, 5 Hz, 4.2 Hz, 7.2 Hz, 5.6 Hz, 5.6 Hz, 5.6 Hz, 4.6 Hz, 5.6 Hz, 4.2 Hz, 3.3 Hz, 3.6 Hz. The amplitude of the antennal movement was rather low and covered in total only 10°. It was only slightly increasing (Fig. 62: 1°, 3°, 6°, 8°, 2°, 3°, 2°, 5°, 6°, 9°, 4°, 6°, 3°, 8°). Hence the highest velocity was only 450° per second. The length of the display was 3.54 seconds.

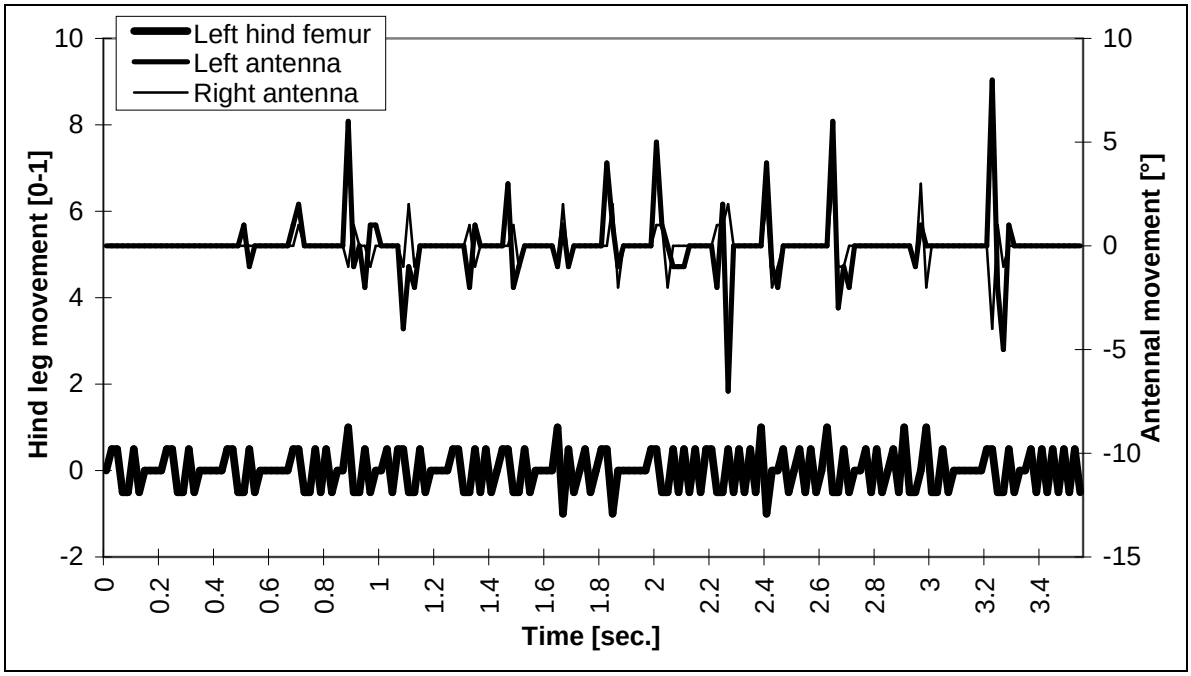


Fig. 63: Relative movements of hind legs and antennae in relation to the preceding position of *A. longicornis* (record 51, 11.02.1998, 15:46, 26°C, dorsal view (slightly from the right), location: leaf litter)

The development of the average frequency of the four records of *A. longicornis* is illustrated in figure 64, including one incomplete record. In the longest record (4.48 seconds) a longer gap (0.92 seconds) occurred in the first half of the display. This caused the high variation, which is visible in interval number 6. The number of observed strokes ranged from 13 to 16 in the complete records (the incomplete record included six strokes). The average number of strokes of the three complete records was 14.67 (s.d.: 1.53), which is quite similar to *A. nguru*. The average frequency of the first twelve intervals varied from 4.2 Hz to 5.3 Hz (the gap was ignored). A decreasing frequency could be observed in the last three intervals (3.8 Hz, 3.3 Hz, 2.8 Hz). The total average of the frequency was 4.1 Hz. The amplitude of the antennal movement was small in all records (usually <10°), but slightly increasing (figure 65). The mean total amplitude was 13° with a standard deviation of 4.8°. The average of the highest velocity per display was only 433° per second. A display lasted in average 3.76 seconds (s.d.: 0.64). In summary, the displays of *A. longicornis* were comparatively long, with many smaller movements of the hind femora and only small amplitudes of the antennae. The frequency was comparatively high, but decreasing in the last intervals.

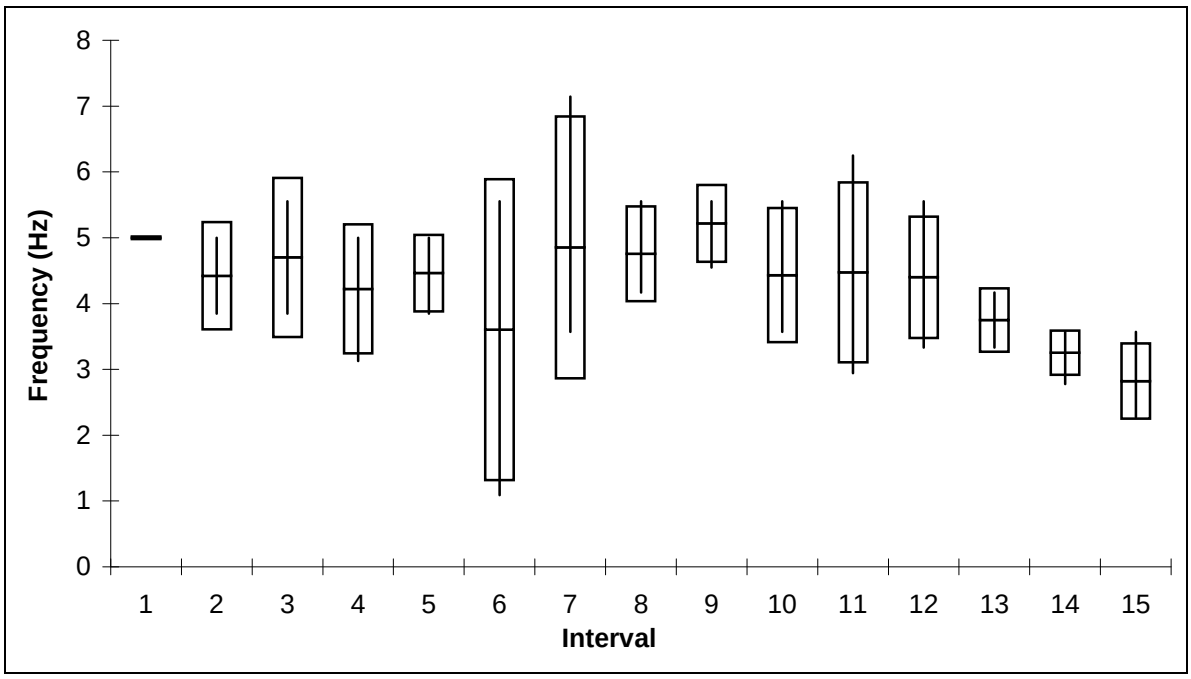


Fig. 64: Development of the frequency of the strokes of *A. longicornis* with each interval (mean, standard deviation, range). The low frequency and high variation in the sixth interval is caused by a longer gap (0.88 seconds) in one display and was not observed in the other records.

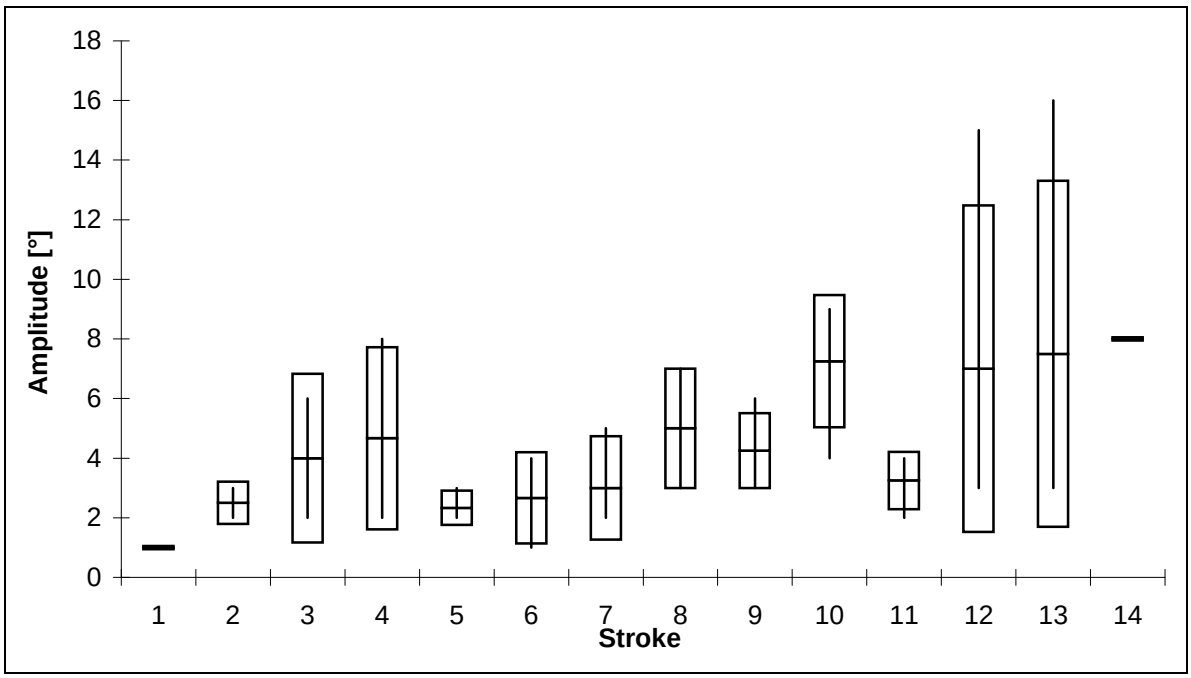


Fig. 65: Development of the amplitude of the antennal movement with each stroke in *A. longicornis* (mean, standard deviation, range). The amplitude is very low, but slightly increasing with each stroke.

P. arachniformis

Six records of *P. arachniformis* displays were obtained and analysed later. The temperature at the location was approximately 25-26°C. In one case the male was sitting behind a female nymph and apparently courting this specimen. In another case a stretching of the hind legs preceded the display without transition. Two displays seemed to be reduced. In one case such a reduced display was

followed by a more extensive one. A typical record (nr. 44) is represented in the figures 66 and 67. Due to the high amplitude of the hind leg movements, the movement is given in an angle and not in categories. The length of the display was 13.42 seconds and thus much longer than in any of the *Afrophlaeoba* records. The number of antennal strokes was 37, while the number of femoral strokes was 45. The femoral movement was characterised by typical dephased waves, in which the right femur started a forward movement, followed by the left femur. At the highest angle, the hind tibiae were spread ("knee waving"), which is not illustrated in the figures. All movements were made in rhythmical strokes, as already presented in *Afrophlaeoba*. A minor movement of the antennae started at the highest amplitude of the first femoral movement. In the central part of the display the antennal amplitude increased and decreased afterwards. The antennal movement stopped with the last major movement of the hind legs, while another smaller movement of the right hind femur occurred afterwards. The palpi were not visible in this record due to the dorsal view. In another record rhythmically synchronous movements of the palpi were visible at a frequency of 7-8 Hz. The frequency of the femoral waving movements was approximately 0.4 Hz, while the frequency of the rhythmically synchronous antennal and femoral strokes varied in this record between 2.9 Hz and 5 Hz. There was a small tendency of a decreasing frequency within the display, but the variability seemed to correlate with the amplitude of the movement. The amplitude of the femoral movement was very high and covered a total range of 133° in the left and 104° in the right hind femur. In contrast, the antennal movement covered only smaller amplitudes with a total range of only 12°. Hence the highest velocity was only 300° per second, while in the femora it was 2,250° per second.

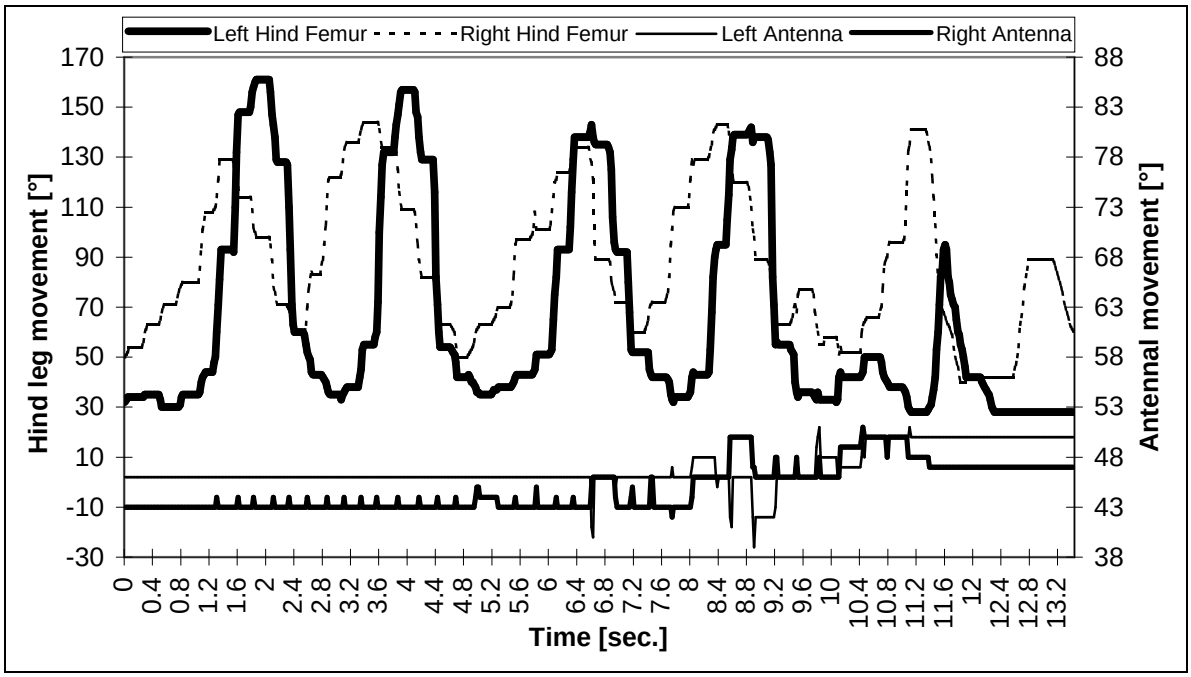


Fig. 66: Characteristic, continuous sequence of the male visual display in *P. arachniformis* (record 44, 29.01.1998, 11:58, c. 25-26°C, dorsal view, location: leaf of a grass)

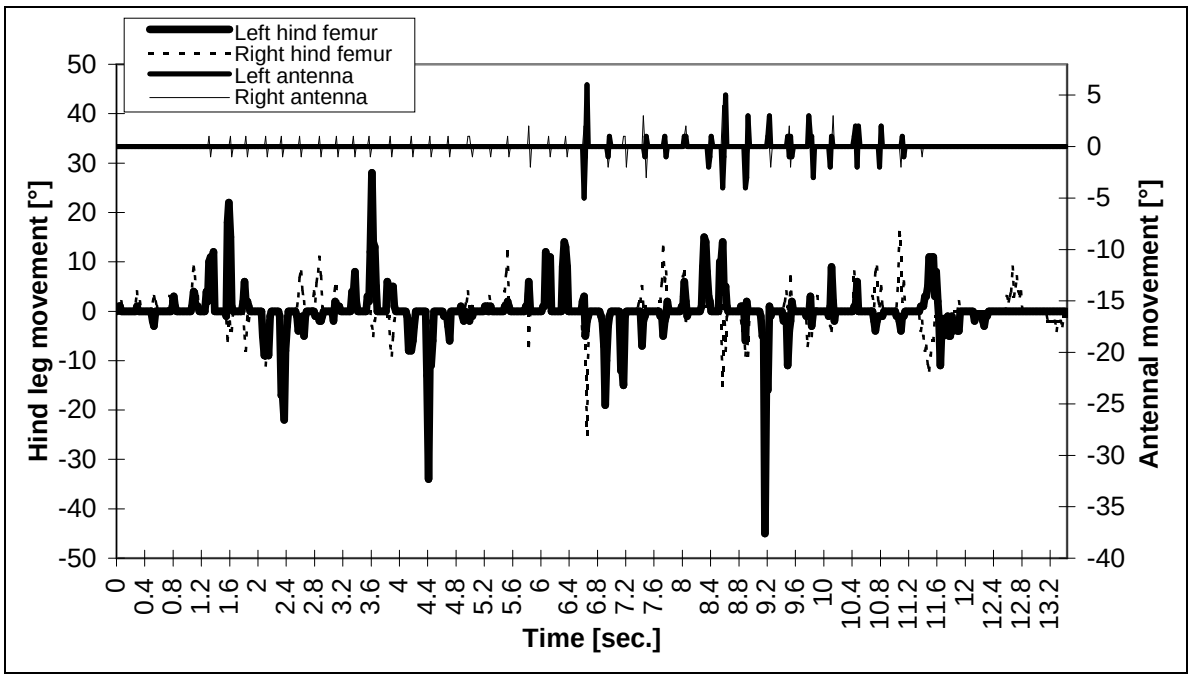


Fig. 67: Relative movements of hind legs and antennae in relation to the preceding position of in *P. arachniformis* (record 44, 29.01.1998, 11:58, c. 25-26°C, dorsal view, location: leaf of a grass)

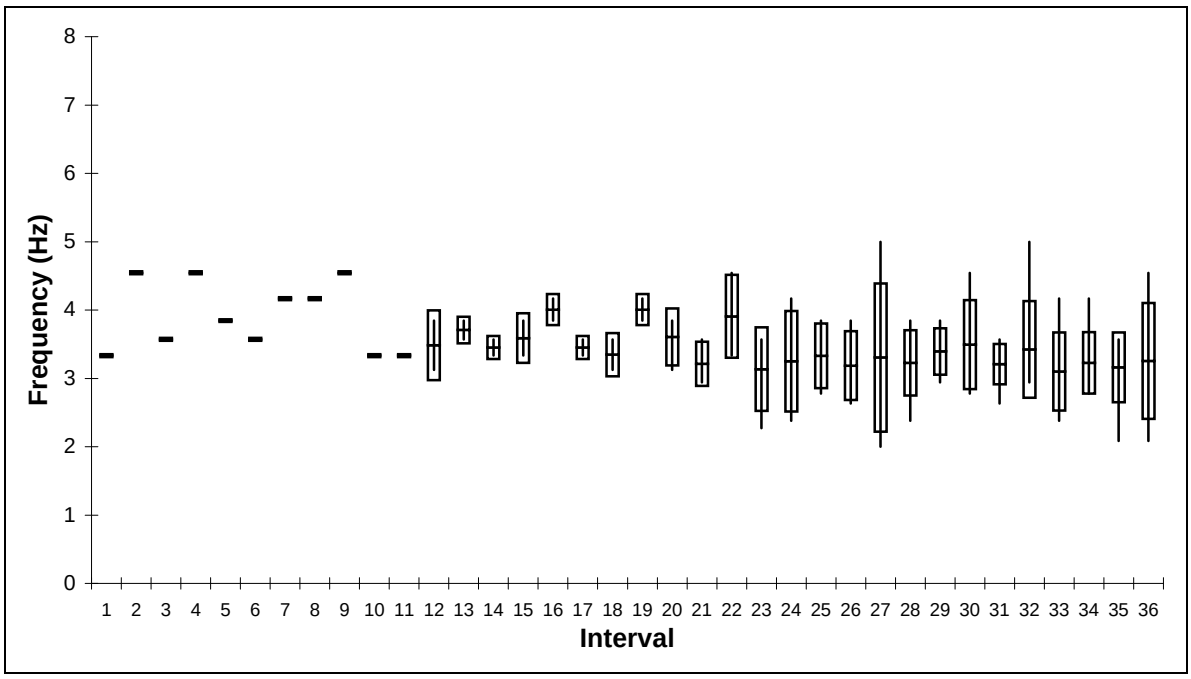


Fig. 68: Development of the frequency of the strokes of *P. arachniformis* with each interval (mean, standard deviation, range). There is only a slight tendency of a decreasing frequency, which seems to be correlated with the amplitude.

The development of the average frequency in the displays of *P. arachniformis* is presented in figure 68. The frequency ranged from 2-5 Hz and slightly decreased with each stroke. The total average of the frequency was 2.75 Hz. The number of observed femoral strokes ranged from 9 to 45. No record was incomplete, but some displays seemed to be reduced. The number of antennal strokes was usually lower (9-37) than the number of femoral strokes. Due to the high variation, no average is

presented here. The lengths of the displays ranged from 2.74 seconds to 13.42 seconds. The total amplitude of the antennal movement was usually small (average 15.7°, s.d.: 17.8°). Up to the 26th stroke it was lower than 5°. In the strokes 27-32 it increased up to 12° and thereafter it decreased to an average of 2.4° in the last stroke (figure 69). The average of the highest antennal velocity per display was only 433° per second, while the average velocity of the femoral movement was 1,534° per second with a maximum velocity of 2,250° per second. In summary, the displays of *P. arachniformis* were characterized by dephased femoral movements of low frequency and high amplitude, combined with knee-waving. The rhythmically synchronous antennal movements of 2-5 Hz had only small amplitudes. In one specimen synchronous high-frequency movements of the palpi were observed. Thus the visual display behaviour of *P. arachniformis* differed substantially from *Afrophlaeoba*.

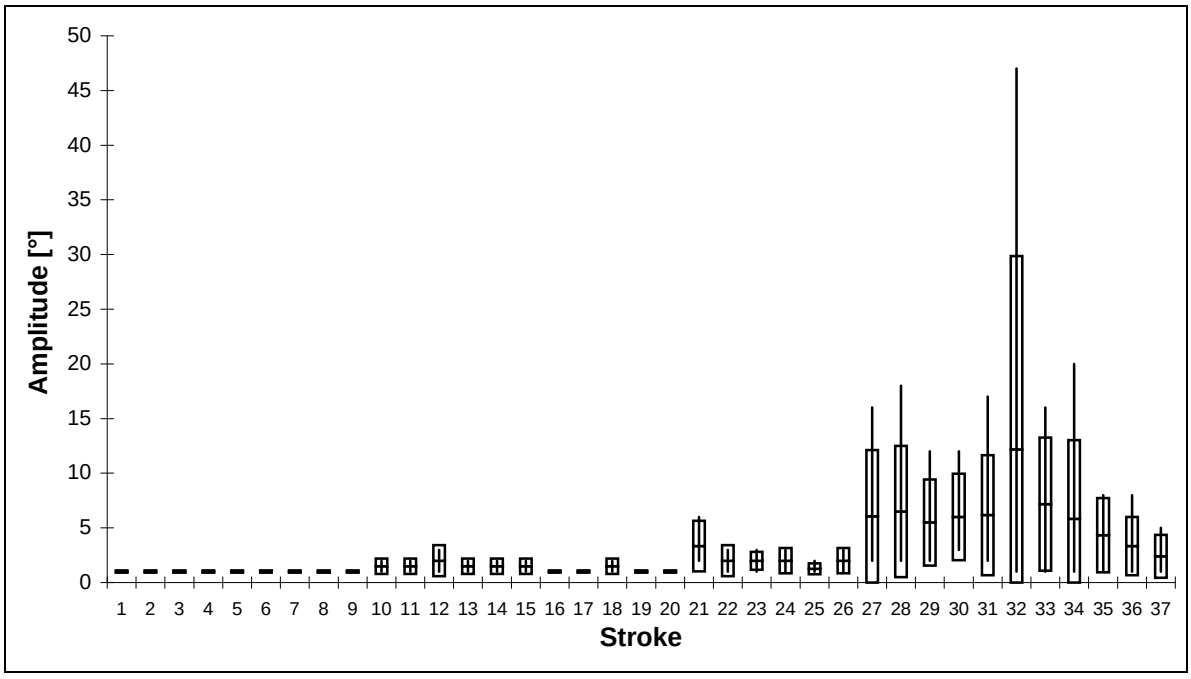


Fig. 69: Development of the amplitude of the antennal movement with each stroke in *P. arachniformis* (mean, standard deviation, range). The amplitude is very low, but slightly increasing in the first two thirds of the display and decreasing afterwards.

6.3.2 Comparison of the Species

Several differences between the visual displays are already obvious from the preceding chapter. In this chapter the main descriptors will be compared to emphasize differences and similarities between the species. From the qualitative point of view *P. arachniformis* differs substantially from the *Afrophlaeoba* species by using high-amplitude, low-frequency movements of the hind femora combined with knee-waving. Although sometimes high-amplitude femoral movements can also be observed in *Afrophlaeoba*, they apparently occur mainly in the stretching and cleaning behaviour and don not seem to be related to communication. *P. arachniformis* is also the only species, in which rapid movements of the maxillary and labial palps were observed. The rhythmical structure of femoral and

antennal movements is similar to *Afrophlaeoba* with short strokes and characteristic intervals in between. Figure 70 illustrates the average duration of a display in the five species. While in *A. euthynota* nearly all displays were shorter than two seconds, displays of *A. usambarica* lasted 2.5 to 4 s (t-test, df: 31, $P < 0.05$). In *A. longicornis* the display lasted 3.3 to 4.3 s, but it only differed significantly from *A. euthynota* (t-test, df: 23, $P < 0.05$). The displays of *A. nguru* had durations from 4.1 to 5.5 s, differing from all *Afrophlaeoba* species except *A. longicornis* (t-test, df: 4, n.s.). The longest displays were observed in *P. arachniformis*, with an average of 6.7 seconds, covering a range from 2.74 to 13.42 seconds.

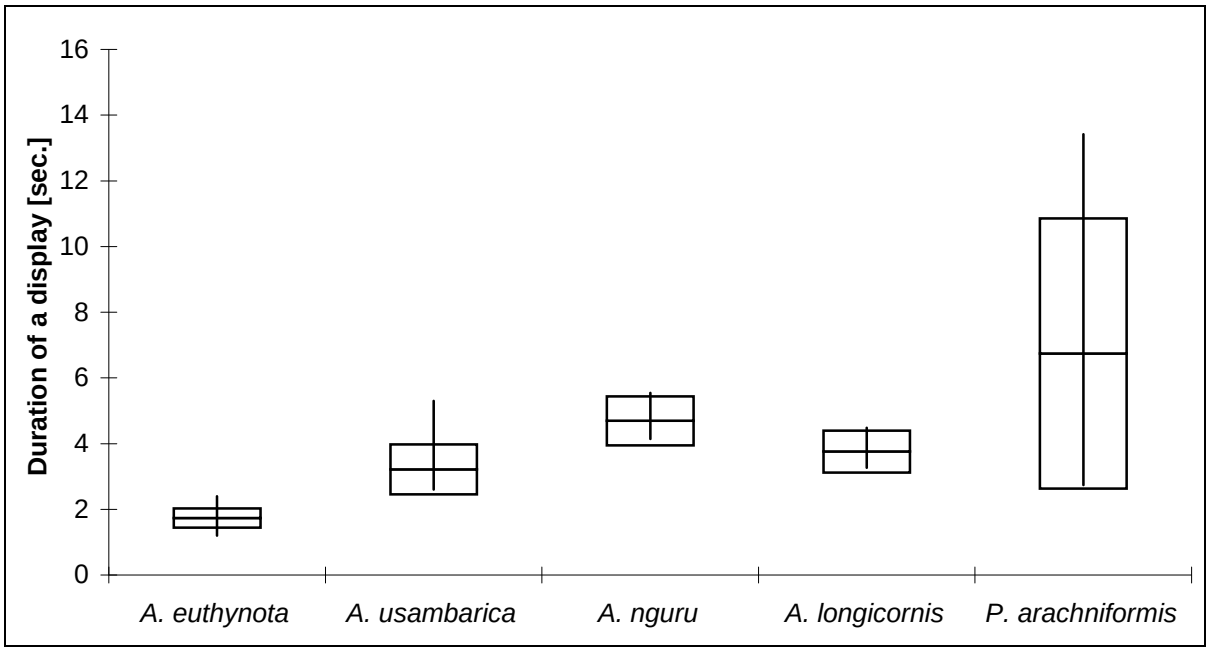


Fig. 70: Duration of the visual displays in the five species examined (mean, standard deviation, range). While the display in *A. euthynota* usually lasts shorter than two seconds, it lasts four to 5.5 seconds in *A. nguru*. *P. arachniformis* has the longest and most complex displays.

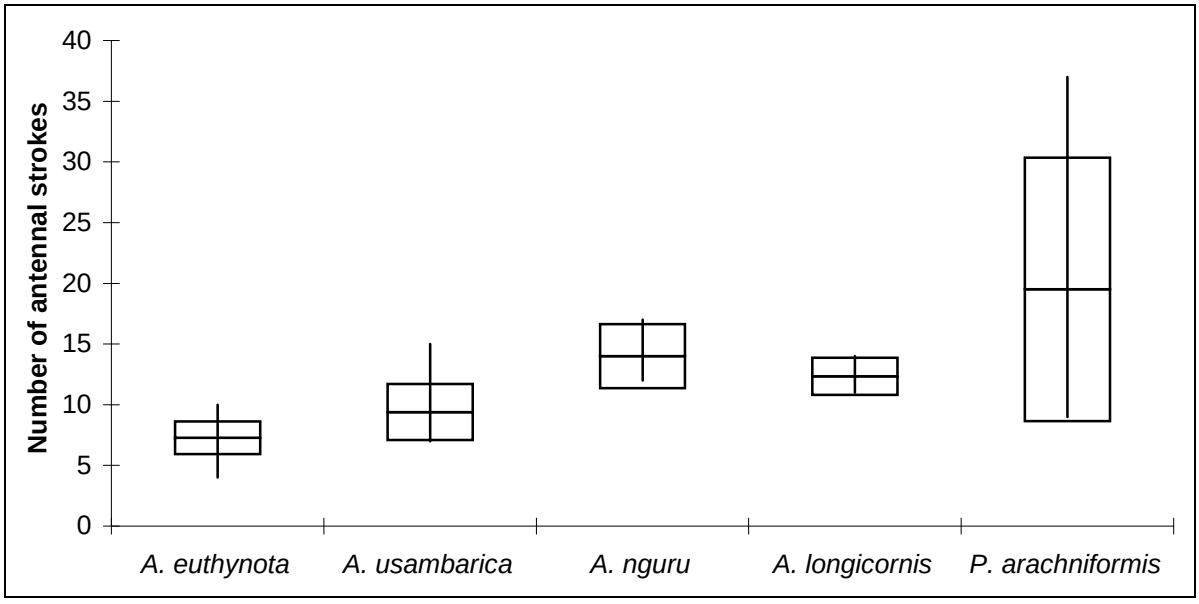


Fig. 71: Number of antennal strokes per display for the five species (mean, standard deviation, range). The number of strokes is highly correlated with the length of the display (see figure 70).

A similar pattern is visible in figure 71, in which the average number of antennal strokes for each species is illustrated. All species differ significantly in this regard (t-test, df: 4-29, $P < 0.05$), with the exception of *A. nguru* and *P. arachniformis*. A high correlation of the number of strokes with the duration of a display can be found (figure 72). Since the number of femoral strokes is usually nearly the same as the number of antennal strokes, this character is not presented here.

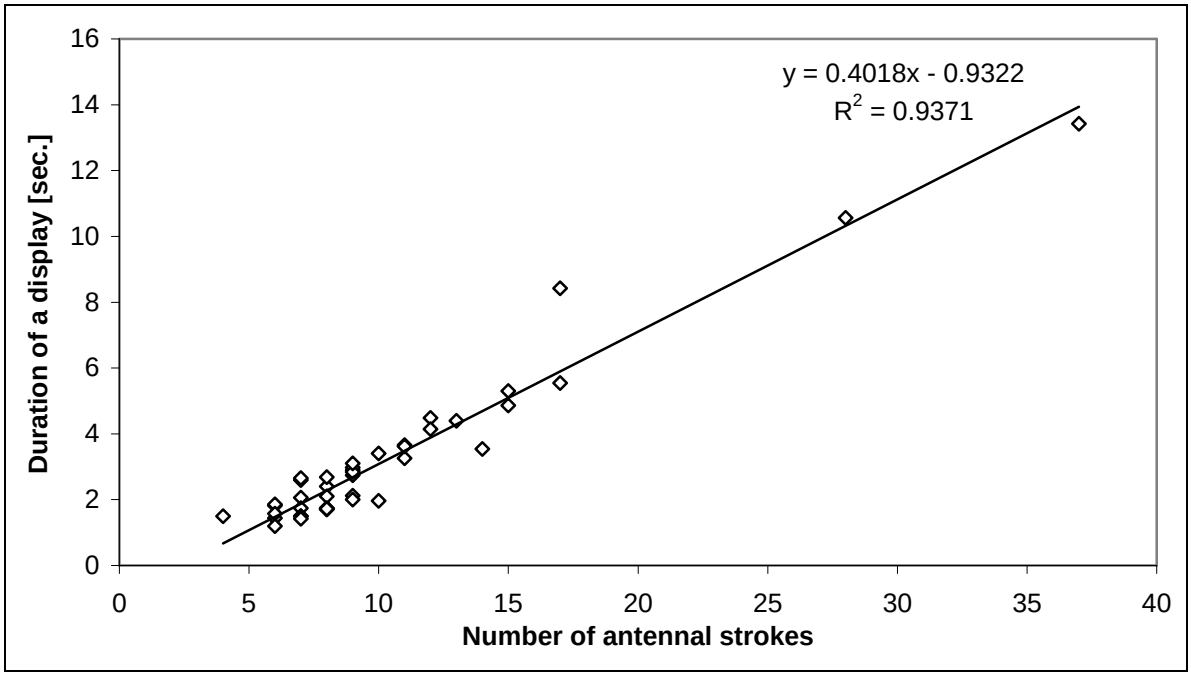


Fig. 72: Correlation between the duration of a display and the number of antennal strokes, including all records.

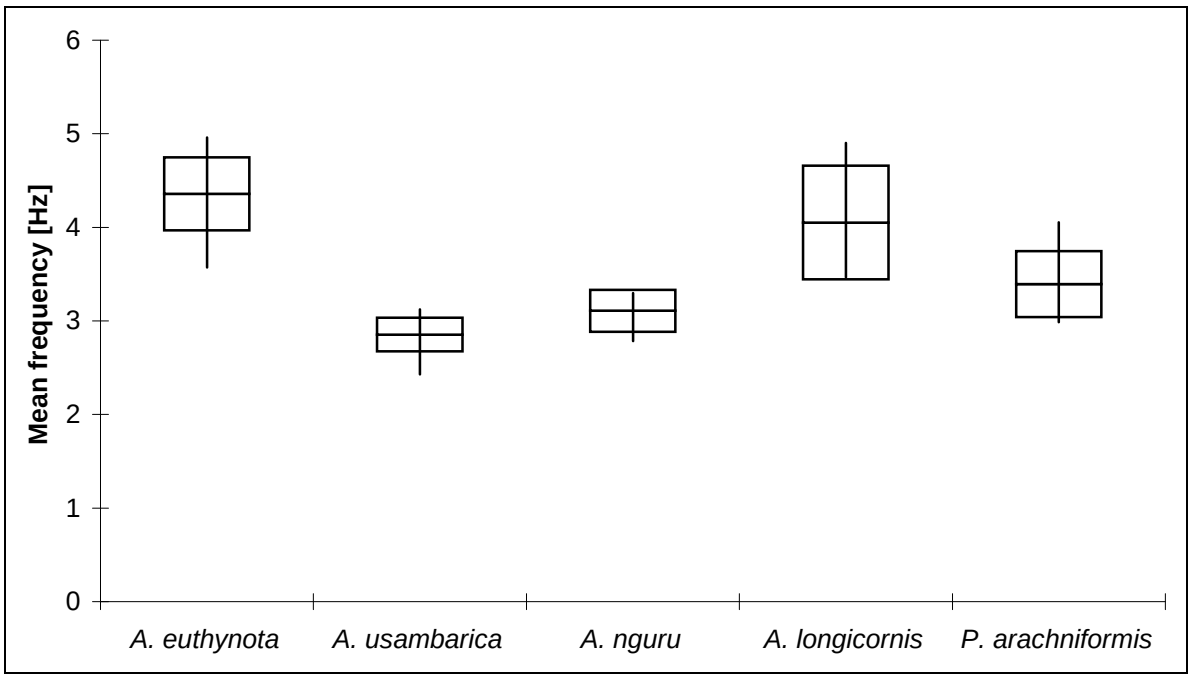


Fig. 73: Mean frequency of the displays of the five species (mean, standard deviation, range). The highest frequency can be observed in *A. euthynota* and *A. longicornis*, while *A. usambarica* and *A. nguru* have lower frequencies.

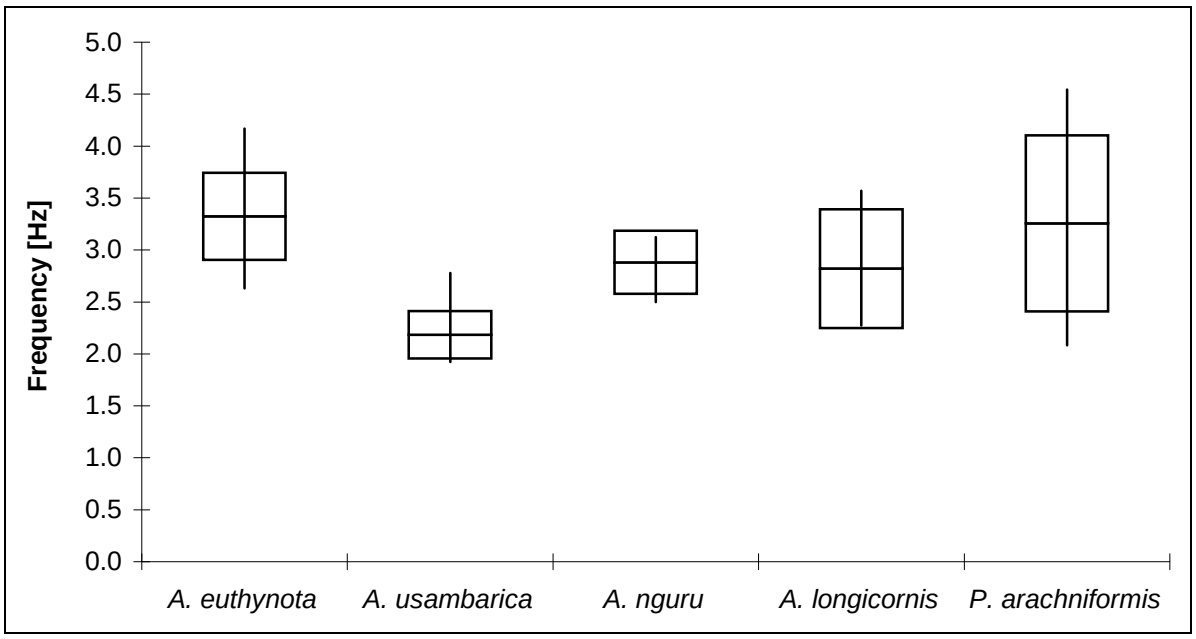


Fig. 74: Mean frequency of the last two strokes per display in the different species (mean, standard deviation, range). The highest frequency can be observed in *A. euthynota*; *A. usambarica* has the lowest and *A. longicornis* and *A. nguru* have medium frequencies.

The mean frequency of the complete display is illustrated in figure 73 for each species. In *A. euthynota* the mean frequency is 4-5 Hz, in *A. usambarica* 2.5-3 Hz, in *A. nguru* 2.8-3.3 Hz and in *A. longicornis* 3.5-4.5 Hz. The display of the outgroup, *P. arachniformis*, has a mean frequency of 3-4 Hz. Nearly all species differ significantly in the average frequency (t-test, df: 6-31, $P < 0.05$). No differences occur between *A. euthynota* and *A. longicornis*, and between *A. nguru* and *P. arachniformis*.

In all *Afrophlaeoba* species there is a general trend of decreasing frequencies in the last five intervals (in *A. euthynota* from 4.9 Hz to 3.3 Hz, in *A. usambarica* from 3.3 Hz to 2.2 Hz, in *A. nguru* from 3.7 Hz to 2.9 Hz and in *A. longicornis* from 4.5 Hz to 2.8 Hz). While *A. nguru* and *A. usambarica* show increasing frequencies in the first two thirds of the display, this was not observed in *A. euthynota* and *A. longicornis*. This pattern causes differences to the above-mentioned average frequencies, if only the frequency of the last two strokes is compared (figure 74). In *A. euthynota* the average frequency of this last interval is 3.3 Hz, in *A. usambarica* 2.2 Hz, in *A. nguru* 2.9 Hz and in *A. longicornis* 2.8 Hz. The outgroup, *P. arachniformis*, has an average frequency of 3.3 Hz in the last interval. No significant differences were found in this regard between *A. longicornis*, *A. nguru* and *P. arachniformis*, and between *A. euthynota* and *P. arachniformis* (t-test, df: 6-31, n.s.), while other pairwise comparisons resulted in significant differences (t-test, df: 13-35, $P < 0.05$).

The total amplitude of the antennal movement is an important descriptor of the displays (figure 75). *A. nguru* performs antennal movements with extremely high amplitudes ($\bar{x}$: 40.7°), while in *A. usambarica* (17.6°) and *A. euthynota* (15.6°) the amplitude is medium. The latter two species do not differ in this regard (t-test, df: 32, n.s.), while all other species of *Afrophlaeoba* differ significantly

(t-test, df: 6-28, $P < 0.05$). *A. longicornis* and *P. arachniformis* differ from the other species by performing only minor antennal movements ($\bar{x}$: 4.7°, rep. $\bar{x}$: 5.6°), while no significant differences were found between those two species in this character (t-test, df: 8, n.s.). The same specific pattern can be observed, if the maximum amplitude, the velocity, the last amplitude or the total amplitude are analysed. The highest antennal velocity (5,000° per second) occurs in the species with the highest antennal amplitude (*A. nguru*). Within a display there is a general trend of increasing antennal amplitudes, which is extremely high in *A. nguru*, medium in *A. euthynota* and *A. usambarica* and low in *A. longicornis*. However, even in the last species this trend is still visible (figure 65).

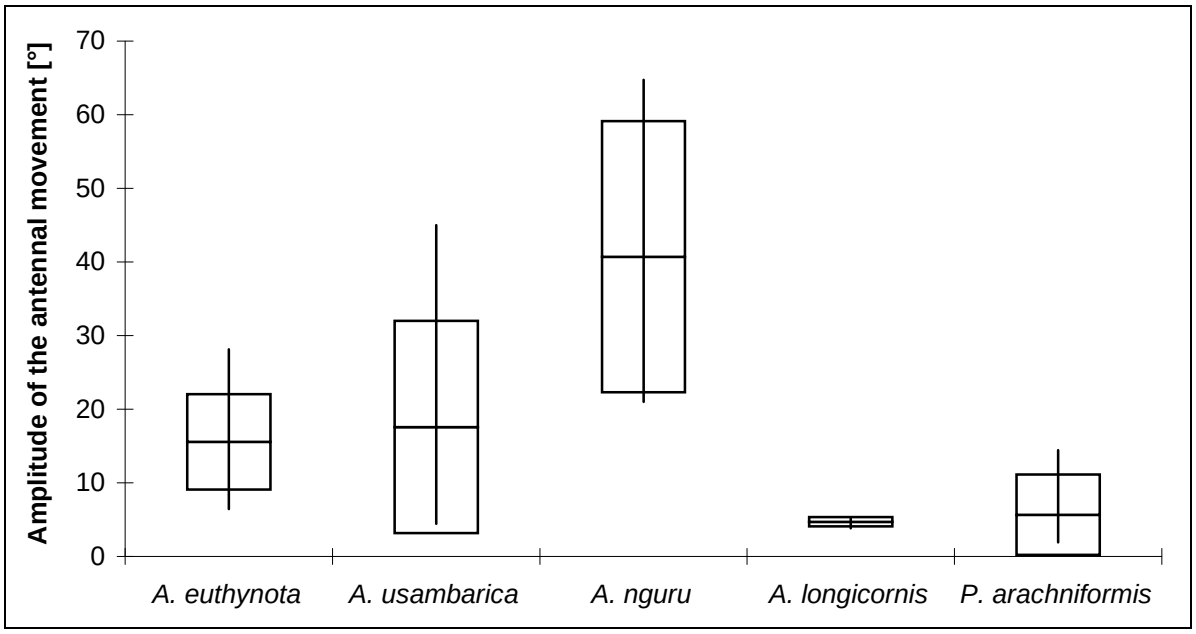


Fig. 75: Mean amplitude of the antennal movement in the displays of the five species (mean, standard deviation, range). *A. nguru* has the highest amplitudes, while in *A. usambarica* and *A. euthynota* the amplitude is medium. *P. arachniformis* and *A. longicornis* have small amplitudes.

6.3.3 Canonical Discriminant Analysis

6.3.3.1 Choice of the Characters

Due to the low number of records in *A. nguru* and *A. longicornis* it was not possible to include more than three variables in the discriminant analysis. It was necessary to exclude those characters which had missing values in these two species (e. g. duration, number of strokes, which unfortunately are quite characteristic for those two species) to allow the inclusion of all four records of both species. Since *P. arachniformis* is characterized mainly by the duration of the display and femoral movements, which have not been analysed for *Afrophlaeoba*, this species was excluded from the canonical discriminant analysis. For the choice of the three most important variables for the discriminant analysis, a stepwise analysis was performed first, including the following nine characters: The average amplitude, the total amplitude, the average frequency, the amplitudes of the last three strokes

and the frequencies of the last three intervals. The univariate F-statistics revealed significant differences for all included characters of the analysis ($P < 0.005$). According to the stepwise analysis, the three most important characters were average frequency, total amplitude and the penultimate frequency (table 44). These three characters have been chosen as variables for a second discriminant analysis. If *P. arachniformis* was included, the penultimate frequency was replaced by the last frequency.

Tab. 44: Included characters in the stepwise discriminant analysis in order to the steps in which they have been included. The value of Wilks' Lambda illustrates the contribution of each value to the analysis – the value is decreasing with each character added.

Step	Character	Wilks' Lambda	estimated F	df1	df2	Significance
1	Average frequency	0.139	74.3	3	36.0	0.0001
2	Total amplitude	0.071	32.2	6	70.0	0.0001
3	Penultimate frequency	0.047	23.0	9	82.9	0.0001
4	Last frequency	0.036	18.5	12	87.6	0.0001

6.3.3.2 Discriminating Power of the Analysis

The high discriminating power of the analysis is illustrated by a very low Wilks' Lambda ($\Lambda = 0.049$; $\chi^2 = 110.2$; df = 9; $P < 0.0001$). For CAN2 to CAN3 it is 0.471 ($\chi^2 = 27.5$; df = 4; $P < 0.0001$), but for CAN3 it is not significant with 0.998 ($\chi^2 = 0.088$; df = 1; $P > 0.77$). Hence, the third canonical function does not contribute anything to the discrimination. The classification statistics assigned 92.7% of the specimens to the correct species. The three wrong assignments included one *A. euthynota* grouping with *A. longicornis*, one *A. nguru* with *A. usambarica* and one *A. usambarica* with *A. nguru*. It should be kept in mind that only three variables were included in the analysis and that these did not include the number of strokes or the duration of the display, which would probably further discriminate between the species.

6.3.3.3 Pairwise Mahalanobis Distances and Discriminant Structure

The multivariate analysis revealed significant pairwise differences between all species (F statistics; $P < 0.0002$). The lowest Mahalanobis distance was found between *A. longicornis* and *A. euthynota* and the highest distance between *A. usambarica* and *A. euthynota* (table 45). This was mainly caused by the high discriminatory power of the frequency in the first function. The two species with higher frequencies had lower distances and were plotted together in the discriminatory topology (figure 76).

Tab. 45: Generalized Mahalanobis distances (D^2) between the species

Species	*A. longicornis*	*A. nguru*	*A. usambarica*
A. euthynota	8.03	31.30	35.43
A. longicornis		29.57	18.32
A. nguru			10.21

The high eigen value of canonical function 1 (CAN1, table 46) demonstrates that this function plays a very important role in discrimination. 88.5% of the variation is explained by this first function, while the second function explains the remaining 11.5% of the variation. All species form distinct groups in the discriminatory topology (figure 76) with the exception of an outlier of *A. nguru*, which is plotted in *A. usambarica*. This outlier (record 49) is caused by a lower total amplitude (67°) and lower frequencies (penultimate stroke: 2.5 Hz, average: 2.8 Hz). Only the first two variates show significant differences between the five species (F statistics; $P < 0.0001$). The group centroids of each species for the four canonical axes are given in table 47, illustrating the good discrimination of *A. usambarica* and *A. euthynota* on the first variate and *A. nguru* and *A. longicornis* on the second variate.

Tab. 46: Eigen values of the four axes; note the high eigen value of CAN1.

CAN	Eigen value	Difference	Proportion	Cumulative
1	8.64	5.52	0.885	0.885
2	1.12	1.12	0.115	1.000
3	0.002	-	0.000	1.000

Tab. 47: Group centroids for the five species on the four canonical axes; note the high distance between *P. arachniformis* and the *Afrophlaeoba* species on the CAN1.

Species	CAN1	CAN2
A. euthynota	-2.22	0.09
A. longicornis	-0.40	-1.62
A. nguru	2.67	2.60
A. usambarica	4.20	-0.61

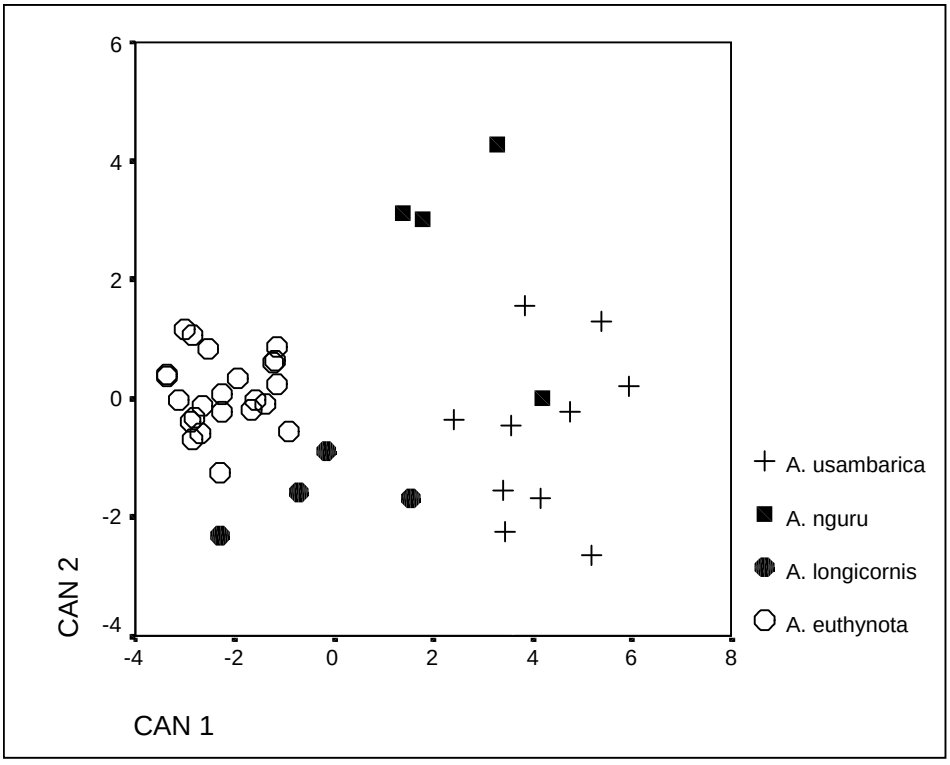


Fig. 76: Plot of canonical variates 1 and 2 of the discriminatory topology, illustrating the good discrimination of the four groups:

6.3.3.4 Contribution of Single Characters

The contribution of single characters to the complete analysis can be extracted from the order, in which they were included in the stepwise discriminant analysis (table 44). The strongest character is the average frequency of a display, which also contributes the most to the first canonical function. In the second step the total amplitude is included, which is the main descriptor of the second function (table 48). Thus, the discriminatory topology can be simplified in a bivariate plot of those two characters against each other (figure 77). This plot already shows the good discrimination by the use of these two variables. *A. usambarica* is characterized by low frequencies and medium amplitudes, *A. nguru* by high amplitudes and medium frequencies, *A. longicornis* by extremely low amplitudes and *A. euthynota* by high frequencies with medium amplitudes.

Tab. 48: Standardized canonical discriminant coefficients for the canonical axes CAN1 and CAN2. The third function has been omitted due to its low significance.

Character	CAN1	CAN2
Average frequency	0.711	0.294
Total amplitude	0.173	0.994
Penultimate frequency	0.555	-0.486

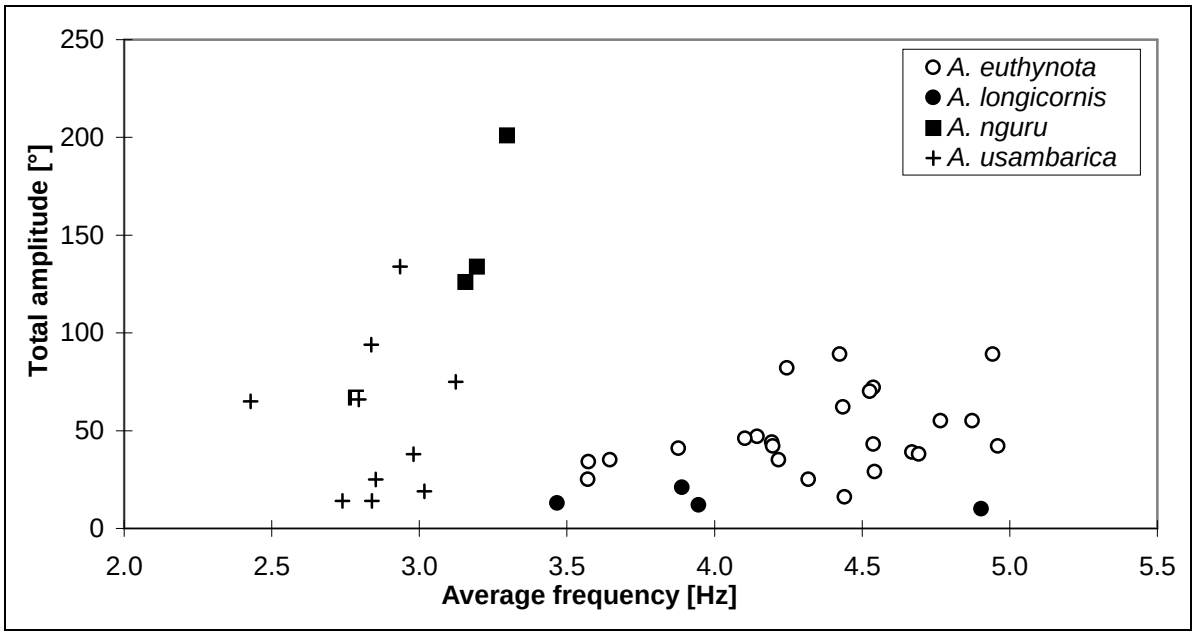


Fig. 77: Bivariate plot of the two most important variables of the stepwise discriminant analysis for the *Afrophlaeoba* species.

The two most important variables of CAN1 are plotted against each other in figure 78. There is a strong correlation between those two characters (R^2 = 0.814, $P < 0.0001$), since the penultimate frequency of the movements influences the average frequency directly. However, it is visible that in *A. longicornis* the penultimate frequency is quite low in comparison to the high average, while in *A. euthynota* the penultimate frequency is higher. The lowest frequencies are found in *A. usambarica*.

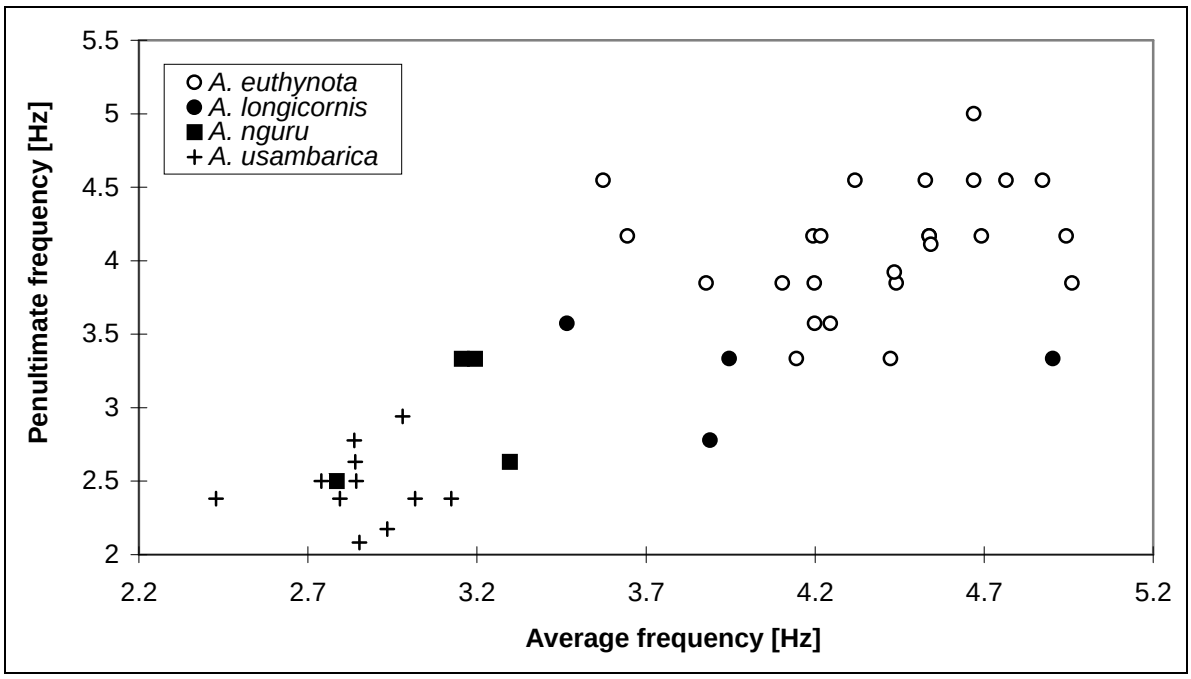


Fig. 78: Plot of the two most important variables of CAN1 against each other. The penultimate frequency is correlated with the average frequency. In both cases *A. usambarica* has the lowest and *A. euthynota* has the highest frequencies. In *A. longicornis* a comparative high variation can be found in the average, but the penultimate frequency is lower than in *A. euthynota*.

6.4 Discussion

6.4.1 Biological Function of the Displays

There is little doubt that the described male displays are part of the communicative behaviour, since in one case a female of *A. euthynota* followed a displaying male and in two cases "vibratory dialogues" were observed between two males. However, the biological function of the visual displays is difficult to unravel. In Gomphocerinae many species perform different song types with different functions. The calling song is produced spontaneously by isolated males to elicit a response from conspecific females or to attract them. If two conspecific male grasshoppers are sufficiently close to each other they often produce a modified song, which is called rivalry song. These rivalry songs are often faster or abbreviated versions of the calling song, but they can also be rather different in character. The courtship song is produced when the male is next to a female. This song is often a modified calling song, which may include some new elements (Ragge & Reynolds 1998). If the "silent majority" of grasshoppers have the same differentiation of visual displays, these display types may superimpose the interspecific differences of *Afrophlaeoba*. In three of the four displays of *A. longicornis* a second male was present close to the displaying one (in visual contact but not located on the same substrate). The last display was recorded after one of the males jumped away. Although this last display showed the same spatial and temporal pattern, it cannot be excluded that these four displays were "rivalry displays" and that the low antennal amplitude of *A. longicornis* is an outcome of this type of display. On the other hand, in *A. usambarica* eight of the eleven displays were recorded in a similar rivalry

situation, but they did not differ from the displays of isolated males. The interspecific differences to *A. longicornis* were higher, even if only the males in a rivalry situation were included. This suggests that the rivalry display in *Afrophlaeoba* does not differ from the normal display of isolated males.
Additionally to the rivalry situation, courtship might be the main function of the displays. Since the observed types of movements are quite common in courtship behaviour of grasshoppers (Riede 1987), there is little reason to suggest something else for *Afrophlaeoba*. The pattern of the hind leg movements closely resembles the pattern exhibited by singing Gomphocerinae. According to Ragge & Reynolds (1998) synchronous gaps in the downstrokes of the hind legs occur often in singing Gomphocerinae, which is quite similar to the synchronous intervals in *Afrophlaeoba*. Additionally many Gomphocerinae species perform a crescendo during the course of an echeme (Ragge & Reynolds 1998), similar to the increasing antennal amplitude in *Afrophlaeoba*. At least in one case of *A. euthynota*, a female was pursuing the displaying male. Amazingly the male kept jumping away, when the female touched it with its antennae and started to display at another location. Thus the courtship function of the visual display still needs confirmation of a successful mating observation. Copulating specimens were found in two cases in the field during the ecological records (chapter 7) (one couple of *A. usambarica* and one of *A. nguru*), but the preceding courtship behaviour was not observed. As discussed for the rivalry situation, it must be discussed, whether the "courtship situation" of *A. euthynota* influences the structure of the display. It is remarkable that the display in a courtship situation was characterized by the longest duration (2.4 seconds, eleven femoral strokes). However, the other main descriptors (amplitude, frequency) were in the range of normal displays. Since in most cases the male *A. euthynota* was displaying isolated, the courtship situation should not overlay the interspecific differences observed.

6.4.2 Perception of the Displays

The observation of two "vibratory dialogues" raises the question of the perception of the displays. Jago (1983) assumed a vibratory transmission of the signal through the substrate. Although vibratory transmissions seem to be common in Orthoptera (Riede 1987), this is not likely to be the sole factor in the observed cases, since in both rivalry situations (*A. usambarica* and *A. longicornis*) the two males involved were situated on different substrates. A second possibility would be the production of ultrasonic sound, which is not audible to man. However, since the hind legs are spread apart slightly from the body during the display, sound production is unlikely to occur. Whether pheromones play a role in communication cannot be answered. According to the current knowledge, a visual signal seems to be the best explanation. The homogenous coloration of the male specimens in *Afrophlaeoba* and *Parodontomelus* confirms this hypothesis. The antennae are probably flattened for the purpose of visual signalling and the hind femora are always orange-brownish with contrasting black knees. The females are much more variable in colour, but always brownish camouflaged.

6.4.3 Environmental Influence

The high temperatures during the displays of *A. euthynota* are of high importance, as they might influence the frequency and duration of the displays (von Helversen 1972). Indeed the displays of *A. euthynota* had the highest frequency and shortest duration. On the other hand substantial interspecific differences in frequency and duration were also found between the other *Afrophlaeoba* species, although the temperature was quite similar in these cases. The displays of *A. nguru* were recorded from a single male with only one hind leg. The loss of hind legs usually occurs during moulting or predation (Hochkirch et al. 1999) and it is known from Gomphocerinae grasshoppers that a missing leg might cause short gaps (4 ms) in the songs (von Helversen & von Helversen 1994). These gaps are usually caused by the reversal of the hind leg movement, which is otherwise overlaid by the slightly out-of-phase movement of the other leg (Greenfield 1997). Gaps between the strokes are a typical element of the display in *Afrophlaeoba* and the hind legs move in perfect synchronicity. Moreover, the gaps are quite long, lasting between 0.2 and 0.6 s, which would certainly obscure a gap of 4 ms. However, in one display of *A. nguru*, a larger gap (approximately twice in length of the usual interval) occurred. Since this was not the case in the other two complete records of *A. nguru*, it must be assumed that the gap is not a result of the missing hind leg. Moreover, larger gaps were also present in the displays of *A. longicornis*. Since the hind femora movements are synchronous in all species of *Afrophlaeoba*, this was also suggested to be true for *A. nguru*. Of course, the extremely high antennal amplitudes in *A. nguru* might be explained as compensation for the missing hind leg – but this remains speculation.

6.4.4 Interspecific Differentiation

An obvious problem of the existing records is their insufficient quantity for assessing the extent of intraspecific variation. Although the observed interspecific differences seem to be striking and much higher than any morphological differences, it remains uncertain whether these differences play an important role for mate recognition and thus may function as prezygotic barriers. Since interbreeding experiments are not available, it remains speculative, whether one species would react on the display of another one.

Species distinction in Orthoptera may be mediated by evaluation of a single (temporal) property of the males' signal or by several factors (Greenfield 1997). Despite of the differences in the amplitude, the duration and the frequencies of the displays, there are also factors, which are quite similar among the *Afrophlaeoba* species. These include the increasing distances between the last five intervals (decreasing frequency) and the increasing amplitude within the display. If the female response to the displays is mainly based on these two relative factors, the observed differences in duration, frequencies or amplitudes might not be substantial enough for specific mate recognition. According to Ragge & Reynolds (1998) the most important component for mate recognition in European

Orthoptera are the rhythmic patterns of the songs and the duration of the gaps between. The enormous amplitudes of the antennal movement in *Afrophlaeoba* suggest that these movements are important visual signals in the species. It is striking that the two morphologically and genetically most closely related species, *A. longicornis* and *A. nguru*, are quite distinctive in the display pattern. Although both perform a similar number of antennal strokes, the amplitudes are much higher in *A. nguru* and the frequency is lower, resulting in a longer duration of the display. In the multivariate analysis the two species have quite high Mahalanobis distances, illustrating the differences between the two species.

6.4.5 Intergeneric Differentiation

The differences between *Afrophlaeoba* and *Parodontomelus* are much more conspicuous than the interspecific differences in *Afrophlaeoba*. *P. arachniformis* performs dephased leg movements with high amplitudes combined with knee waving, and high frequency vibrations of the white palps. A small time lag between the movements of the left and right legs is also quite common in singing Gomphocerinae (Ragge & Reynolds 1998). The contrasting white coloration of the palps and black hind knees suggests that visual signals play a major role in the communicative behaviour of *P. arachniformis*. Many grasshopper species which perform visual signals have conspicuously coloured antennae, palps or femora (Riede 1987). In contrast to the small antennal vibrations of *P. arachniformis*, *Afrophlaeoba* has usually more pronounced antennal movements with high amplitudes and velocities. The hind femora produce only small vibratory bursts, which are usually referred to as “femur shaking” (Otte 1970).

7 Habitat Preferences

7.1 Introduction

Our general knowledge on the habitat requirements of grasshoppers usually includes two factors: microclimate and vegetation. Concerning microclimate, several affects on eggs, nymphs and adults are known. The water demands of the eggs are usually correlated with the microclimate of the habitat of a species (Ingrisch 1983, 1988), while temperature affects mainly the developmental speed (Ingrisch 1979, 1980). Grasshoppers are often divided into geophilous and phytophilous species, since they are usually associated to typical vegetation structures (Uvarov 1977). The significance of vegetation structure for grasshoppers is partly explained by its influence on the microclimate. Many species, however, have also behavioural adaptations to the vegetation structure, which is often expressed in their orientation (Sänger 1977). Frequently the sexes exhibit different utilization of microhabitats, due to sex-specific differences in energy budgets and behaviour (Hochkirch 1999a).

The ecological information of the East African forest insects, or tropical forest insects in general, is extremely low. Lawton (1993) wrote: "Intriguingly, I have never seen anybody discuss what we actually know about the 1.7 million [species] that do have names. Overwhelmingly the answer will be nothing, except where they were collected and what they look like." For the Eastern Arc grasshoppers, only some rough data on the habitats were available (Phipps 1966), until more detailed studies followed in the 1990s (Hochkirch 1995, 1996a, 1998). The vicariant distribution patterns of grasshoppers of the Eastern Arc are thought to be also illustrated by their ecology. Congeneric allopatric species seem to have similar habitat requirements in different distribution areas (Vesey-Fitzgerald 1964, Hochkirch 1998), while sympatric species of the East Usambara Mts. can be clearly distinguished ecologically (Hochkirch 1995, 1996a). This suggests that equilibrium models are better explanations for the high within-habitat-diversities than non-equilibrium models (Linsenmair 1990).

The main motivation for the study of the habitat requirements of *Afrophlaeoba* was the reconstruction of the ancestral ecology, to gain information about the necessary habitat connections for gene flow. Additionally, the outgroup species *P. arachniformis* was studied to emphasize the intergeneric differences of the sympatric species *A. usambarica* and *P. arachniformis*. These data will also add something to the general ecological information of the Eastern Arc grasshopper fauna, which is certainly necessary for conservation purposes (Hamilton 1989b).

7.2 Methods

The practical fieldwork was performed from 06.12.1997 to 13.02.1998. The ecological data was recorded during a short period to avoid ecological differences caused by seasonality. The data were obtained on sunny days from 8:00 to 18:00. The research periods and localities for the single species are presented in table 49. Climatic data are directly comparable only between *Parodontomelus arachniformis* and *Afrophlaeoba usambarica*, as they were recorded at the same location during the same period.

Tab. 49: Date, localities and altitude, from which the analysed videos were recorded.

Species	Dates	Locality	Altitude
A. euthynota	06.12. to 13.12.1997	Uluguru Mts. near Morogoro	800 m to 1400 m
A. usambarica	02.01. to 15.01.1998	East Usambara Mts. near Amani	600 m to 1100 m
P. arachniformis	06.01. to 18.01.1998	East Usambara Mts. near Amani	600 m to 850 m
A. nguru	30.01. to 02.02.1998	Nguru Mts. near Mhonda	500 m to 900 m
A. longicornis	08.02. to 13.02.1998	Rubeho Mts. near Kilosa	500 m to 600 m

A total of 1,500 ecological records were obtained, including 300 records for each species. The following data were recorded:

- Date, time, study site
- Sex: female, male, nymph
- Vegetation cover: In a circle of 50 cm diameter surrounding the grasshopper the vegetation density was estimated (plant cover in percentage). In addition, the coverage of grasses, forbs, mosses, leaf litter and open soil was noted. Mosses were not taken into consideration for the analysis, since they hardly ever occurred.
- Vegetation height: recorded by measurement of the highest plant in a circle of 50 cm diameter surrounding the specimen with a folding rule. It was classified in 10 cm-classes (0 cm, 1-10 cm, 11-20 cm, 21-30 cm, etc.). Vegetation higher than 200 cm was summarized to a class >200 cm.
- Height of situation: measured with a folding rule from the ground and noted in classes of 10 cm as with vegetation height.
- Orientation: horizontal, vertical
- Item of location: stem, leaf
- Location: plants, litter, soil. Soil was not considered for the analysis, since it hardly ever occurred.
- Plant: The plant, where the specimen sat on was recorded: trees, bushes, climbers or lianas, forbs, grasses, ferns, dead leaves and mosses. Only grasses and forbs occurred often enough for a statistic analysis.
- Temperature of location occupied by the grasshopper: The temperature was measured by a digital thermometer at the location of the specimen. It was recorded to two decimal points, but rounded up or down to 1°C for the analysis.
- Shade: The following categories were divided: sunny, semi-shaded, shaded, clouded, and lightly clouded.
- Radiation: Radiation at the location was recorded with a luxmeter.
- Weather in the following categories: sunny, sunny to clouded, clouded with sunny periods, clouded, clouded with rain, rainy, night
- Behaviour: Special behaviour of specimens was noted. When feeding was observed, the plant type was recorded as in plant.

Crosstabulation tests (χ^2) were used for the statistical analysis of distributions within nominal categories (Precht 1979). Since the metric data were not normally distributed, Mann-Whitney U-tests and Kruskal-Wallis H-tests adjusted for large sample size were used to test data on the habitat (Sachs 1974). The highest P accepted was 0.01. Renkonen's coefficient for niche-overlap was calculated according to Mühlenberg (1989). Q1 and Q3 refer to the quartiles one and three.

7.3 Results

7.3.1 Nymph Ratio

No significant differences were found in the ratio of nymphs to adults between *A. usambarica*, *A. nguru* and *A. longicornis* during the research period (χ^2 test, n. s., df = 2, Renkonen's coefficients > 0.93). In the species, which was studied first, *A. euthynota*, significantly more nymphs were found than in all other *Afrophlaeoba* species (χ^2 test, df = 1). *P. arachniformis* had significantly more nymphs than all *Afrophlaeoba* species (χ^2 test, df = 1, figure 79, Renkonen's coefficients < 0.6).

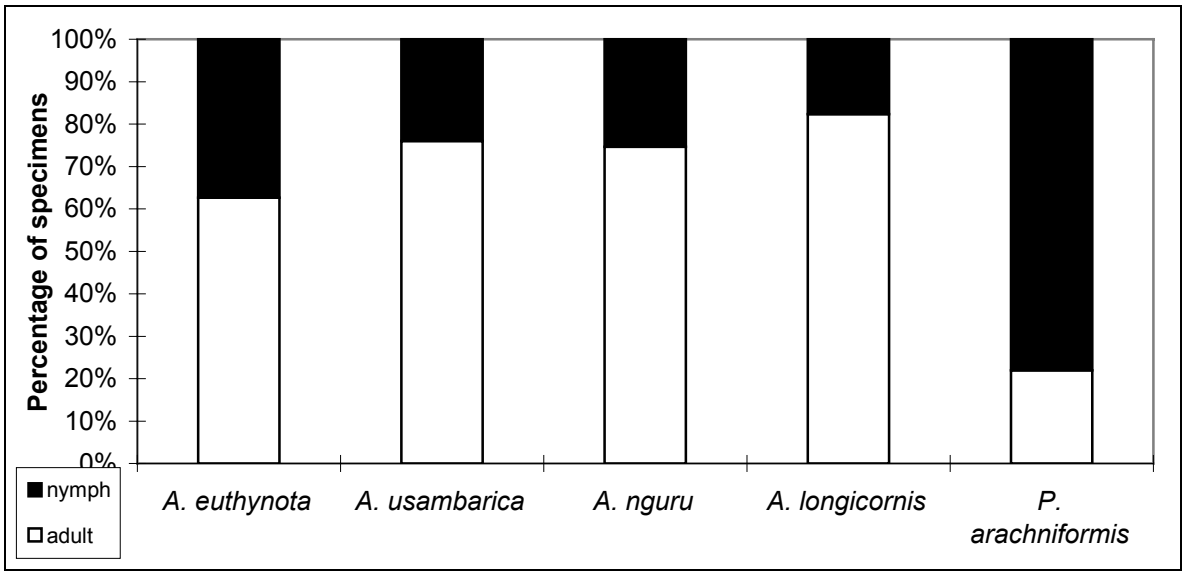


Fig. 79: Percentage of nymphs for the investigated species (*Afrophlaeoba* arranged according to the date of record, for each species n = 300). Significant differences were found between *A. euthynota* and the other species, as well as between *P. arachniformis* and the other species (χ^2 test, df = 1).

Tab. 50: Renkonen's coefficients for the nymph/adult ratio

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.923			
A. euthynota	0.803	0.88		
A. usambarica	0.937	0.987	0.867	
P. arachniformis	0.397	0.473	0.593	0.46

7.3.2 Sex Ratio

The sex ratio was similar among the five species (χ^2 test, n. s., df = 1, Renkonen's coefficients > 0.94, table 51). The percentage of recorded males varied between 85% and 92% (figure 80).

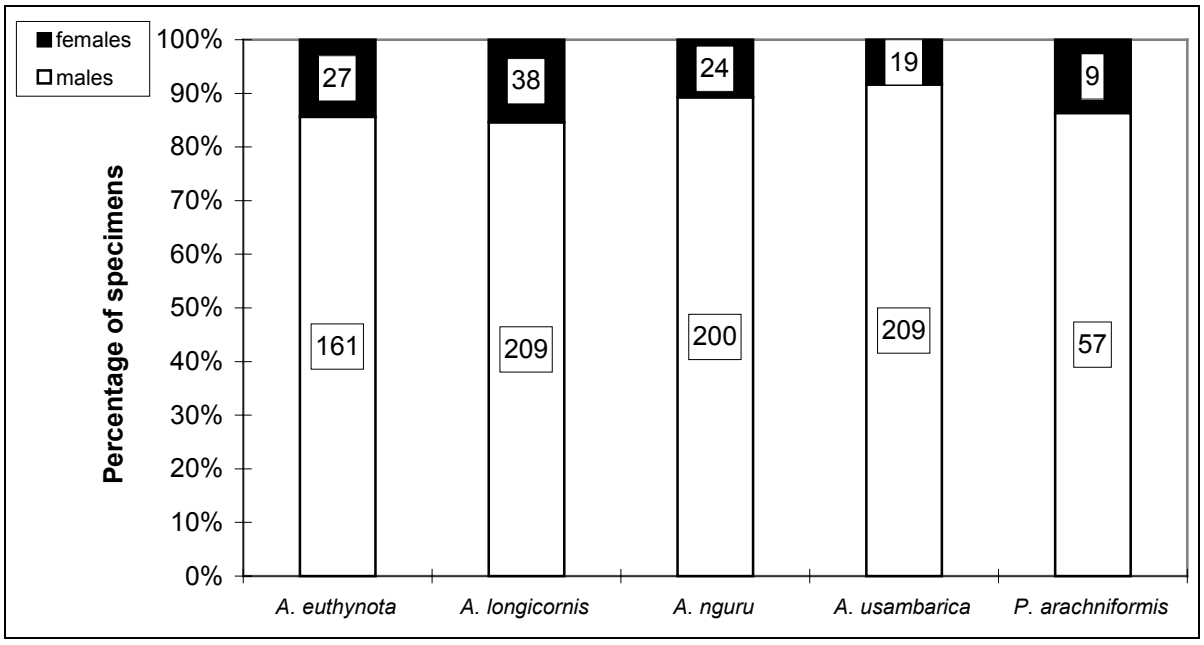


Fig. 80: Sex ratio of adults (*Afrophlaeoba* arranged according to the date of record). No significant differences were found (χ^2 test, n. s., df = 1).

Tab. 51: Renkonen's coefficients for the male/female ratio

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.953			
A. euthynota	0.99	0.964		
A. usambarica	0.929	0.976	0.94	
P. arachniformis	0.983	0.971	0.993	0.947

7.3.3 Vegetation Height

P. arachniformis was found in significantly lower vegetation heights than the *Afrophlaeoba* species (Mann-Whitney-test, Renkonen's coefficients: 0.61-0.80). Most specimens were found in vegetation heights between 30 cm (Q1) and 70 cm (Q3). The median was 50 cm, while it was 70 cm for nearly all *Afrophlaeoba* species, with the exception of *A. usambarica* (60 cm). Among *Afrophlaeoba* species, only the vegetation height in the habitat of *A. longicornis* and *A. euthynota* was not differing (Mann-Whitney-test, n. s., Renkonen's coefficient: 0.88). All other species differed significantly in this variable (Mann-Whitney-test, Renkonen's coefficients: 0.73-0.86). *A. nguru* was found in the highest vegetation (Q3: 90 cm; max.: 200 cm, figure 81).

Tab. 52: Renkonen's coefficients for the vegetation height

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.81			
A. euthynota	0.88	0.86		
A. usambarica	0.84	0.733	0.813	
P. arachniformis	0.727	0.613	0.713	0.8033

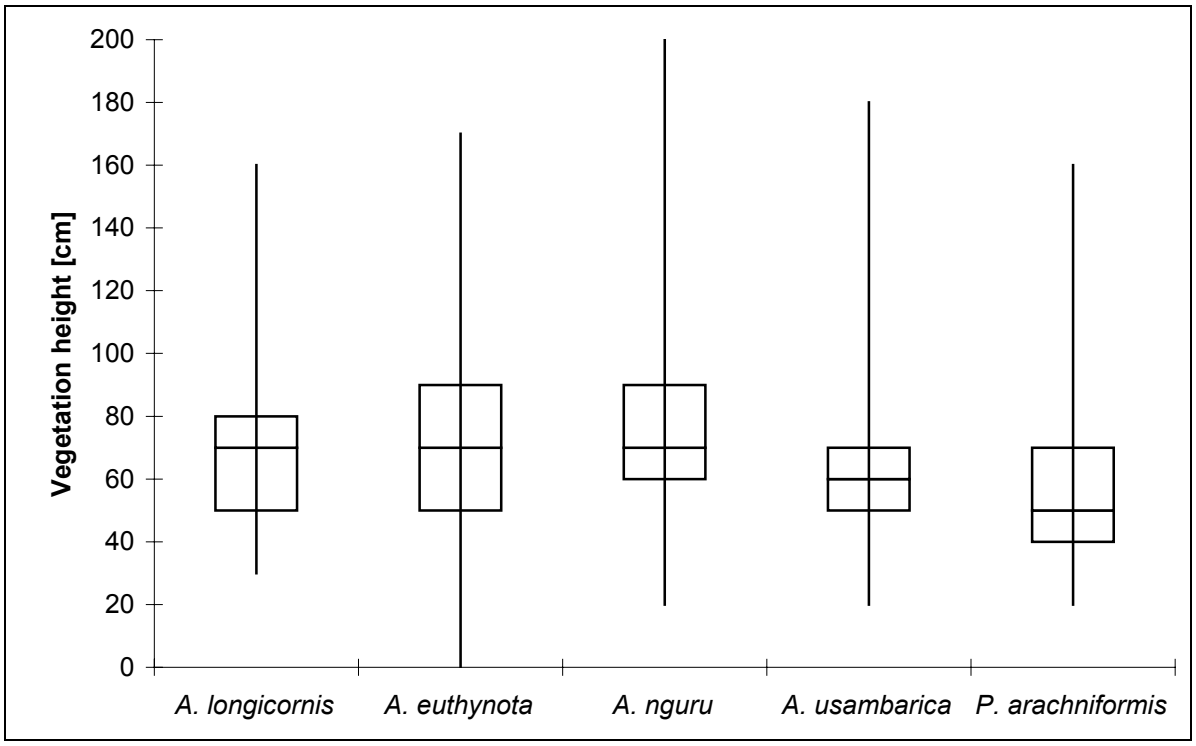


Fig. 81: Vegetation heights in the habitats of the five species (median, Q1, Q3, range). The vegetation height in the habitat of *A. euthynota* and *A. longicornis* can be regarded as similar (Mann-Whitney-test, n. s.), while all other species differ significantly from each other in this parameter (Mann-Whitney-test).

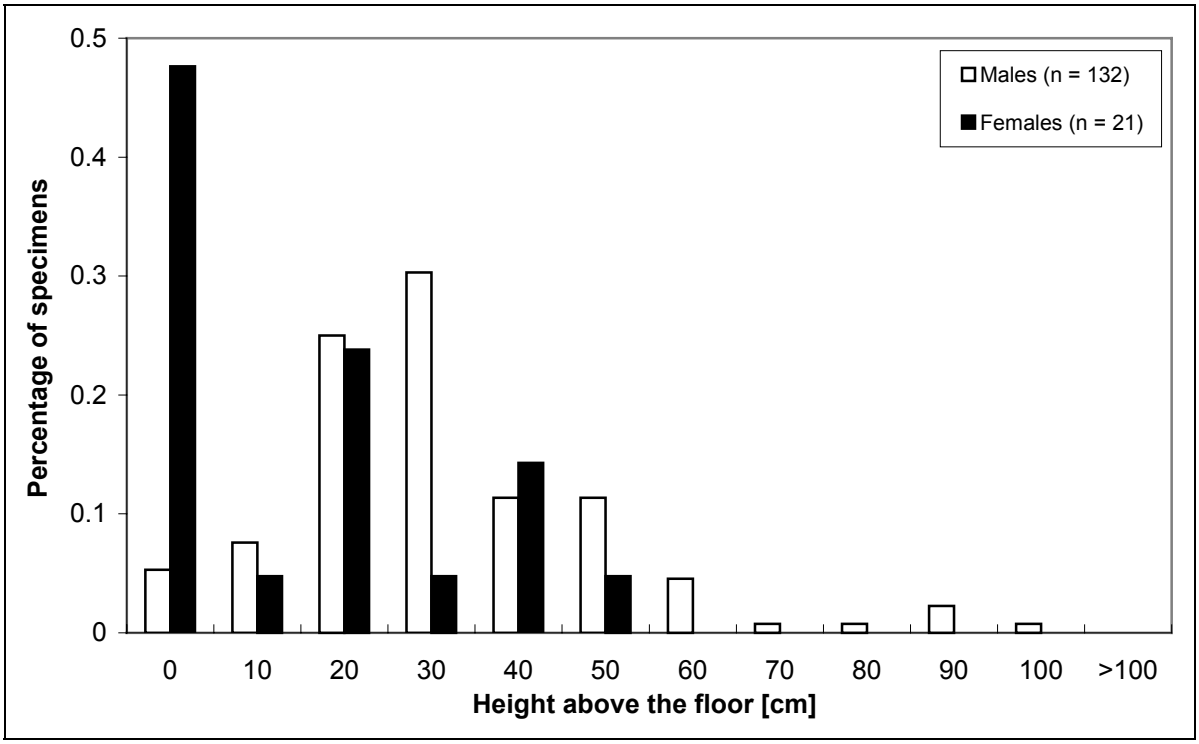


Fig. 82: The height of situation for males and females of *A. euthynota* differs significantly from each other (Mann-Whitney-test).

7.3.4 Height of Situation

The height of situation was dependent on the sex. Females were found more often on the floor than males (figure 82). Between 30 and 50% of the females were resting on the floor, in *A. longicornis* even more than 80% (Renkonen's coefficients for sex-specific differences: 0.18-0.6). This was tested only for *A. euthynota* on significance (Mann-Whitney-test), since in this species the most data on height of situation of females were recorded (n = 21). Between nymphs and males, a significant difference was found for nearly all species (Mann-Whitney-test), except for *A. euthynota* and *P. arachniformis*. For interspecific comparison only data on males were used. Males were usually found between 10 cm (Q1) and 50 cm (Q3), in mean (median) 20 to 30 cm. No significant interspecific difference was found (Kruskal-Wallis-test, n. s., Renkonen's coefficients: 0.61-0.85, figure 83).

Tab. 53: Renkonen's coefficients for the height of situation in males

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.773			
A. euthynota	0.724	0.718		
A. usambarica	0.792	0.765	0.849	
P. arachniformis	0.613	0.785	0.737	0.7564

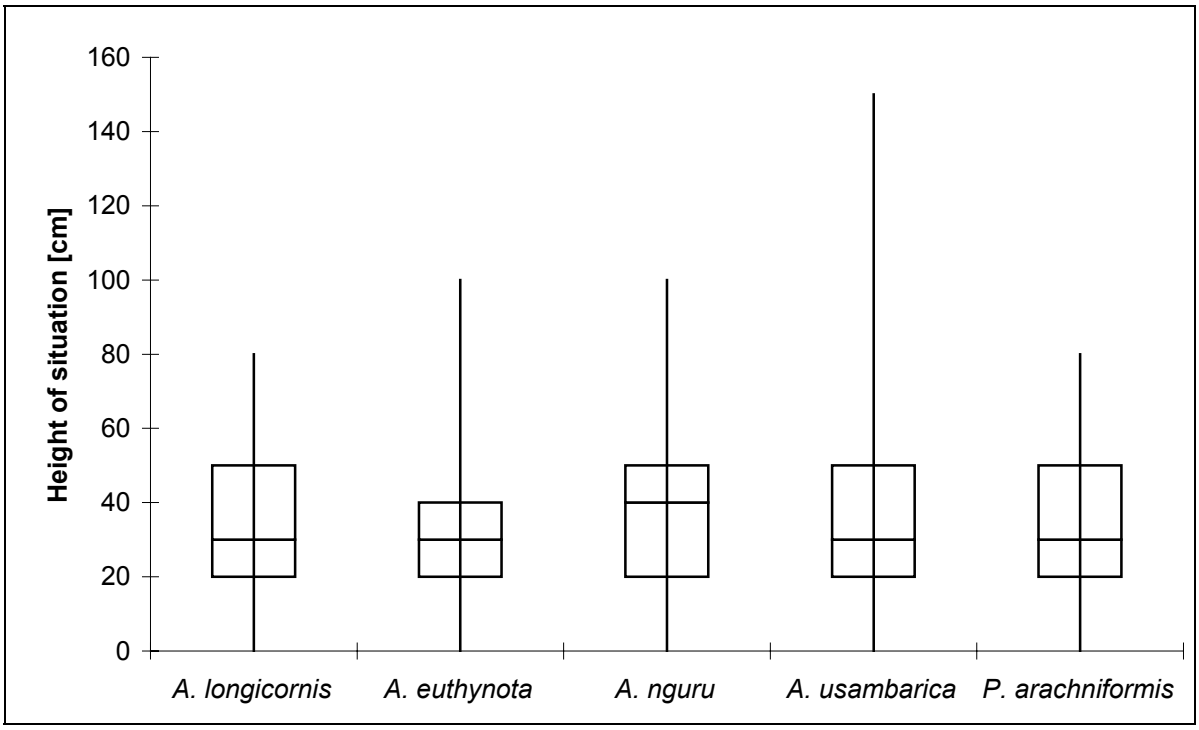


Fig. 83: Heights of situation of the five species (median, Q1, Q3, range). The situation heights of males do not differ significantly (Mann-Whitney-test, n. s.; Kruskal-Wallis-test, n. s.).

7.3.5 Orientation

All species were usually seated horizontally (76% to 89% of the specimens, figure 84). The difference between the species was not significant (χ^2 test, n. s., df = 4). The Renkonen's coefficients were exceptionally high (0.88-0.99, table 54).

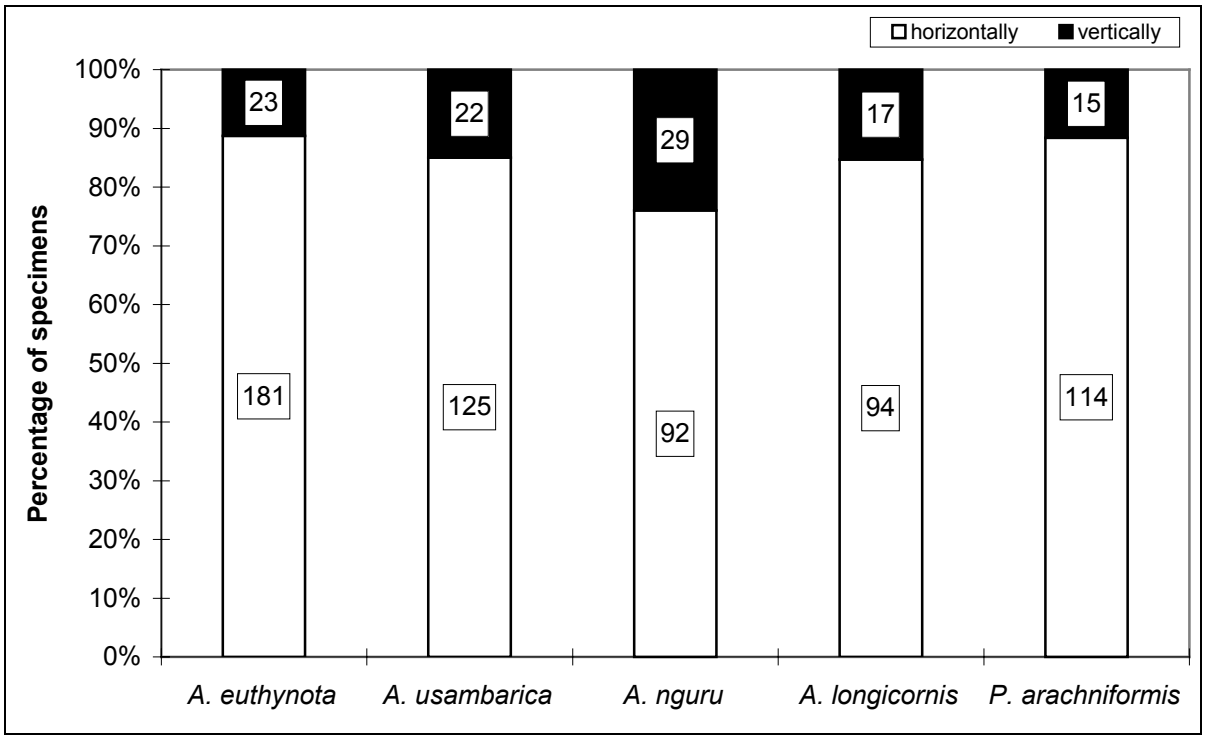


Fig. 84: Percentage of specimens resting horizontally and vertically in the vegetation. In all species horizontally orientated specimens dominate. The difference between the species is not significant (χ^2 test, n. s., df = 4)

Tab. 54: Renkonen's coefficients for the orientation

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.913			
A. euthynota	0.96	0.873		
A. usambarica	0.997	0.91	0.963	
P. arachniformis	0.963	0.877	0.996	0.9666

The orientation of the species was correlated with the location (figure 85). The majority (88% to 96%) of the horizontally resting specimens were found on leaves, while specimens resting vertically were found more often on stems (35% to 80%). No significant interspecific difference was found (χ^2 test, n. s., df = 4), while the difference between the two states of orientation was significant for all species (χ^2 test, df = 1, Renkonen's coefficients: 0.91-1, table 55).

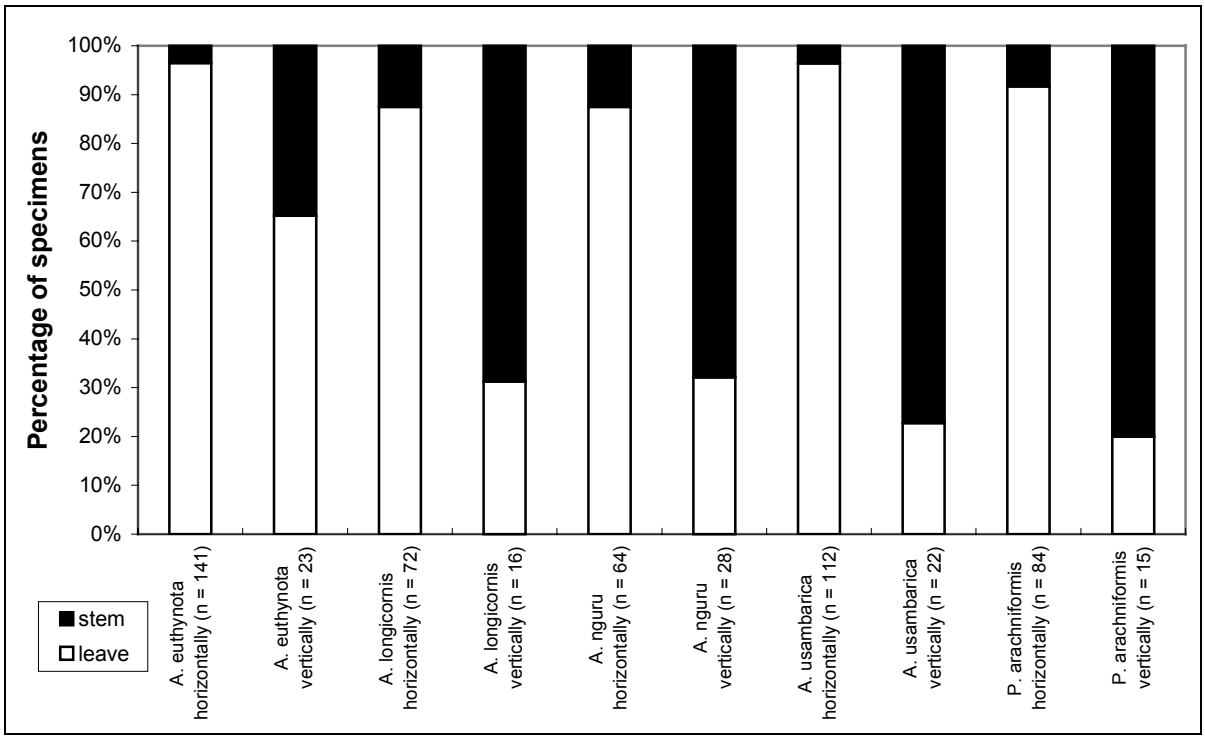


Fig. 85: Percentage of specimens resting on stems and on leaves, divided into specimens resting horizontally and vertically. In all species the specimens resting horizontally were mainly found on leaves, specimens resting vertically were mainly found on stems.

Tab. 55: Renkonen's coefficients for the stem-leaf ratio of horizontally sitting specimens

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	1			
A. euthynota	0.91	0.91		
A. usambarica	0.911	0.911	1	
P. arachniformis	0.958	0.958	0.952	0.9524

7.3.6 Locations

In figure 86 the locations of males, females and nymphs are sorted in order of the proportion of specimens located on grasses. This arrangement demonstrates that females were found more often on litter, while males were nearly exclusively found on plants. In all species the locations of males, females and nymphs were differing significantly (χ^2 test, df = 4). No interspecific differences were found in each gender (χ^2 test, n. s., df = 4, table 56).

Tab. 56: Renkonen's coefficients for the locations of males

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.903			
A. euthynota	0.936	0.966		
A. usambarica	0.978	0.881	0.915	
P. arachniformis	0.888	0.985	0.951	0.866

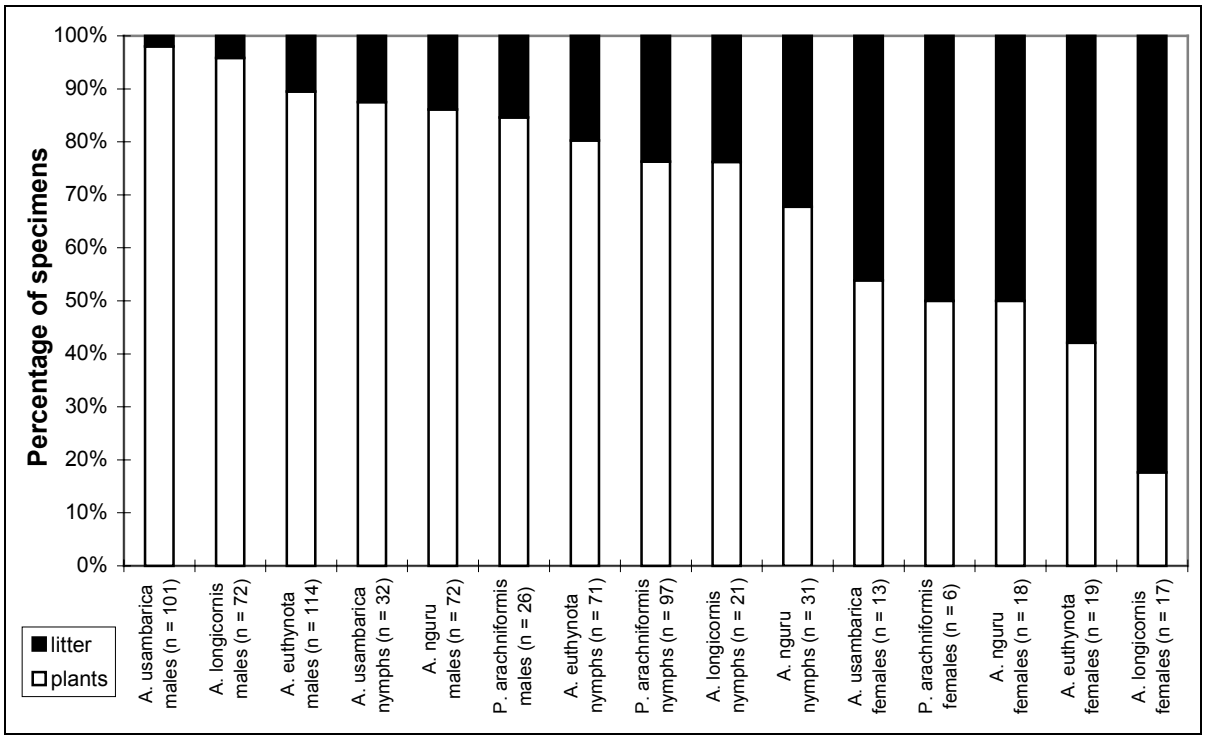


Fig. 86: Locations of males, females and nymphs of the species, in order of the percentage of specimens sitting on living plants. Note the grouping of males, nymphs and females.

7.3.7 Plants

The species were found more often on grasses than on forbs (51% to 86% on grasses, figure 87). In all species nymphs were found more often on grasses than males. This difference was significant in *A. longicornis*, *A. usambarica* and *P. arachniformis*, for the latter species also between males and females (χ^2 test, df = 1). Consequently, interspecific differences were tested separately for males, females and nymphs. Regarding females or nymphs no differences were found among the species. For most of the males no differences were found as well. The only significant difference was found between *A. nguru* and *A. euthynota* (χ^2 test, df = 1). *A. nguru* was found more often on grasses than *A. euthynota*. The Renkonen's coefficient varied for males between 0.71 and 0.99 (table 57), for nymphs between 0.79 and 0.98 and for females between 0.61 and 0.96. The low values for females are mainly caused by the small sample sizes (n = 9-14).

Tab. 57: Renkonen's coefficients for the grass-forb ratio (males)

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.846			
A. euthynota	0.861	0.707		
A. usambarica	0.991	0.837	0.87	
P. arachniformis	0.891	0.736	0.971	0.8993

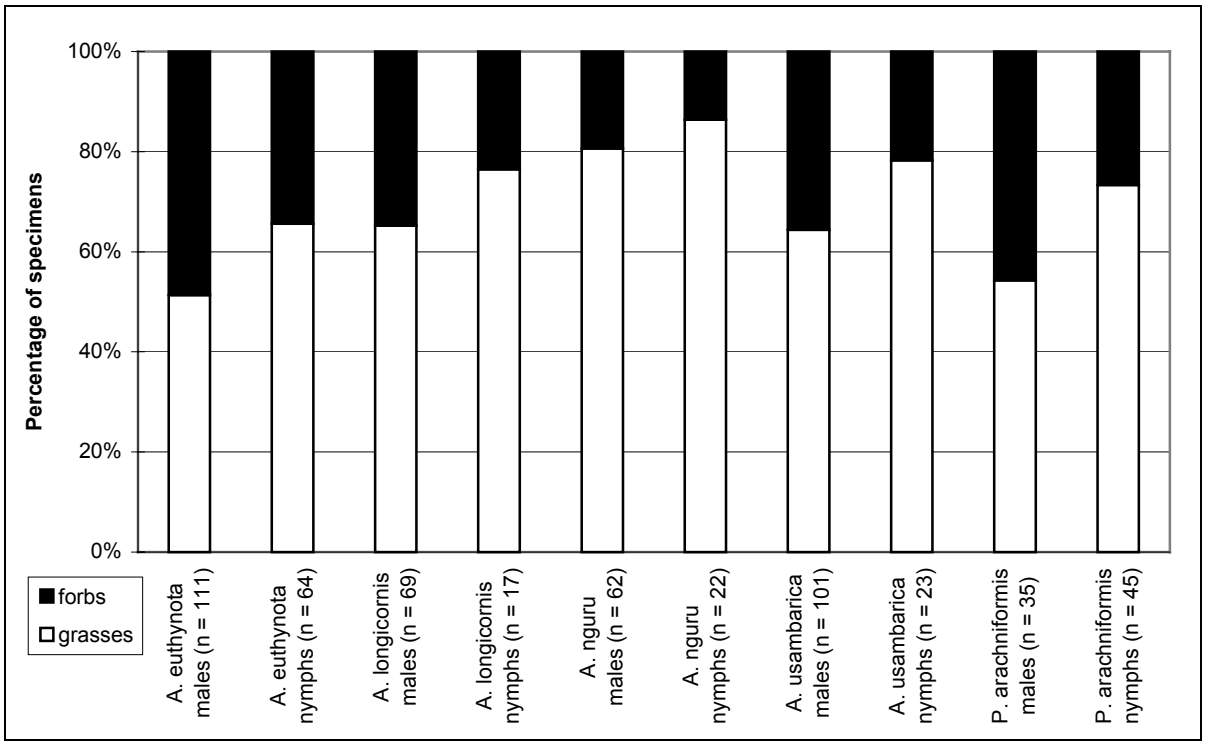


Fig. 87: Locations of males and nymphs of the species, divided into forbs and grasses. All species were found more often on grasses than on forbs (51% to 86%). Nymphs were always found more often on grasses than males. Females are not included, because of the small sample size.

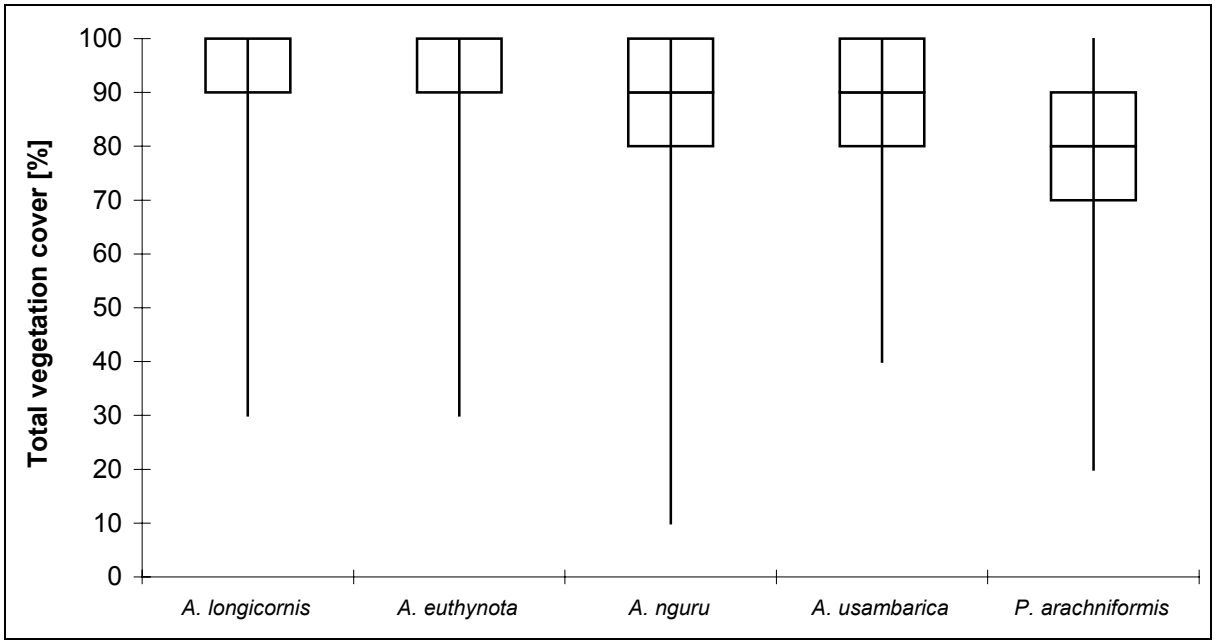


Fig: 88: Vegetation cover in the habitat of the five species (median, Q1, Q3, range). All species were found mainly in dense vegetation (>70% vegetation cover). The vegetation density differed significantly between *Afrophlaeoba* species and *P. arachniformis* (Mann-Whitney-test). *A. longicornis* and *A. euthynota* do not differ in this parameter, as well as *A. nguru* and *A. usambarica*, while the differences between those two groups are significant (Mann-Whitney-test).

7.3.8 Vegetation Cover

No significant differences were found in the vegetation cover between the sexes or between nymphs and adults (χ^2 test, n. s., df = 28). All species were found mainly at locations with dense vegetation (80-100%, figure 88). However, the vegetation density in the habitat differed significantly among some of the species (Mann-Whitney-test, Renkonen's coefficients: 0.53-0.9, table 58). Species pairs, in which no significant difference was found included *A. nguru* / *A. usambarica* (Mann-Whitney-test, n. s.) and *A. longicornis* / *A. euthynota* (Mann-Whitney-test, n. s.). *P. arachniformis* was found significant more often at sites with lower vegetation densities than any of the *Afrophlaeoba* species (Mann-Whitney-test).

The vegetation cover was divided into grasses, forbs, leaf litter and open soil. Grasses usually made up 60 to 80% of the vegetation cover (figure 89). No significant differences were found between *A. usambarica*, *A. nguru* and *A. euthynota* (Mann-Whitney-test, n. s.), while *A. longicornis* differed significantly from all other species in this parameter (Mann-Whitney-test). *A. longicornis* was found more often at sites with 100% of grasses in the vegetation. *P. arachniformis* was found significantly more often at sites with lower densities of grasses (Mann-Whitney-test), but it differed not from *A. euthynota* (Renkonen's coefficient: 0.88, table 59).

Tab. 58: Renkonen's coefficients for the vegetation density

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.737			
A. euthynota	0.893	0.807		
A. usambarica	0.793	0.9	0.84	
P. arachniformis	0.46	0.703	0.53	0.6667

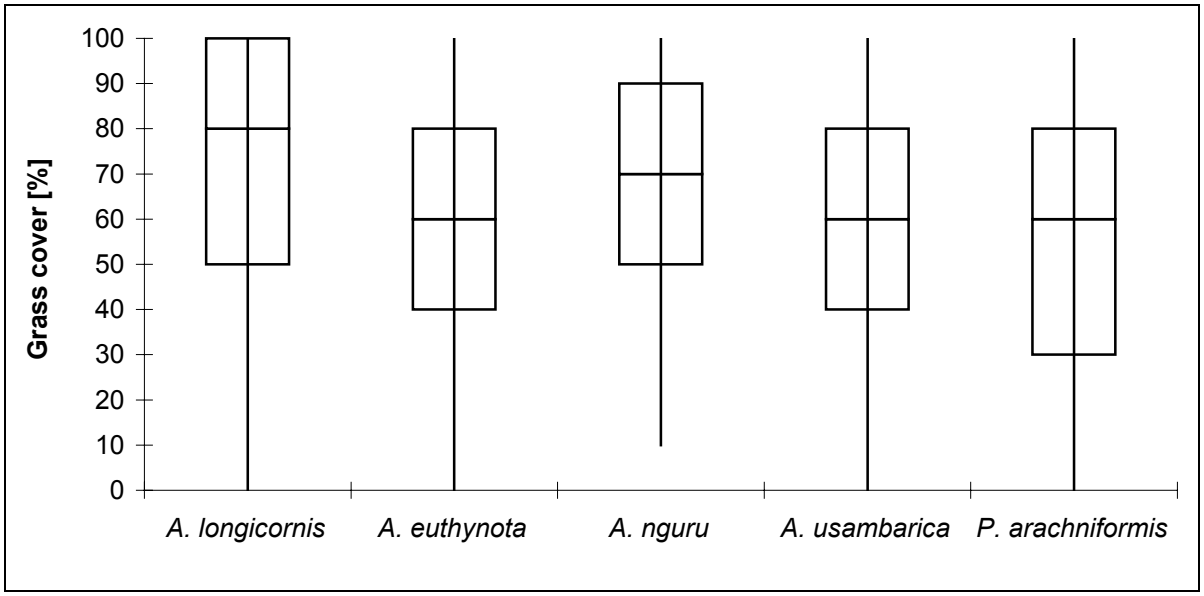


Fig: 89: The grass cover in the habitat of the five species (median, Q1, Q3, range). No significant differences were found between *A. usambarica*, *A. nguru* and *A. euthynota* (Mann-Whitney-test, n. s.), while *A. longicornis* differs from all other species significantly in this parameter (Mann-Whitney-test).

Tab. 59: Renkonen's coefficients for the grass cover in the habitat

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.723			
A. euthynota	0.687	0.86		
A. usambarica	0.697	0.87	0.913	
P. arachniformis	0.68	0.82	0.883	0.86

The density of forbs in the habitat of the species usually varied from 10 to 30% (figure 90). *P. arachniformis*, *A. nguru* and *A. longicornis* differed not in the percentage of forbs (Mann-Whitney-test, n. s., Renkonen's coefficients: 0.75-0.88, table 60). No significant difference was also found between *A. usambarica* and *P. arachniformis* (Mann-Whitney-test, n. s., Renkonen's coefficient: 0.86) and between *A. usambarica* and *A. euthynota* (Mann-Whitney-test, n. s., Renkonen's coefficient: 0.91). All other pairwise comparison proved to be significant (Mann-Whitney-test, Renkonen's coefficients: 0.72-0.85).

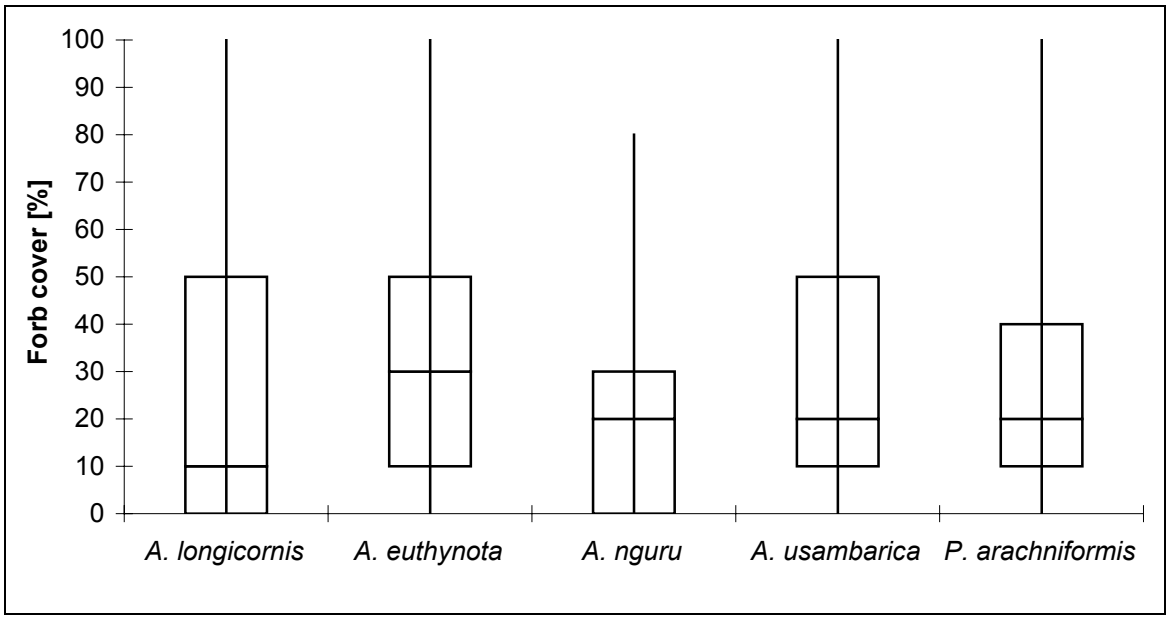


Fig: 90: The forb cover in the habitat of the species (median, Q1, Q3, range).

Tab. 60: Renkonen's coefficients for the forb cover in the habitat

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.753			
A. euthynota	0.757	0.817		
A. usambarica	0.717	0.85	0.913	
P. arachniformis	0.793	0.887	0.807	0.86

The percentage of leaf litter in the habitat usually ranged from 0 to 10%. In *P. arachniformis* it was significantly higher than in any of the *Afrophlaeoba* species (Q1 and median: 10%, Q3: 30%; Mann-Whitney-test, Renkonen's coefficients 0.45-0.80, table 61). No difference was found between *A. longicornis* and *A. usambarica* and between *A. longicornis* and *A. euthynota* (Mann-Whitney-test, n. s.). All other *Afrophlaeoba* species differed significantly from each other (Mann-Whitney-test). The percentage of open soil in the habitat was even lower than that of leaf litter (median, Q1 and Q3 = 0 in all species) and, therefore, it was not considered for this analysis.

Tab. 61: Renkonen's coefficients for the percentage of leaf litter in the habitat

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.687			
A. euthynota	0.763	0.62		
A. usambarica	0.893	0.783	0.76	
P. arachniformis	0.627	0.797	0.45	0.67

7.3.9 Diet

All feeding specimens observed, were exclusively feeding on grasses. Altogether 17 specimens were recorded feeding on grasses (*A. euthynota*: two males, six nymphs; *A. nguru*: one male, one female; *A. usambarica*: four males, one female, *P. arachniformis*: two males).

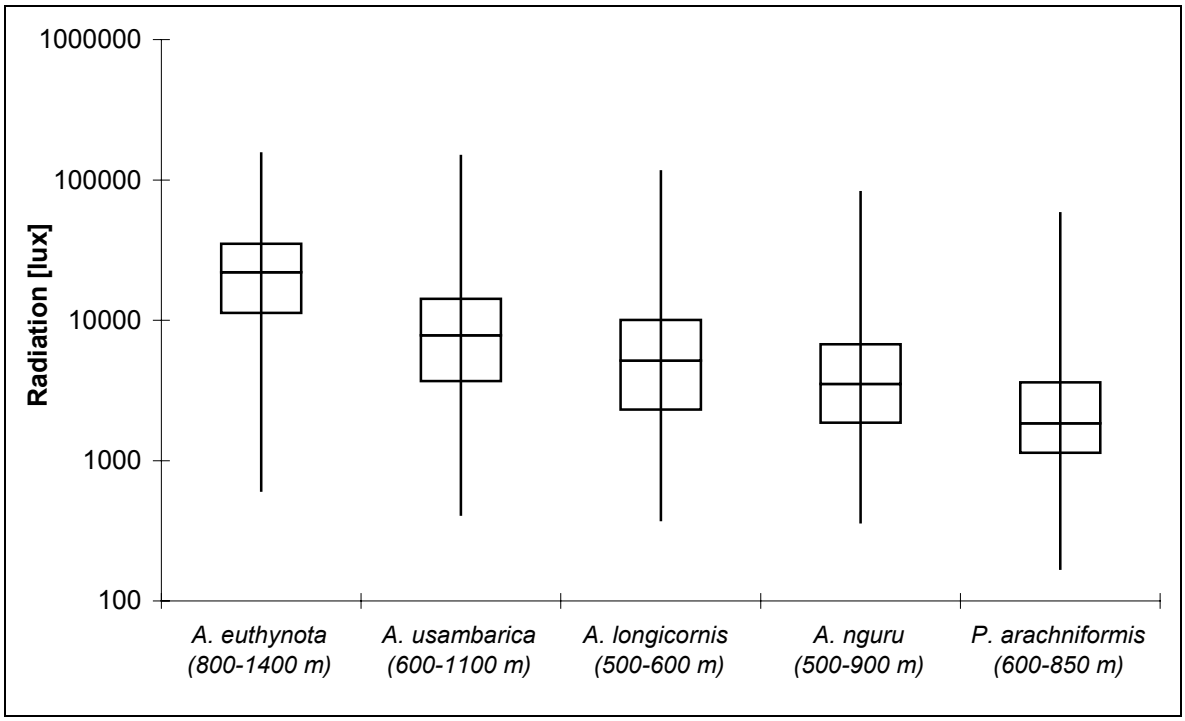


Fig. 91: Radiation at the location of the grasshoppers (median, Q1, Q3, range). Note the high radiation in the habitat of *Afrophlaeoba* species, which were studied at higher elevations and the low radiation at the locations of *P. arachniformis*.

7.3.10 Radiation

The radiation differed significantly between all species (Mann-Whitney-test, Renkonen's coefficients: 0.29-0.91, table 62). The lowest values were found in *P. arachniformis*, of which the median was 1,843 lux and Q3 was 3,615 lux. Only 5% of the specimens were found at locations with light intensities higher than 10,000 lux. In *A. nguru* the median was 3,513 lux. and in *A. longicornis* 5,150 lux. In these two species the highest similarity was found (Renkonen's coefficient: 0.91) with 16% respectively 25% of the specimens found at patches with light intensities higher than 10,000 lux. In *A. usambarica* even 36% were found at those lighter patches (median 7,805 lux.) and in *A. euthynota* the median was 22,000 lux.

These values correlate with the height of the study sites (figure 91). Species studied at lower elevations (*A. longicornis* and *A. nguru*) were found at darker patches than those of higher elevations. The only exception was *P. arachniformis*, which was studied only slightly lower than *A. usambarica*, but still it was found more frequently at darker places. Sometimes *P. arachniformis* was found only several metres away from *A. usambarica*. In these cases *A. usambarica* was always at lighter places (forest edge, clearings), while *P. arachniformis* was more associated with the forest interior.

Tab. 62: Renkonen's coefficients for the light intensities (four classes: <1,000 lx; 1,000-10,000 lx; 10,000-100,000 lx; >100,000 lx).

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.907			
A. euthynota	0.49	0.397		
A. usambarica	0.867	0.773	0.623	
P. arachniformis	0.8	0.893	0.29	0.6667

7.3.11 Shade

Comparing the percentage of specimens sitting in shade, semi-shade or sun, no significant differences were found between *Afrophlaeoba* species (χ^2 test, n. s., df = 6, figure 92). All species were found mainly in the shade of trees (>60%) and only <20% of the specimens were found at sunny locations (Renkonen's coefficients: 0.84-0.96). The difference was significant, when *P. arachniformis* was included (χ^2 test, df = 8). The percentage of *P. arachniformis* specimens sitting in shade, semi-shade or sun differed not significantly from *A. nguru* (χ^2 test, n. s., df = 2, Renkonen's coefficient: 0.98), but from all other *Afrophlaeoba* species (χ^2 test, df = 2, Renkonen's coefficients: 0.81-0.93, table 63). In *P. arachniformis* only 1.4% were found in the sun, while 71% were found at shaded patches.

Tab. 63: Renkonen's coefficients for the shade conditions

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.944			
A. euthynota	0.921	0.872		
A. usambarica	0.885	0.835	0.963	
P. arachniformis	0.929	0.979	0.851	0.814

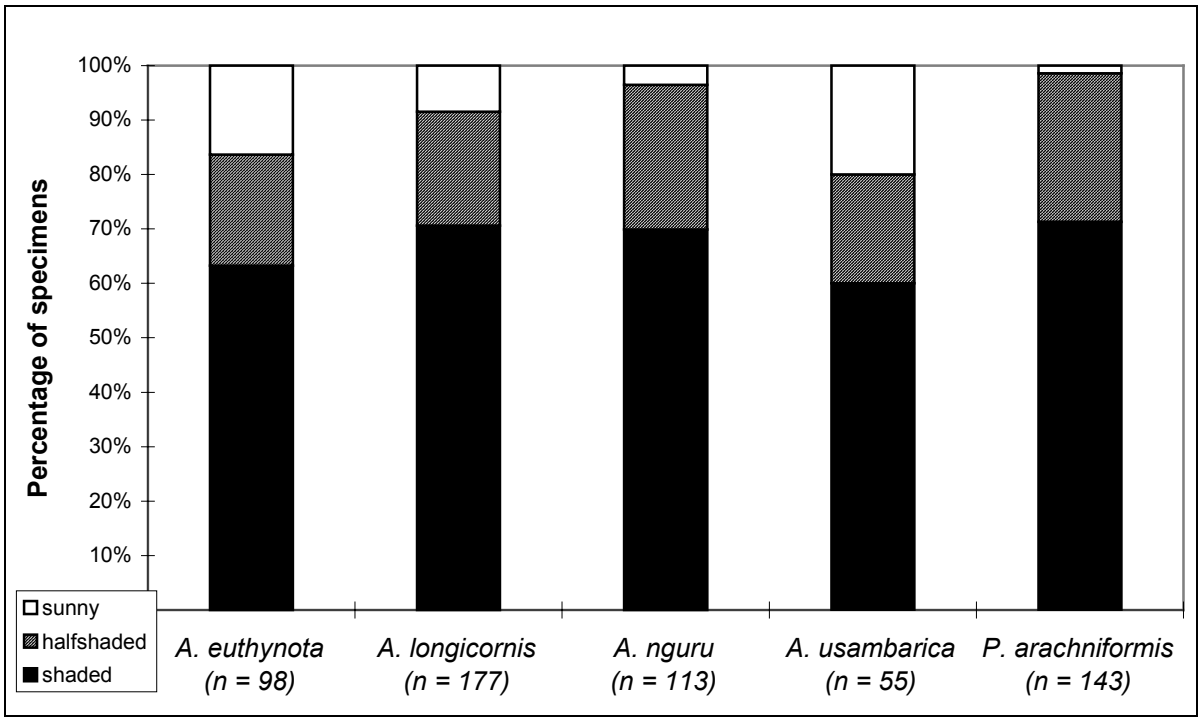


Fig. 92: Shade at the location of the grasshopper specimens. The higher radiation of species studied at higher elevations is visible, although the differences are not significant. In *P. arachniformis* the most specimens were found at shaded or semi-shaded locations. The species differs significantly from nearly all other species, with the exception of *A. nguru* (χ^2 test, df = 1)

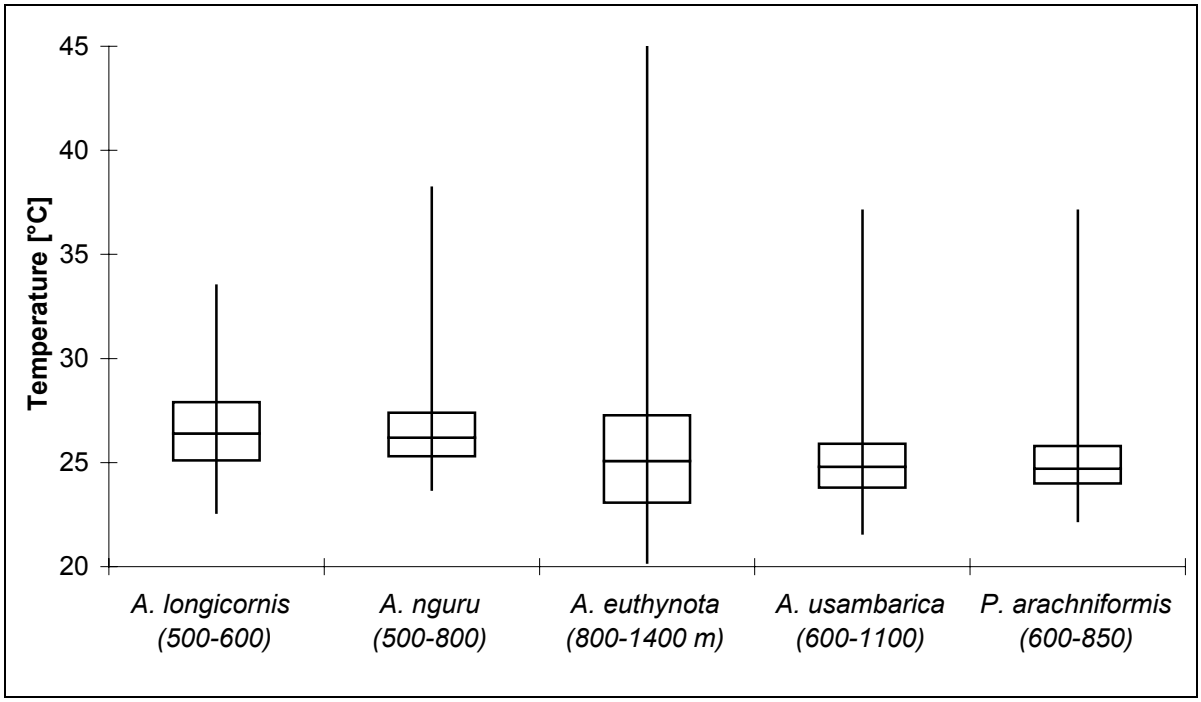


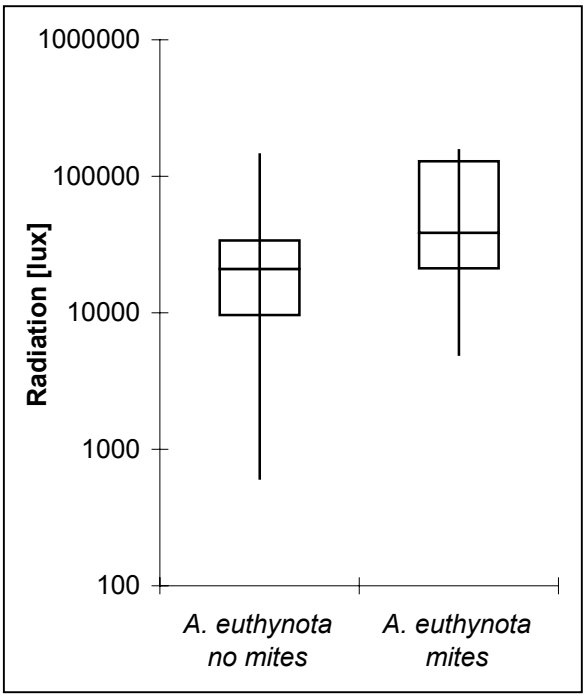
Fig. 93: Temperatures at the location of the grasshopper specimens. Note the two groupings, with *A. longicornis* and *A. nguru* at warmer locations and the other species at cooler locations.

7.3.12 Temperature

A comparison of the temperatures at the locations of the grasshoppers showed that two groups can be distinguished: *P. arachniformis*, *A. usambarica* and *A. euthynota*, of which the median ranged from 24.7°C to 25.1°C (Renkonen's coefficients: 0.52-0.83, table 64) and *A. longicornis* and *A. nguru*, of which the median was 26.4°C respectively 26.2°C (Renkonen's coefficient: 0.87; figure 93). Significant differences were found between those groups (Mann-Whitney-test), but not within them (Mann-Whitney-test, n. s.). The temperatures correlated with the height of the study sites. *A. longicornis* and *A. nguru* were studied at the lowest elevations, while the other three species were studied at higher locations (figure 93).

Tab. 64: Renkonen's coefficients for the temperature in the habitat

Species	*A. longicornis*	*A. nguru*	*A. euthynota*	*A. usambarica*
A. nguru	0.867			
A. euthynota	0.61	0.577		
A. usambarica	0.62	0.593	0.687	
P. arachniformis	0.61	0.603	0.52	0.83



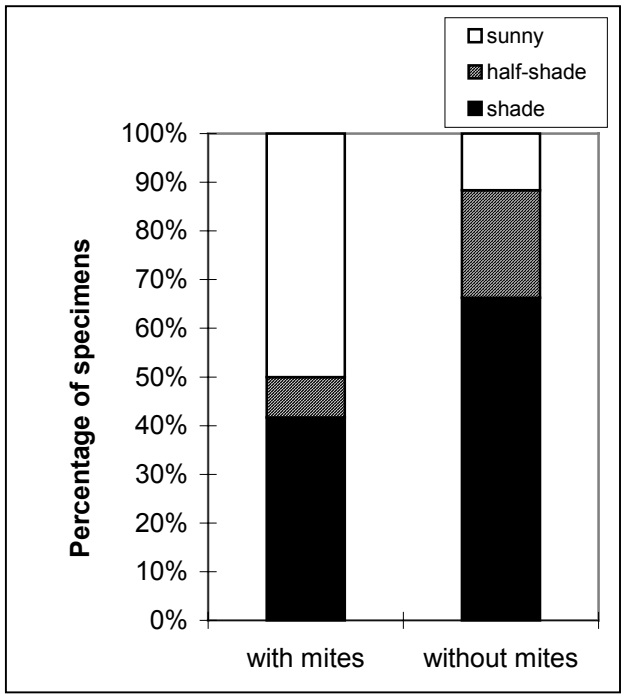


Fig. 94 & 95: Radiation at locations of *A. euthynota* specimens attacked by mites and shade conditions of those specimens.

7.3.13 Ectoparasites

African grasshoppers are often hosts for ectoparasitic mites (e. g. Erythraeidae, Podapolipidae). Species in open habitats seem to be more often attacked than species inside the forest (Cloudsley-Thompson 1969). During the field work mites were found exclusively on *A. euthynota*, and only at some study sites, which were rather treeless with only some shrubs around. The figures 94 and 95

illustrate the radiation and shade conditions of those specimens, which were attacked by mites. They were characterized by significant higher radiation (median 38,600 lux in comparison to a median of 20,950 lux for specimens without mites; Mann-Whitney-test). Specimens with mites were also more often found at sunny places (50%, n = 12) than the other specimens (12%, n = 86; χ^2 test, df = 2). The attacked specimens were also found in higher vegetation compared to the other specimens.

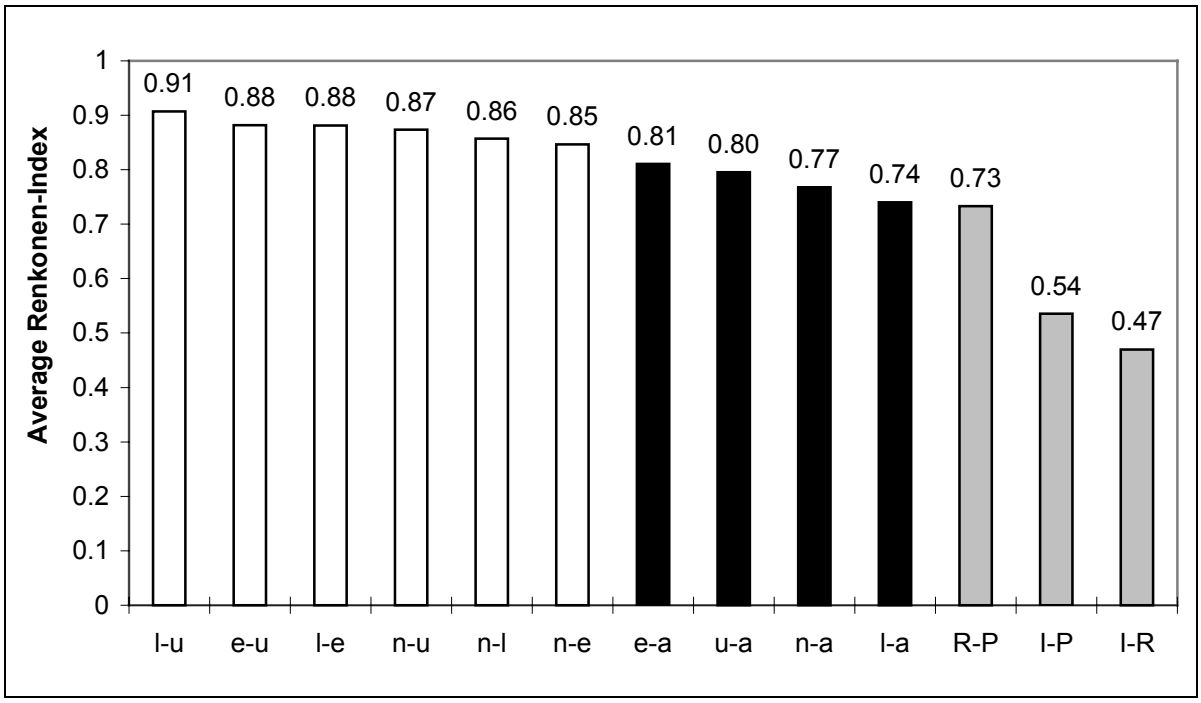


Fig. 96: Average pairwise Renkonen's coefficients; the pairwise niche overlap for *Afrophlaeoba* is indicated by white bars (l: *A. longicornis*, u: *A. usambarica*, e: *A. euthynota*, n: *A. nguru*); the intergeneric comparisons are represented by black bars (a: *P. arachniformis*); the grey bars indicate the average niche overlap for three sympatric species of the East Usambara Mts. (Hochkirch 1995; R: *Rhainopomma usambaricum*, I: *Ixalidium transiens*; P: *Parepistaurus pygmaeus*).

7.3.14 Average Renkonen's Indices

Due to the nominal and interval scales of many variables, it was not possible to perform a multivariate analysis. To illustrate an overview of the habitat affinities, an average of the Renkonen's coefficients was calculated for each species pair (figure 96). Following parameters were considered for calculating this average: sex ratio, nymph-adult ratio, total vegetation cover, vegetation height, height of situation, orientation, location and plants. Climatic parameters were not considered, as they depend too much on the geographic situation, daily fluctuations and the research period. To avoid higher weighting of the vegetation cover, only the total vegetation cover was included, while the parameters grass cover, forb cover, leaf litter and open soil were excluded from the analysis. In addition, the average niche overlap of three sympatric species of the East Usambaras was included (figure 96) to illustrate the high values of the allopatric *Afrophlaeoba* species. The highest niche overlap was observed in *Afrophlaeoba* species (0.85-0.91). The niche overlap between *Afrophlaeoba* species and *P. arachniformis* ranged from 0.74 to 0.81, although *P. arachniformis* occurs sympatric with *A. usambarica*.

7.4 Discussion

7.4.1 Ecological Differentiation on Single Mountains

Compared to the clear differences between three sympatric grasshopper species (*Ixalidium transiens*, *Parepistaurus pygmaeus*, *Rhainopomma usambaricum*) studied in 1994 in the East Usambaras (Hochkirch 1995), the four allopatric *Afrophlaeoba* species have quite similar habitat preferences. The lower Renkonen's indices between *P. arachniformis* and *Afrophlaeoba* suggest, that niche differentiation within a single mountain block is generally higher than between congeneric species of different mountain blocks. This supports equilibrium models, suggesting that niche specialization plays a major role in the generation of the high within-habitat-diversities of rainforests (Linsenmair 1990). The five species of the East Usambara Mts., which were studied intensely so far, can be clearly distinguished ecologically: *Ixalidium transiens* occurs in the ground litter of the forest, feeding on litter; *Rhainopomma usambaricum* occurs in dense and high vegetation of smaller gaps in the forest, feeding on forbs; *P. arachniformis* occurs in sparse and low vegetation of forest gaps, feeding on grasses; *Parepistaurus pygmaeus* and *A. usambarica* occur syntopous in dense and low vegetation of the forest edge, but while *P. pygmaeus* feeds on forbs, *A. usambarica* is a graminivorous. Generally, the *Afrophlaeoba* species were most similar to *P. pygmaeus* in ecology (based upon the Renkonen's indices). This is illustrated by a similar height of situation of *P. pygmaeus* compared to *Afrophlaeoba* (Mann-Whitney-test). The vegetation height in the habitat of *P. pygmaeus* was not differing from *P. arachniformis* and the vegetation cover was similar to *A. nguru* (Mann-Whitney-test).

A comparison of the habitat preferences of *P. arachniformis* and *A. usambarica* might illustrate the main differences between the two related genera. Although the species were studied close to each other during the same period and in similar temperatures, *P. arachniformis* was found at darker sites than any other *Afrophlaeoba* species. This illustrates the association of *P. arachniformis* with the forest interior (Hochkirch 1996a). The two species were only rarely found syntopous. Usually there was a transition from darker patches with *P. arachniformis* to more sun-exposed patches with *A. usambarica*. These differences were confirmed by the higher amount of leaf litter in the habitat of *P. arachniformis* and by the lower and less dense vegetation. *Parodontomelus luci*, the sister species from the Udzungwa Mts., was also found in a grassy clearing (Hochkirch 1999b).

7.4.2 Ecological Differentiation of *Afrophlaeoba*

The high niche overlap in *Afrophlaeoba* confirms the former impression that the species composition of the single mountain blocks within the Eastern Arc is very similar, with vicariant species of the same genera occurring at the forest edge (Hochkirch 1998). *Afrophlaeoba* is such a typical forest edge genus. It is confined to grassy forest edges and large clearings of submontane rainforests, as already suggested prior to this study (Hochkirch 1996a). Sometimes it was found at open locations (*A. euthynota*), but always near trees, shrubs or steep slopes, which provided shade at least for some

time of the day. Populations of open locations are affected by higher radiation and drought. They were often attacked by parasitic mites, which might indicate "environmental stress" of those grasshoppers. However, this might be also an effect of the ecological preferences of the mites. According to Cloudsley-Thompson (1969) parasitism is not developed to any great degree in tropical forests.

In the vegetation structure of the habitat many significant differences were found, but the similarities are more striking. Based upon the medians the vegetation can be characterized as follows. The vegetation usually is 60-70 cm high (in *P. arachniformis* it is 50 cm high) and has a cover of 90-100% (in *P. arachniformis* 80%). Grasses dominate in the habitat (60-80%), while forbs usually cover only 10-30% of the ground and leaf litter 0-10%. The median percentage of open soil is 0 in all species. Thus *Afrophlaeoba* species are typical for dense and low vegetation, which is dominated by grasses. *P. arachniformis* occurs in slightly lower and less dense vegetation, probably due to the occurrence inside the forest. The amount of leaf litter in the habitat of *Afrophlaeoba* is usually low, but in the habitat of *P. arachniformis* it is higher.

The microclimatic data have to be compared carefully, since the data were recorded at different locations and during different periods. Due to the allopatric occurrence of the four *Afrophlaeoba* species a direct comparison is only possible between *A. usambarica* and *P. arachniformis*. It has to be considered that *A. nguru* and *A. longicornis* were studied at lower elevations and later in the year, when the average temperature is generally higher. These two species were closer associated with shaded areas than those, which have been studied at higher altitudes. They were recorded from darker sites with higher temperatures, mainly under trees with a dense canopy, such as mango trees. Sometimes nearly a complete forest edge association occurred under such mango trees (Hochkirch 1998). Since the few *A. euthynota* specimens found in the lowlands at Morogoro were also situated under mango trees, it is reasonable to conclude that the genus *Afrophlaeoba* as a whole is more closely associated with dense canopy trees under the high temperature conditions of the lowlands. Thus, humidity might be a more important factor for the habitat preferences of *Afrophlaeoba* than temperature.

7.4.3 Sex-specific Microhabitats in Relation to Behaviour

The similar habitat preferences of the four *Afrophlaeoba* species are particularly well illustrated by the locations of the sexes. Males were usually found on plants, mainly horizontally on leaves of grasses, between 10 and 30 cm above the floor. In these characters nearly no differences occurred between the species or between *Afrophlaeoba* and *P. arachniformis*. 85 to 92% of the recorded specimens were males. Females were mainly recorded from leaf litter and nymphs more frequently on grasses. These locations are probably affected by sex-specific differences in behaviour. All *Afrophlaeoba* species and *P. arachniformis* seem to feed exclusively on grasses. This food specialization is of high significance, as it clearly restricts the species to grassy patches, which are less common in forests than in open habitats. According to Jago (1973) grass feeders are rare among rainforest grasshoppers, due to the

rareness of grasses and their bad palatability. This was confirmed by the feeding behaviour of *Rhainopomma usambaricum*, *Ixalidium transiens* and *Parepistaurus pygmaeus* (Hochkirch 1995). Apparently food specialization was underestimated by various authors (Hochkirch et al. 2000). The higher importance of grasses for nymphs can easily be interpreted by their higher energetic requirements in comparison with adults. This should also be true for females, which are bigger than males and nymphs and need to produce eggs (Hochkirch 1999a), but predation avoidance seems to be another important factor (Hochkirch 1998, 1999a), superimposing this expectation. In contrast to the small and inconspicuous nymphs, the females are bigger and would be easily seen when resting on grasses. Their stick-like appearance provides a useful camouflage in the ground litter, where they are nearly invisible. This might be the main cause for the high percentage of recorded males. During this study, records were made during the day to allow a better comparison of the environmental factors. It is reasonable to suggest that females supply their greater energetic costs during the night, since feeding females were observed during a short night excursion at Amani.

The high importance of predator avoidance for tropical forest insects seems to have a major influence on the life strategies (Linsenmair 1990, Hochkirch 1998, 1999a). In all *Afrophlaeoba* species, the males vary less in colour than the females. Typically they have a straw-like colouration with dark lateral stripes from the antennal bases to the end of abdomen. This striped colour might be interpreted as adaptation to the vertical structure of the habitat, which is dominated by grasses. The uniform straw-like to orange colour of the hind femora with contrasting dark knees is probably of importance for courtship (chapter 6). Additional behaviour, which might be of significance for habitat preferences are thermoregulation, mating and oviposition (Hochkirch 1996c, Hochkirch et al. 1999). Little is known in this regard due to the sparse occurrence of these behaviours (Hochkirch 1998).

7.4.4 Life Cycle

The nearly equal percentage of nymphs in the four *Afrophlaeoba* species indicates that the life cycle within the genus is rather similar, while *P. arachniformis* differs substantially from *Afrophlaeoba*. A higher number of adult *P. arachniformis* was found in March 1997. Thus the main adult emergence might be from March to May, which is the main rainy season and warmest period in the East Usambaras (figure 2). In *Afrophlaeoba* adults were dominant already during the research period from December to February. The slowly decreasing percentage of nymphs from the first studied species to the last one might be due to the ongoing moult of last-instar nymphs. Nevertheless adults of *A. usambarica* were recorded throughout the year. This might be explained by long life spans, which is known for other rainforest grasshoppers of the East Usambaras (Hochkirch 1995), but also by a continuous life cycle (Hochkirch 1996a). Unless quantitative studies are available, the life history of *Afrophlaeoba* remains a matter of speculation.

8 Final Discussion

8.1 Palaeoendemics or Neoendemics?

The Eastern Arc Mountains are known for their exceptionally high rates of endemism, including species, which are thought to be relicts of a former pan-African forest ("palaeoendemics") and recently evolved groups ("neoendemics"). The latter radiated during the Pleistocene climatic fluctuations and dominate (Fjeldså & Lovett 1997). Jago (1983) assumed that the members of the *Afrophlaeoba* genus group are evolutionary relicts of "long past climatic patterns", while the morphologically similar genus *Odontomelus* includes species "apparently still in a state of active evolution." The latter genus was studied merely as an outgroup to the genus *Afrophlaeoba* and only two of the 33 species described (Jago 1994a) were included in the analysis. Thus it is not possible to present any final evidence to Jago's (1983) comments on *Odontomelus*. However, it is striking that the genetic distance between the two species of *Odontomelus* that were studied is substantially higher (6%) than the distances between *Afrophlaeoba* species (0.8-2.5%). The low pairwise distances within *Afrophlaeoba* indicate that the species of this genus have to be regarded as neoendemics. Although the literature data on molecular clocks are highly variable, calculations of a possible time of divergence using medium substitution rates (0.01-0.03 per my) did not exceed 2.3 my. In contrast to the low distances between *Afrophlaeoba* species, the distance between the two species of *Parodontomelus* that were analysed was substantially higher (4.2%). These differences were possibly caused by the greater geographical distance between the two species of *Parodontomelus* (350 km), but also by their stronger preference for closed forest. As long as only parts of the *Afrophlaeoba* genus group have been studied, it is difficult to prove Jago's (1983) remarks completely. It would be interesting to study the phylogeny of the remaining genera of the *Afrophlaeoba* genus group and to test, if Jago's hypothesis is true for them. Since all species of the group are restricted to forested areas of eastern Africa and Madagascar, such a study would probably contribute a lot to the knowledge of the natural history of this region. It might clarify the significance of the arid corridor or the Indian Ocean as separating elements and the affinities of the Eastern Arc elements to the forests near Lake Victoria, the southern montane regions of Malawi, or the forests of the off-shore islands of Zanzibar, Mafia and Madagascar.

8.2 The Eastern Arc as Refuge Area

The Eastern Arc is thought to have served as a major refuge area during periods of drought. This hypothesis is mainly based on the high number of endemic species. Climatic stability is important for the survival of relictual taxa and for initial speciation (Lovett & Friis 1996). The peak concentrations of neoendemics are located in the same areas as those of relictual species (Fjeldså & Lovett 1997). Regarding grasshoppers the same pattern can be observed. This includes the recent radiation of *Afrophlaeoba* as well as relictual species, such as *Anischnansis burtti* (UVAROV, 1941), with uncertain systematic affinities (Dirsh 1959) or *Loveridgacris* REHN, 1954, with affinities to *Parapetasia*

I. BOLÍVAR, 1884 occurring in the West Central refuge in West Africa (Cameroon, Gabon, Nigeria). This confirms the hypothesis of the high importance of climatic stability for the survival of endemics (Anderson 1994). Since nowadays the Eastern Arc receives the highest rainfall of East Africa, there is no reason to suggest anything else for the past (Lovett & Friis 1996). Marine drill-core data suggest that the coastal waters of Tanzania were less influenced by Pleistocene climatic fluctuations (1-2°C) than other oceans (Prell et al. 1980). Even when aridity spread over large parts of Africa, forest taxa were possibly able to survive in small humid patches within the Eastern Arc. The low number of endemic mammals in the Eastern Arc (Stanley et al. 1998) suggests, that these refuge areas were rather small. According to Fjeldså & Lovett (1997) the Eastern Arc served as a principal centre of evolution during the Plio-Pleistocene period. The case of *Afrophlaeoba* confirms this hypothesis, but multiple waves of dispersal and speciation (Rodgers 1998, Johanson & Willassen 1997) cannot be confirmed. The restriction of both genera, *Parodontomelus* and *Afrophlaeoba* to the Coastal Forests and the Eastern Arc suggests that either no sufficient habitat connections to the volcanic mountains of northern Tanzania existed during the period of dispersal, or that populations in these areas became extinct due to drought events or volcanic eruptions.

8.3 The Significance of Ecological Studies

The genus *Afrophlaeoba* apparently represents a young radiation in the Eastern Arc. Based on the small ranges of all four species and on literature data (Jago 1983), one would expect them to be restricted to evergreen rainforests. Although this is true on a broader scale, they rather occur at forest edges and in clearings. From the small-scale ecological point of view, no species seems to be restricted to closed forest. They do not occur in treeless habitats, but a single tree with a dense (and probably evergreen) canopy might suffice for the existence of the species, if grasses are available as food plants. Such a situation has been observed frequently under mango trees, which spend enough shade and humidity and often serve as small refuges for forest edge species or even "real" forest species (Hochkirch 1998). Since the habitat requirements of the four *Afrophlaeoba* species are rather similar, there is no reason to suggest a different ancestral biology. This has an enormous consequence for the natural history of the genus, since a postulation of a closed forest connection is not necessary to interconnect the populations of *Afrophlaeoba*. This might illustrate the high importance of ecological studies for phylogeographical conclusions. On the other hand it is striking that the species are apparently restricted to the Eastern Arc and do not occur in the Coastal Forests. Even among material from 30 Coastal Forests of Tanzania, recently collected by "Frontier Tanzania" and kept at the Zoologisk Museum in Copenhagen, there is no record of *Afrophlaeoba*, although approximately 100 species occur in this collection, including three species of *Parodontomelus* (Hochkirch unpubl.). A restriction by macroclimatic factors, such as rainfall or temperatures seems likely. Although the study sites of *A. longicornis* at Kilosa only receive about 1,000 mm rainfall per year, they might be influenced by the local climate of Mkondoa River and the Rubeho / Ukaguru Mountain massif. While

the river might influence the local humidity and the groundwater regime, the Rubeho and Ukaguru Mountains probably receive higher rainfall. The Coastal Forests of Tanzania receive 800-1,300 mm rainfall per year and the average temperatures are higher (Hawthorne 1993, Burgess et al. 1998b, Clarke 2000b). Experimental studies on the requirements of eggs, nymphs and adults of *Afrophlaeoba* regarding the temperature and the humidity would possibly explain the absence of *Afrophlaeoba* from the Coastal Forests.

8.4 A Scenario of the Evolutionary Divergence

Acridinae are a comparatively young group of Orthoptera, related to the Gomphocerinae (Rowell & Flook 1998). They are specialized on grasses in their diet (Jago 1973), while more "primitive" groups are usually herbivorous. Since there are apparently no exceptions in Acridinae and in the sister group Gomphocerinae, it is reasonable to suggest this food specialization to be a plesiomorphous character. The origin of Poaceae in Africa is dated to the Palaeocene (60-55 my BP) and a major spread of grass-dominated habitats to the middle Miocene (c. 15 my BP, Jacobs et al. 1999), supporting the view that Acridinae are a comparatively young group. The position of the Phlaeobini within the Acridinae remains unknown, but the forest specialization suggests them to be a comparatively ancient group of Acridinae (Jago 1983).

The most established evolutionary concept for tropical forests is the refuge concept, which assumes that forest species evolved allopatric by isolation in areas, which remained stable (Haffer 1974). This mechanism of segregation of an ancestral species into geographically separated populations caused by climatic or geological events that lead to speciation, is usually referred to as vicariance. Generally it is likely that both vicariance and dispersal scenarios interact, but the natural history of eastern Africa and the flightlessness of *Afrophlaeoba* suggest that vicariance is of a higher importance, although at some time in the past the ancestral dispersal must have occurred. Despite the evidence for periodic aridity alternating with humid periods (Hamilton 1982), it remains doubtful whether this caused several waves of dispersal and speciation in refuge areas (Brühl 1997). This can be concluded from the fact that each mountain block of the Eastern Arc usually has only one endemic species of a genus (Hochkirch 1998). According to Hamilton (1982) it is likely that many disjunct "montane" taxa once occurred in lowland forests, either during wetter periods or during initial phases of forest expansion. A lowland origin of many endemic montane taxa is also assumed by Harmsen (1989) and Kingdon & Howell (1993). For flightless species there is naturally no other way to disperse than via lowland connections. Moreover, forest grasshoppers of the Eastern Arc do not exhibit a steep altitudinal gradient of species replacement (Hochkirch 1996a), which is typical for plants (Lovett et al. 2000).

Since most of the Eastern Arc forests are separated by dry savannah and woodland, two elements have to be distinguished for the connection of the ancestral range from the ecological point of view: Coastal Forests and Riverine Forests. According to Clarke (2000a) some existing gallery forests include Swahilian (Coastal) elements, representing transitions to the Coastal Forests. The Coastal Forests on the other hand represent a mixture of different vegetation types, including Coastal Dry Forests, Coastal

Scrub Forest, Coastal *Brachystegia* Forest, Coastal Riverine / Groundwater / Swamp Forest and Coastal / Afromontane Transition Forests, which represent a transition to the Eastern Arc (Clarke 2000a). The genus *Afrophlaeoba* seems to be mainly dependent on the existence of evergreen trees rather than on a dense forest cover. Therefore, different Coastal Forest types and riverine forests possibly served as habitats during humid periods. In this context it is interesting to mention that a female *Afrophlaeoba* was collected 10 km north of Mbwewe (Jago 1983), a village close to the Wami River situated between the East Usambaras and the Ulugurus (figure 97). This female was assigned to *A. usambarica* (Jago 1983), although it is certainly difficult or even impossible to identify females. A genetic analysis of specimens from Mbwewe would be of high interest for the phylogenetic conclusions, since the area represents a node between the *euthynota-usambarica* group and the *nguru-longicornis* group. Although *Afrophlaeoba* was missing in the Coastal material collected by "Frontier Tanzania", the genus might occur in the region of Pangani (Hemp pers. comm.), which is situated at the coast close to the Usambara Mts. Additional sampling would provide yet a more complete understanding of the phylogenetic history of the genus.

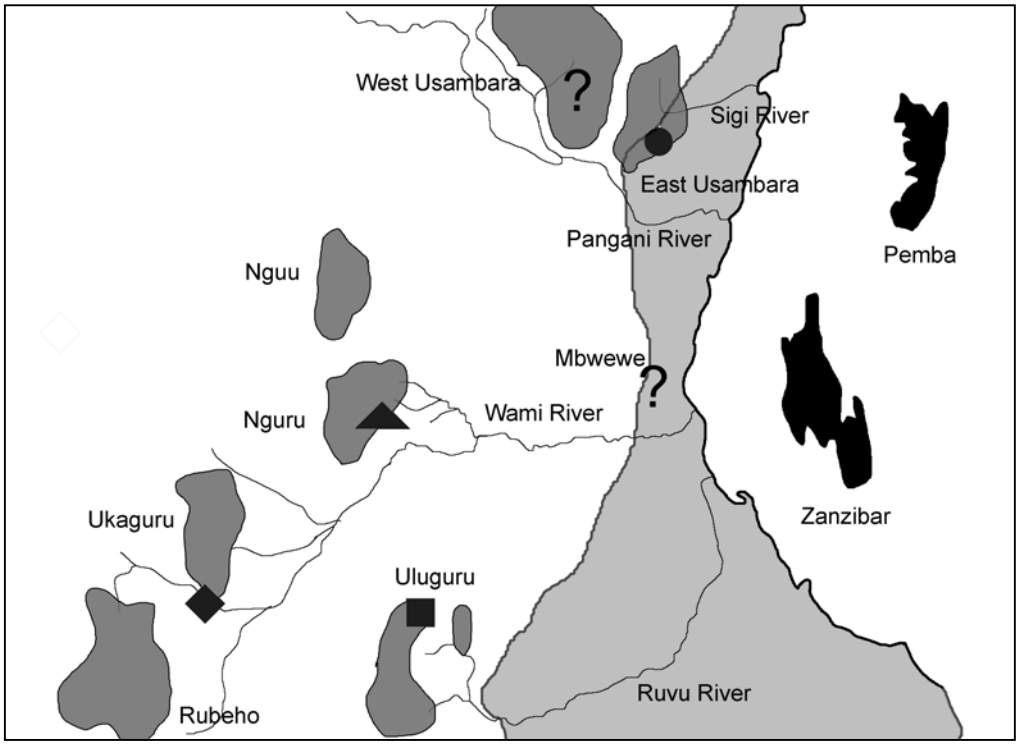


Fig. 97: Present knowledge on the distribution of *Afrophlaeoba*; circle: *A. usambarica*; square: *A. euthynota*, triangle: *A. nguru*; rhombus: *A. longicornis*. Question marks represent unconfirmed records of *Afrophlaeoba*. The lightly shaded area is the coastal part of the Zanzibar-Inhambane regional mosaic sensu White (1983); the dark shaded areas are blocks of the Eastern Arc; the offshore islands of Pemba and Zanzibar are black. Note the possible interconnection of *A. usambarica* and *A. euthynota* through the Coastal Forests and the possible connections of *A. nguru* and *A. longicornis* through the Wami River basin.

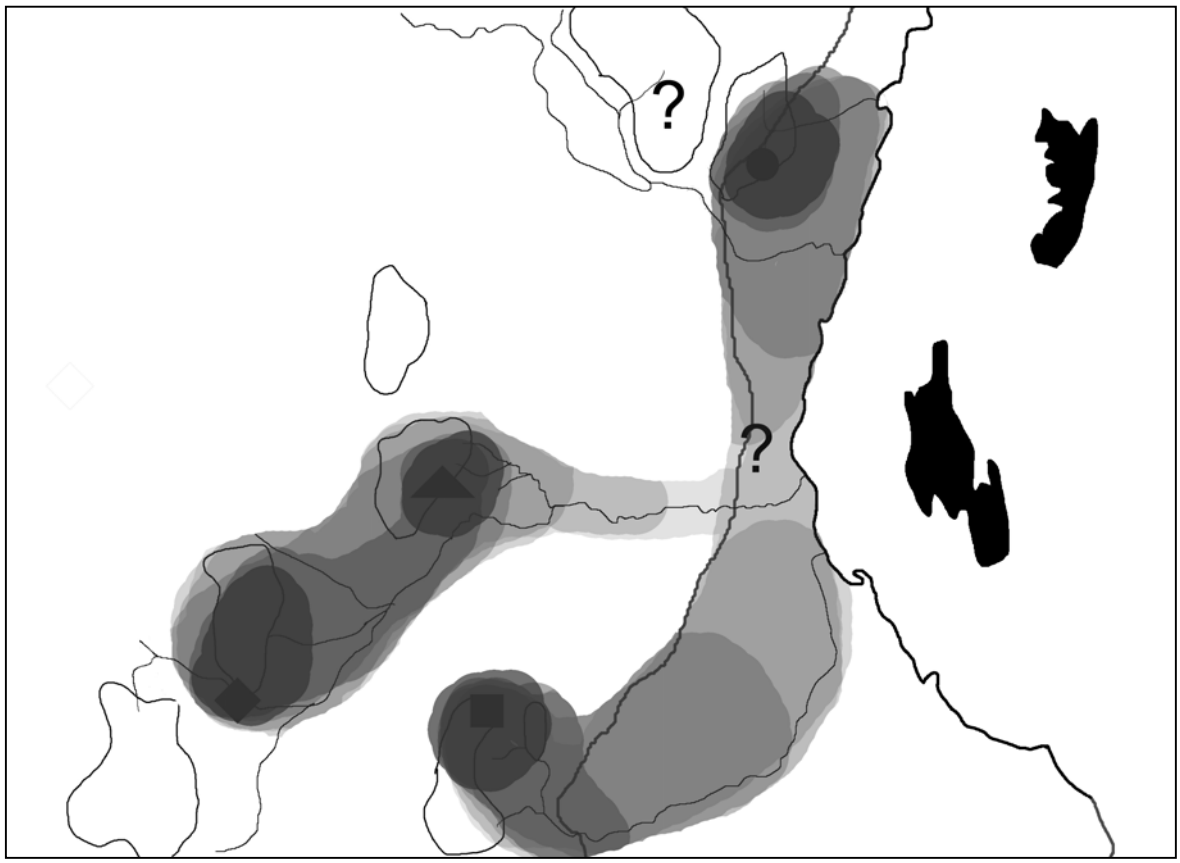


Fig. 98: Synopsis of a vicariance scenario for the separation of populations of *Afrophlaeoba* (question marks represent unconfirmed recent records, lighter shadings represent a higher ages; the off-shore islands of Zanzibar and Pemba are black). The first habitat split occurred in the rainshadow of the island Zanzibar, separating the inland group from the coastal group and then the northern from the southern populations. The connection of the *nguru-longicornis* group to the coastal group follows the Wami River, while *A. euthynota* was connected to the northern populations via the Ruvu River and the Coastal Forests.

If a scenario of the evolutionary divergence of *Afrophlaeoba* has to be set (figure 98), the ancestor of the species would be found in the formerly widespread Coastal Forests, which included those parts of the Eastern Arc, which are situated close to the coast (E. Usambaras, Ulugurus) and those which were connected via the basin of Wami River. Although the distance between the Ulugurus and the coast is substantially higher than that of the East Usambaras, the phytogeographical regions in Tanzania connect the Ulugurus with the coast through the Zanzibar-Inhambane regional mosaic sensu White (1983), which also includes the East Usambaras (figure 97). Moreover, this phytogeographical region includes the record of *Afrophlaeoba* from Mbwewe. In addition, a connection of the populations of the Ulugurus may have been possible through the Ruvu River basin. The Wami River basin drains the Rubehos, the Ukagurus and the Ngurus and thus might have connected the populations of those mountain ranges with each other and with the Coastal Forest zone. The phylogenetic hypotheses suggest an initial fragmentation of the "Wami River Connection" close to the coast, followed by a fragmentation of the "Coastal Forest Connection" shortly after that (figure 98). The short period between these two steps of divergence would be a sufficient explanation for the lower reliability of the *euthynota-usambarica* branch and the similar genetic and morphological distances between those two

species on the one hand and the *nguru-longicornis* group on the other hand. The instability of the *euthynota-usambarica* group thus has only minor effects on the main phylogeographic conclusions. A basal *euthynota*-branch would just suggest that the initial fragmentation occurred some kilometres further south, but still in the same area and in the same period of time. The Wami River Connection between the Nguru Mountains and the Rubeho Mountains must have remained stable for a longer time and might even still exist, but a connection via the Ukaguru Mountains is also feasible.

The dating of the habitat fragmentation remains a matter of uncertainty. If medium evolutionary rates are assumed, the basal divergence occurred 753 thousand years to 2.26 my BP. Despite this high variation, at least a Pleistocene radiation of *Afrophlaeoba* is likely, as has been suggested for other recent radiations within the Eastern Arc (Fjeldså & Lovett 1997). The loss of habitats in the lowlands were possibly affected by natural desiccation as well as by human disturbance. Although the use of fire in East Africa started 1.5 my BP, anthropogenic bushfires were not reported prior to 150,000 y BP. Hence, it is reasonable to suggest a natural cause for the initial split. The node between the three groups, *A. euthynota*, *A. usambarica* and the *nguru-longicornis* group, is located exactly in the rainshadow of Zanzibar. Since this area is still characterized by a lower rainfall pattern, it is likely that the natural desiccation during the Pleistocene had more severe effects on the vegetation here than in other areas. The present north-south disjunctions in the distribution patterns of plants confirms this hypothesis (Hawthorne 1993). Fragmentation possibly started further inland, since the coastal area generally receives a higher rainfall. There is unequivocal evidence that Coastal Forests were once more widespread in areas that are now woodland (Clarke & Karoma 2000). However, Clarke & Karoma (2000) point out that both factors, human influence and natural desiccation interacted and Hawthorne (1993) states that moist forests probably occurred naturally only sporadic along the coast, while dry forest types previously occurred in extensive blocks.

A second event of habitat loss possibly occurred between the Ngurus and the Rubehos, but the low genetic distances between *A. nguru* and *A. longicornis* suggest that this happened either recently or not at all. The estimates for the time of divergence of the two species vary from 285 thousand years to 855 thousand years. If the higher relative error at lower substitution rates is considered, it is rather doubtful to present a minimum time of separation (otherwise the two specimens of *Parodontomelus luci* would have been separated for 17,000 to 50,000 years, which is certainly not the case, since they were collected from the same site). At least the very low distances between *A. nguru* and *A. longicornis* suggest a fairly recent divergence, probably not sooner than the late Pleistocene. The part of the Wami River valley connecting the Nguru and the Rubeho Mountains is situated south of the Zanzibar rainshadow and thus possibly received a higher rainfall. Gained from the present knowledge on the natural history of eastern Africa, four hypotheses are presented here that might explain the close relationships between *A. nguru* and *A. longicornis*.

1. A connection between both species still exists along the Wami River or the Ukaguru Mountains. The missing records from the area between the Nguru Mountains and the Rubeho Mountains would then be a result of lacking collection activities.
2. A connection was present during a minor climatic fluctuation in the Holocene, e. g. during the "Little Ice Age" in the 17th and 18th century (Verschuren et al. 2000).
3. A connection was present during major climatic fluctuations within the Pleistocene or Holocene. The warm interglacials were probably wetter and forests spread during those periods. The last major wet period is believed to have lasted from 9,000 to 8,000 y BP (Hamilton 1982).
4. The connection has vanished during the last thousands of years due to anthropogenic events such as logging and burning. The use of fire in Tanzania started 1.5 my BP (Clarke & Karoma 2000)

8.5 The Zanzibar Rainshadow as Separating Component

The habitat loss in the coastal area of the Wami River basin is quite reasonable, if the present climatic regime is considered. Even today the rainshadow of Zanzibar Island seems to separate the northern Coastal Forests from the southern Coastal Forests (Robbrecht 1996, Clarke 1998, Tattersfield 1998). According to Hawthorne (1993) the low rainfall around Bagamoyo (and the Msangasi River) explains the abrupt change in the flora across this area. In many cases northern and southern groups of taxa are also distinguished in the Eastern Arc (Lindquvist & Albert 1999), which might confirm the general importance of the Zanzibar rainshadow for the isolation of Eastern Arc and Coastal Forest species. In the past the Afromontane element of eastern Africa was divided into a northern Usambara-Imatong section and a southern Uluguru-Malanje section (White 1978), until the Eastern Arc was considered as a floristic unit (Lovett 1988). According to Scharff (1993) the linyphiid faunas of the Uluguru Mts. and the Usambaras do not share a single true forest species. This can only be explained by strong habitat barriers between those two mountain ranges. This also is true for grasshoppers, in which the number of shared species increases with the distance from the forest, while in the forest even endemic genera can be found (Hochkirch 1998). Additionally, there are only few connections between the millipede faunas of the Usambaras and Ulugurus (Hoffman 1993). Based upon a study of nuclear DNA sequences (ITS) of the African Violets (*Saintpaulia*), Möller & Cronk (1997a) conclude that the Uluguru Mts. became isolated prior to the isolation of the northern mountain ranges, while Lindqvist & Albert (1999) suggest that an initial north-south disjunction must have occurred, separating the Uluguru-Ukaguru-Nguru ranges from the Taita Hills and Usambaras. Burgess et al. (1998b) assume that the high number of single-site endemics in the Coastal Forests have to be interpreted as relicts due to fragmentation. They note a predominance of old species within the Coastal Forests contrasting with the Eastern Arc, where a considerable proportion of endemic species are believed to be recent radiations (Fjeldså & Lovett 1997). However, the *Saintpaulia* studies elucidated a recent radiation in the Coastal Forests (Lindqvist & Albert 1999).

8.6 Riverine Forests as Connecting Components

The "Wami River Connection" is supported by distribution patterns of several other forest taxa as well. The millipede genus *Pseudotibiorus* DEMANGE, 1978 has several species in the Eastern Arc and the Coastal Forests, including one in the East Usambaras, one in the Ulugurus and one in the Wami River valley east of Ngurus (Hoffman 1993). Pongwe Mountain in the Wami River plain is occupied by a subspecies of the millipede *Rhododesmus planus* (KRAUSS), which has another subspecies in the Ulugurus and one in the northern Udzungwas (Hoffman 1993). Based upon the distribution pattern of the millipede genus *Ceratodesmus*, Hoffman (1993) already assumed that some continuity of rain forest must have existed between the Usambaras, Pongwe Mountain and the Ngurus. He also suggested a relatively recent disjunction of the habitat in the late Pleistocene. This hypothesis can be confirmed from the *Afrophlaeoba* data. The importance of riverine forests for contact between forest faunas has already been suggested by Hamilton (1982) and Brandl et al. (1996). Kellman et al. (1994) found typical forest floras in savannah gallery forests. According to Möller & Cronk (1997a) spread along river systems may be of significance for the typical Eastern Arc and Coastal Forest genus *Saintpaulia* (African Violets). Riverine forests possibly served also as refuges for forest taxa in periods of drought, since they are more dependent on groundwater than on rainfall. The Tana River forests in Kenya are situated in semi-arid land with annual rainfall of 470 mm (Medley 1995). Nevertheless some typical forest species occur here, including *Pachystela msolo*, a tree, which is found outside West Africa only in the East Usambaras and along Tana River (Medley 1998). Even small remnants of gallery forests were possibly enough to allow the survival of certain populations of arthropods (Scharff 1992). The possible significance as refuges is supported by observations of Evans (1997), who found the riverine forest in the East Usambaras to be particularly rich in species. Fjeldså & Lovett (1997) suggested patches near waterfalls or local mist zones to be of high importance as forest refuges. The Wami River valley probably played a major role for gene flow between the different rainforest areas, as it connects three Eastern Arc mountain ranges with the Coastal Forests.

8.7 Isolation or not?

The species of *Afrophlaeoba* were described based on morphological differences (Jago 1983), which proved to be not reliable. Although the species are genetically distinct and also distinct in morphometric space, this does not help to answer the question whether the species are isolated or whether they should be regarded as subspecies or populations of one species. A slight degree of differentiation will be found between most populations (Gries et al. 1973) and in the comparatively old habitats of East African mountains a genetic differentiation is likely to occur. The problem arises mainly as a result of the allopatric distribution of the *Afrophlaeoba* species. A promising approach was the study of the communicative behaviour, which revealed clear differences between the species. Amazingly those two species, which are genetically and morphologically most close to each other (*A. longicornis* and *A. nguru*), appear to have the highest differences in communicative behaviour.

This indicates the possibility of a higher selective pressure on the displays due to recent interconnections between these two species. On the other hand, the intraspecific variability is not yet known well enough to present any final conclusions. However, it should be kept in mind that the *Chorthippus biguttulus* group in Europe is not isolated genetically, but nevertheless interbreeding does not occur in nature due to the specific mate recognition systems based on different songs. Obviously, interbreeding experiments are the only solution to the question, whether isolation of the *Afrophlaeoba* species may be given or not. In many species groups of the Eastern Arc, which are regarded as neoendemics, the taxonomic status seems to be a matter of opinion (Lovett & Friis 1996, Möller & Cronk 1997a).

8.8 Comparison with other Phylogenetic Analyses

Only a few phylogenetic studies of Eastern Arc taxa are available. Knox & Palmer (1998) studied the phylogeny of giant lobelias (*Lobelia*, subgenus *Tupa*, section *Rhynchopetalum*) based on chloroplast DNA sequences. This genus radiated geographically and altitudinally in the mountains of Eastern Africa, but in contrast to *Afrophlaeoba* it is wind-dispersed. According to Knox & Palmer (1998) the Uluguru Mts. represent the site of origin of the African giant lobelias. They have been colonized approximately 20 my ago from Southeast Asia. From the Uluguru Mts. two species dispersed to the Nguru Mts. and one from there to the East Usambaras. Another species dispersed from the Ulugurus to the Ukaguru Mts. and from there to the Nguru Mts. It is obvious that these dispersal routes follow the main wind directions (SE monsoon, Hamilton 1982). Hence, any conclusions to the natural history drawn from the phylogeny of such a wind dispersed taxon should be taken with caution. At least, the Eastern Arc origin of the high number of giant lobelias is interesting, as it confirms the high age and persistence of this mountain chain.

Several recent studies of the phylogeny of the African Violets (*Saintpaulia*) based on DNA sequences are available (Möller & Cronk 1997a, 1997b, 1999, Möller et al. 1999, Lindqvist & Albert 1999). Möller & Cronk (1997a) studied the phylogeny of African Violets (*Saintpaulia*) based on nuclear DNA sequences (ITS), while later (Möller et al. 1999) they included a nuclear developmental gene (Gcyc – the putative homologue of cycloidea). Lindqvist & Albert (1999) studied the same genus based on faster evolving nuclear ribosomal 5S non-transcribed spacer sequences. The obtained phylogenies of both studies are wellmatched, but since the latter authors included more species and faster evolving sequences, they were able to present a higher resolved phylogeny. An interesting similarity to the *Lobelia* study (Knox & Palmer 1998) is the possible origin of *Saintpaulia* in the Uluguru Mts. (Möller & Cronk 1997a) or a complex of Uluguru, Ukaguru and Nguru Mts. (Lindqvist & Albert 1999), while the high number of species in the Usambaras and Coastal Forests proved to be recent radiations, which are regarded as taxonomically critical due to wide cross-fertility (Möller & Cronk 1997a). The rapid recent radiation close to the East Usambaras is probably caused by the close location of this mountain range to the coast and multiple waves of repeated separation events in this

smaller area. In contrast to the Eastern Arc Mountains, the coastal area near the Usambaras is also characterized by a high number of congeneric species of grasshoppers, although *Afrophlaeoba* does not follow this pattern. The Coastal Forests of this area are also known to be particularly rich in endemic species (Clarke 1998). Möller & Cronk (1997a) interpret this radiation as the result of Pleistocene climatic changes. In comparison to *Lobelia* the genus *Saintpaulia* is probably not capable of long-distance dispersal, which allows a better comparison with *Afrophlaeoba*. In the study of Lindqvist & Albert (1999) the Uluguru Mts. cluster with the Ukaguru Mts. and the Nguru Mts., while in *Afrophlaeoba* the Usambaras and the Ulugurus cluster together. Apparently the collapse of habitat connections in the Zanzibar rainshadow had an effect on the central connection paths of the Eastern Arc and thus leading to different evolutionary patterns in different taxa. The habitat loss in the coastal Wami River basin interrupts three major regions, the northern part (Usambaras, Pares, Taitas), the western part (Ngurus, Ukagurus, Rubehos) and the southern part (Ulugurus, Udzungwas).

An analysis of the bird genus *Andropadus* (greenbuls) based upon cytochrom b gene sequences of the mtDNA suggests complex historical interchanges between different montane areas, including the Cameroon Mountains and East Africa (Roy et al. 1997). This is probably caused by the high dispersal power of birds, like in the giant lobelias. Generally, the birds of the Usambaras seem to have stronger biogeographic affinities to the northern volcanic mountains than to the southern highlands (Dowsett 1986). Although some insect genera of the Eastern Arc also reach Kilimanjaro (e. g. *Ixalidium*), most of them are restricted to the Coastal Forests and the Eastern Arc. Eleven forest-dwelling species of *Andropadus* occur in Africa, of which four species are strictly montane and represent a recently radiated monophyletic group (Roy et al. 1997). The basal branch of the *Andropadus* gene tree is represented by *Andropadus milanjensis*, a species mainly distributed in the southern Eastern Arc (Roy et al. 1997), like in the *Lobelia* and *Saintpaulia* examples. The recent radiation of *Andropadus* seems to conform to the *Afrophlaeoba* data, but the differing dispersal power of the taxa does not allow any further comparison.

8.9 The Need of Future Faunistic Research in Tanzania

Although the faunistic exploration of Tanzania started more than 100 years ago, there is still a mass of undescribed species, waiting to be studied. Taxonomy is the basis for any biological research, but the number of taxonomists is decreasing, even though the dimensions of biodiversity are estimated to be enormous in the tropics (Erwin 1982). Although the high importance of insects for biodiversity is generally well known, most of the present research activities in the Eastern Arc and Coastal Forests of Tanzania focus on plants and vertebrates (Lovett & Wasser 1993). A short examination of some Orthoptera collected from Coastal Forests of Tanzania by “Frontier Tanzania” and kept at the Zoologisk Museum in Copenhagen included approximately 100 species with 10-15 yet undescribed species, although Orthoptera are taxonomically comparatively well known. This might illustrate that taxonomical research on insects is urgently needed in the area. Some grasshopper groups are in need

of radical revision, including some genera with major radiation in the Eastern Arc and Coastal Forests (e.g. *Ixalidium*, *Eupropacris*, *Chromomastax*, *Euschmidtia*). There is still a lot of material in the museums to be studied and some areas are also badly collected. Faunistic studies in Tanzania should focus on insects in separated ecological zones, such as the evergreen forest areas, including not only the Eastern Arc, but also the Southern Highlands, Mbizi Mts. and Mahali at Lake Tanganyika, the Lake Victoria region, the islands of Pemba, Zanzibar and Mafia and the Coastal Forests. Additionally, the arid zones in the rainshadow of those mountain ranges seem to possess a high number of endemics (Lovett 1988). The fauna of the canopy is still badly known and some mountain ranges (e. g. Udzungwa Mts., Nguu Mts., Rubeho Mts., Pare Mts.) have rarely been visited by entomologists. Obviously there is also a need for studies of the faunas of gallery forests, which might interconnect the forest refuges. It seems appropriate to include the gallery forests of Tanzania into future research, since they might represent important links and provide answers to the history of interconnections. The crucial need for faunistic exploration of Eastern Africa is illustrated by the rapid deforestation. Habitat fragmentation is known to cause extinction (Samways & Sergeev 1997). Probably many species will become extinct, before they are described. Jago (1973) stated that forest speciation and adaptation are an evolutionary dead end and the forest ecosystem a species sink, “continuously seducing species into its confines and then eliminating them by fading away during dry periods.” At present, deforestation by man is a much more severe problem than natural desiccation, thus confirming Jago’s assumptions in a frightening way.

9 Summary

The Eastern Arc Mountains in Tanzania are considered to be one of the principal biodiversity hotspots of mainland Africa. A high number of endemics with very limited ranges are known from here and many species have vicariant distribution patterns. According to the refuge concept these patterns are mainly derived through allopatric speciation of populations during periods of habitat fragmentation. The genus *Afrophlaeoba* is a typical example of such a group. It consists of four geographically separated species, which occur in the East Usambara Mountains, Uluguru Mountains, Nguru Mountains and Rubeho Mountains, respectively. Due to their restricted distributions the species have been regarded as relicts of past forest expansions. Four separate lines of evidence have been studied here, namely the molecular systematics, morphology, ecology and communication behaviour, in an attempt to elucidate the evolutionary history of these species.

Three mtDNA segments have been sequenced from the four species of *Afrophlaeoba*, two species of the sister genus *Parodontomelus*, and two species of the morphological similar, but less related genus *Odontomelus*. The phylogenetic inference was obtained by two methods, neighbor joining and maximum parsimony. With both methods a structurally similar gene tree was obtained, rooted with the outgroup *Odontomelus*. *Parodontomelus* proved to be a monophyletic group in this gene tree, as did *Afrophlaeoba*. In the latter genus, two distinct groups were distinguishable. The first group, the *nguru-longicornis* group, was supported by high bootstrap values and extremely low genetic distances. The second group, the *euthynota-usambarica* group, was less stable in the gene trees, but was still supported by quite high bootstrap values.

Twenty-seven body dimensions were measured from thirty males of each of the four *Afrophlaeoba* species and *P. arachniformis*. Additionally, sixty-six discrete morphological characters were studied, including external characters and internal features of the male genitalia. The discrete characters provided no reliable differences between the four species, and thus a classical phylogenetic analysis was not possible. A phenetic analysis of morphometric discontinuity was made by means of a discriminant analysis. The four species were clearly discriminated from *P. arachniformis* in a first analysis, illustrating the high intergeneric distances. In a second analysis the intraspecific multivariate structure was analysed. The smallest species, *A. euthynota* was discriminated in the first function and *A. usambarica* in the second. The species of the *nguru-longicornis* group had the lowest distances, but the differences were significant and the discriminating power still high. Based upon the roots of the squared generalized distances, a neighbor joining phenogram was obtained, to illustrate the morphometric affinities. This phenogram did not differ structurally from the gene trees inferred from molecular data. An evaluation of the present taxonomical descriptions and keys demonstrated that most species are insufficiently described and not identifiable on the basis of single characters alone.

In the third part of the study, male communicative behaviour of the *Afrophlaeoba* species and *P. arachniformis* has been examined by means of videography of field records. The frame-by-frame analysis of the visual displays showed that significant differences are present between all species.

P. arachniformis included dephased low-frequency hind leg movements with knee waving, palpi movements and minor antennal movements in the display, while *Afrophlaeoba* species performed medium-frequency hind femur vibrations with major antennal movements. The movements were performed in typical short strokes with intervals between. In all *Afrophlaeoba* species the frequency of the strokes decreased within the display. Average frequencies, duration and antennal amplitudes differed significantly between species. The highest difference in antennal amplitudes occurred in the two closely related species *A. nguru* and *A. longicornis*. However, the extent of intraspecific variation has not yet been adequately studied and the significance of these differences in mate recognition remains unknown.

For the ecological part, the habitat preferences of the four *Afrophlaeoba* species and *P. arachniformis* were studied to gain some information about niche differentiation between the species and possible ancestral ecology. The four *Afrophlaeoba* species had rather similar habitat preferences, while *P. arachniformis* differed more strongly from these. All *Afrophlaeoba* occurred in low and dense vegetation, which is dominated by grasses. They were not found in closed forest, but rather at the forest edge, in clearings and even under single trees with dense canopy. In contrast, *P. arachniformis* is associated with the forest interior. Both genera feed exclusively on grasses, but they show substantial differences in phenology. While *Afrophlaeoba* females were closely associated with the ground litter, males were often located on grasses. Species which were studied at higher altitudes were found in environments with lower temperatures but higher radiation. Species, studied in the lowlands, experienced higher temperatures, but lower radiation. This trend was consistent with a higher restriction to dense canopy trees in the lowlands. At study sites with relatively high disturbance, parasitic mites were recorded more frequently.

The main conclusions from these results can be summarized as follows. Based upon the possible ancestral biology of *Afrophlaeoba*, a closed forest connection between the species was not necessary for gene flow, but the presence of trees with dense canopy is required. Possible interconnections may have existed via riverine forests or through the Coastal Forest zone. A scenario for species divergence is presented, starting with an ancestral species, distributed in the coastal zone, riverine forests and some of the Eastern Arc mountains. An initial split is proposed for the area in the Zanzibar rainshadow, which separated first the *nguru-longicornis* group from the *euthynota-usambarica* group and shortly after that the two species *A. euthynota* and *A. usambarica*. The exact dating of this event is not possible, but a rough estimate suggests that the fragmentation occurred during the Plio-Pleistocene. The two species *A. nguru* and *A. longicornis* remained in contact for much longer. The divergence did not start before the Pleistocene, if those two species were isolated at all. The higher genetic distances within the genus *Parodontomelus* suggest that this genus might have been isolated more effectively, due to its restriction to closed forest. In conclusion, the genus *Afrophlaeoba* has to be regarded as a recently radiated group of forest edge species, which are morphologically difficult to disentangle but morphometrically and genetically distinct.

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