66 (1) 3–17

A Molecular and Morphological Phylogenetic Analysis of Afrotropical *Monolepta* Species and Related Galerucinae (Coleoptera: Chrysomelidae)

Heidi Stapel [1], Bernhard Misof [2] & Thomas Wagner [3*]

[1] Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Adenauerallee 160, 53113 Bonn, Germany [heidi.ehrentraut@ukb.uni-bonn.de]
[2] Biozentrum Grindel und Zoologisches Museum, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany [bernhard.misof@uni-hamburg.de]
[3] Universität Koblenz-Landau, Institut für Integrierte Naturwissenschaften – Biologie, Universitätsstr. 1, 56070 Koblenz, Germany [thwagner@uni-koblenz.de]

* Corresponding author (28th contribution to the taxonomy, phylogeny, and biogeography of Afrotropical Galerucinae)

Received 3.i.2007, accepted 25.i.2008.
Published online at www.arthropod-systematics.de on 30.vi.2008.

> Abstract

The phylogenetic status of Afrotropical galerucines was investigated with molecular and morphological analyses. The taxon sample analysed comprised 15 species within *Monolepta*, three within *Afrocandezea*, two each within *Afrocrania* and *Barombiella* and one *Pseudocrania* species; all were originally placed in "Monoleptites". Further galerucines outside the "Monoleptites" are *Diacantha* sp., *Exosoma polita*, *Exosoma* sp., *Galerudolphia tenuicornis*, and *Parasbecesta ruwensorica*. The chrysomeline *Linaeidea nubila* was included as outgroup. 35 morphological characters including 16 characters on genital morphology were analysed. A 540 bp mitochondrial DNA NADH dehydrogenase subunit 1 (ND1) fragment and the entire second internal transcribed spacer region ITS2 (519–709 bp) of the nuclear ribosomal DNA were sequenced from 22 and 24 taxa, respectively. Both molecular data sets were characterized by a high average A-T content of 86.4% (ND1) and 62.7% (ITS2). Trees of separate and combined data sets were reconstructed with Maximum Parsimony (MP) and Maximum Likelihood (ML) approaches. The congruent tree topologies of both morphological and molecular data sets strongly support the monophyly of *Monolepta*, *Afrocrania* and *Afrocandezea* with regard to recently revised Afrotropical representatives. *Barombiella* emerged as polyphyletic, on species showing close relationship to *Galerudolphia tenuicornis*, which is traditionally placed in the "Scelidites". "Monoleptites" is most likely polyphyletic since its decisive character, the elongated metatarsus, obviously evolved more than once in the Galerucinae. Understanding of the phylogenetic position and delimitation of the taxa primarily based on morphological characters could be significantly improved by molecular data.

> Key words

Monolepta, Galerucinae, Afrotropis, molecular phylogeny, nuclear ITS2, mitochondrial ND1, morphology.

1. Introduction

The Galerucinae comprise about 5500 described species and have a worldwide distribution with a concentration in the tropical and subtropical regions. They are closely related to the flea beetles, Alticinae, and both taxa form a well supported monophyletic group (e.g. Reid 1995; Lingafelter & Konstantinov 2000). Alticinae are recognized as a well supported monophyletic group by many coleopterists, having a specific metafemoral jumping mechanism as the most important apomorphic character (Furth 1990; Furth & Suzuki 1994; Konstantinov 1994). Galerucinae are presumably paraphyletic with Alticinae as a subordinated clade (Lingafelter & Konstantinov 2000), while a recent molecular analysis of some taxa leads to a reverse result with Galerucinae as monopyhletic crown group and Alticinae as paraphyletic base (Kim

et al. 2003). Generally, the phylogenetic relationships within the Galerucinae are not well understood, and the usual suprageneric classification (WILCOX 1973; SEENO & WILCOX 1982) might be mostly typological.

Our recent studies focus on the taxonomy and phylogeny of the species-rich Afrotropical galerucines that are traditionally placed in the section "Monoleptites" as in the most recent catalogue (WILCOX 1973). These taxa can be distinguished from others by slender legs and a basi-metatarsus much longer than the remaining tarsomeres. 306 species from the Afrotropical region (excluding Madagascar) have been described in this group, mostly in *Monolepta* Chevrolat, 1836 (180 spp.), *Candezea* Chapuis, 1879 (39 spp.) and *Barombiella* Laboissière, 1931 (42 spp.). Smaller genera are *Afrocrania* Hincks, 1949 (with its junior homonym *Pseudocrania* Weise, 1892) (16 spp., MIDDELHAUVE & WAGNER 2001, WAGNER 2007a), and *Afrocandezea* Wagner & Scherz, 2002 (12 spp., SCHERZ & WAGNER 2007). Many *Monolepta* and *Candezea* species are also known from Asia and Australia, while *Barombiella*, *Afrocrania* and *Afrocandezea* are restricted to Africa. Regional species richness and endemism of Afrotropical *Monolepta* correlate with isolated montane zones, especially with the Ethiopian Highlands, mountains in southern Kenya and northern Tanzania, along the Albertine Rift in Uganda, Rwanda, and Kivu, and finally montane areas in Cameroon (WAGNER 2001b).

Due to the unsatisfactory α-taxonomy of most "Monoleptites" a revision was started some years ago (e.g. WAGNER 2000; STAPEL & WAGNER 2000; WAGNER 2001a,c, 2002, 2003a,b, 2005; HASENKAMP & WAGNER 2000). Many of the 180 Afrotropical species originally described in *Monolepta* were found to be not closely related to the type species of the genus, *Monolepta bioculata* (Fabricius, 1781) (WAGNER 2007c), and consequently, these species were transferred to other genera (HASENKAMP & WAGNER 2000; SCHMITZ & WAGNER 2001; STAPEL & WAGNER 2001; WAGNER & SCHERZ 2002; FREUND & WAGNER 2003; WAGNER & KURTSCHEID 2005). Additionally, many synonyms were identified and only about 50 valid species remain in the genus *Monolepta* (WAGNER 2003a). The same number of new species was recently described or awaits description (WAGNER 2000, 2001a,c, 2002, 2003b, 2005). The shape of the median lobe of the male genitalia and its endophallic structures are decisive characters for both taxonomic delimitation of species and recognition of monophyletic taxa then considered genera. We also found strong similarities between taxa in "Monoleptites" and others placed outside this group, such as *Galerudolphia* Hincks, 1949 from "Scelidites" (WILCOX 1973; SEENO & WILCOX 1982). The latter genus was also revised recently (BOLZ & WAGNER 2005) and representatives are included in the present study.

The main objective of the study presented here is to establish a set of suitable molecular markers which can either confirm or decisively contradict phylogenetic results based on morphological characters. We expect that the comparative analysis of molecular and morphological character sets will further increase our taxonomic knowledge in this notoriously difficult group. Finally, the establishment of suitable molecular markers will be an important step towards a molecular phylogeny within galerucines. The molecular analysis was based on a 540 bp fragment of the mitochondrial NADH dehydrogenase subunit 1 (ND1) and the adjacent $tRNA^{Leu}$, and the entire nuclear ITS2 region with portions of flanking 5.8S and 28S rRNA coding regions. Several phylogenetic studies in arthropods were based on ITS2 (TAUTZ et al. 1988; PASKEWITZ et al. 1993; SCHLÖTTERER et al. 1994; GÓMEZ-ZURITA et al. 2000; ROKAS et al. 2002; CLARK et al. 2001; MALLOCH et al. 2001; DEPAQUIT et al. 2000), and ND1 (VOGLER & DESALLE 1993; TAMURA 1992).

2. Materials and Methods

2.1. Taxon sample

Representatives of 23 species of the Galerucinae traditionally assigned to the "Monoleptites" sensu WILCOX (1973) were included. This comprises about 10% of the known Afrotropical species: 12 of about 100 species of *Monolepta* s.str.; 2 of 17 species of *Afrocrania* from eastern Africa; 3 of 8 species of *Afrocandezea* from East- and Central Africa; *Monolepta advena* and *Monolepta duplicata* from East Africa, which represent a distinct, but not yet described genus of about 15 species; and 2 of about 14 species which have been placed in *Barombiella* up to now, but represent a new species-group different from the genus type of *Barombiella* (WAGNER & FREUND 2003). Since this taxonomic change is not yet published, the species are named in the old combination throughout the manuscript. Additionally, five further Afrotropical Galerucinae species (*Diacantha* sp., *Exosoma politum*, *Exosoma* sp., *Galerudolphia tenuicornis*, *Parasbecesta ruwensorica*) were included. The African Chrysomelinae species *Linaeidea nubila* was included as outgroup, since Chrysomelinae are most likely the sister-group to the Galerucinae. The entire taxon sample is listed in Tab. 1.

In our morphological data set 35 characters of 27 species were scored. 16 characters were based on male

Tab. 1. List of studied taxa, origin of specimens, and Genbank accession numbers. Morphological characters were studied in listed taxa with exception of *Afrocandezea vicina*, *Exosoma* sp., and *Monolepta chiron*. Taxonomic assignment (TA) in 2nd column: Subfamily-Tribe (after SEENO & WILCOX 1982); Gal = Galerucinae; Chr = Chrysomelinae; Age = Agelasticites; Aul = Aulacophorites; Exo = Exosomites; Mon = Monoleptites; Scl = Scelidites.

Taxon	TA	Collecting place	Genbank Accession Numbers	
			ND1	ITS2
Agelastica alni (Linnaeus, 1758)	Gal-Age	Germany (Bonn)	-	-
Afrocandezea rostrata (Laboissière, 1920)	Gal-Mon	Kenya (Kaimosi)	AY116119	AY116089
Afrocandezea tutseki Scherz & Wagner, 2002	Gal-Mon	Kenya (Kakamega Forest)	AY116118	AY116088
Afrocandezea vicina (Gahan, 1909)	Gal-Mon	Uganda (Budongo Forest)	-	-
Afrocrania kakamagaensis Middelhauve & Wagner, 2001	Gal-Mon	Kenya (Kakamega Forest)	AY116116	AY116086
Afrocrania pauli (Weise, 1903)	Gal-Mon	Kenya (Aberdare Mountains)	-	AY116110
Barombiella acutangula (Weise, 1903)	Gal-Mon	Kenya (Kakamega Forest)	AY116136	AY116103
Barombiella vicina Laboissière, 1931	Gal-Mon	Kenya (Kakamega Forest)	AY116124	AY116094
Diacantha sp. Chevrolat, 1836	Gal-Aul	Kenya (Teita Hills)	AY116112	AY116083
Exosoma polita (Jacoby, 1882)	Gal-Exo	Uganda (Budongo Forest)	-	-
Exosoma sp. Jacoby, 1903	Gal-Exo	Kenya (Teita Hills)	AY116139	AY116106
Galerudolphia tenuicornis Jacoby, 1899	Gal-Scl	Ivory Coast (Comoe NP)	AY116122	AY116092
Linaeidea nubila (Weise, 1912)	Chr	Uganda (Budongo Forest)	-	-
Monolepta advena Weise, 1909	Gal-Mon	Kenya (Teita Hills)	AY116120	AY116090
Monolepta alluaudi Laboissière, 1920	Gal-Mon	Kenya (Mt. Kenya)	-	AY116109
Monolepta citrinella Jacoby, 1899	Gal-Mon	Kenya (Kakamega Forest)	AY116134	AY116101
Monolepta chiron Wilcox, 1973	Gal-Mon	Kenya (Kakamega Forest)	AY116123	AY116093
Monolepta clienta Weise, 1907	Gal-Mon	Ivory Coast (Comoe NP)	AY116130	AY116098
Monolepta comoeensis Wagner, 2000	Gal-Mon	Ivory Coast (Comoe NP)	-	-
Monolepta deleta Weise, 1903	Gal-Mon	Kenya (Mt. Kenya)	AY116128	AY116097
Monolepta duplicata (Sahlberg, 1829)	Gal-Mon	Kenya (Kakamega Forest)	AY116121	AY116091
Monolepta elegans Chevrolat, 1837	Gal-Mon	Kenya (Aberdare Mountains)	AY116127	AY116096
Monolepta ephippiata Gerstaecker, 1871	Gal-Mon	Kenya (Mpala)	AY116131	AY116099
Monolepta leuce Weise, 1903	Gal-Mon	Kenya (Aberdare Mountains)	AY116126	AY116095
Monolepta naumanni Wagner, 2005	Gal-Mon	Kenya (Kakamega Forest)	AY116133	AY116100
Monolepta panicea Bryant, 1948	Gal-Mon	Ivory Coast (Comoe NP)	AY116132	-
Monolepta togoensis Laboissière, 1920	Gal-Mon	Kenya (Kakamega Forest)	AY116135	AY116102
Monolepta vincta Gerstaecker, 1871	Gal-Mon	Kenya (Teita Hills)	-	AY116108
Parasbecesta ruwensorica (Weise, 1920)	Gal-Aul	Kenya (Kakamega Forest)	AY116113	AY116084
Pseudocrania semifulva Bryant, 1956	Gal-Mon	Kenya (Kakamega Forest)	AY116115	AY116085

and female genitalic morphology (see some examples in Figs. 3–13), 19 on adult external morphology and coloration (Figs. 14–22) (Tab. 2). Morphometrics have been carried out for external characters. For each species eight males and eight females were measured. All characters were treated as unordered and given equal weight (Tab. 3). Therefore, state "0" does not a priori imply an ancestral condition. Details on some genitalic and external morphological characters are given, for further illustrations see WAGNER (2003a).

Materials for our molecular studies were recently collected in West, Central and East Africa. We were able to obtain ND1 sequences of 22 species and ITS2 sequences of 24 species (Tab. 1). For *Afrocrania pauli*, *Monolepta alluaudi*, and *Monolepta vincta* ND1 could not be amplified, for *Monolepta panicea* ITS2 is missing.

2.2. DNA extraction and amplification

DNA was extracted using either a Chelex-protocol or CTAB extraction. Depending on the size of specimens thorax and/or legs were homogenized. For Chelex extraction 10–25 mg of tissue was ground in 500 µl Chelex solution (Sigma-Aldrich), 50–75 µl of which were Chelex beads, and material was incubated for 2 h at 65°C, then 5 min at 95°C and vortexed 10–15 sec. CTAB DNA extraction method was performed after GUSTINICICH's et al. (1991) protocol. Tissue was transferred into warm 500 µl CTAB-Buffer and heated up to 65°C. 10 µl Proteinase K was added and the solution was incubated for 1–3 h at 65°C. The aqueous phase was separated using a chloroform-isoamyl-solution followed by an ethanol precipitation of the DNA.

The ND1 fragment was amplified using the forward primer 5'-TAG AAT TAG AAG ATC AAC CAG C-3', named N1-J-12201 (WELLER et al. 1994) and the reverse 5'-ACA TGA TCT GAG TTC AAA CCG G-3', named LR-N-12866 (SIMON et al. 1994). The PCR reaction mixture contained 16.9 µl ddH_2O, 2.5 µl 10x PCR buffer (Sigma), 2.0 µl $MgCl_2$ (25 mM), 0.2 µl of each primer (20 pM/µl), 0.5 µl dNTPs (2mM), 1 U of Sigma Taq Polymerase and 2.5 µl template DNA. PCR-touchdown conditions were as follows: 94°C for 4 min; 15 cycles of 92°C for 30 s, 52°C (reduced for 1°C each cycle until 37°C was reached) for 30 s, 72°C for 1 min; 25 cycles of 92°C for 30 s, 50°C for 30 s, 72°C for 1 min; and a final extension of 72°C for 8 min. PCR was performed on Biometra T-gradient and UnoII cyclers (BIOMETRA).

ITS2 was amplified by using two different primer pairs. One pair included the ITS4 primer 5'-TCC TCC GCT TAT TGA TAT GC-3' (GÓMEZ-ZURITA et al. 2000) and ITS3 primer 5'-GCA TCG ATG AAG AAC GCA GC-3' (WHITE et al. 1990). Using this primer pair required adding formamide and performing a hotstart-PCR. A master mix included 16.5 µl ddH_2O, 2.5 µl 10 x PCR buffer (Sigma), 1.75 µl $MgCl_2$ (25 mM), 0.4 µl of each primer (20 pM/µl), 0.5 µl dNTPs (2mM/µl), 1 U of Sigma Taq Polymerase, 2.5 µl template DNA and 0.25 µl formamide (final concentration 2%). PCR cycling conditions were 94°C for 3 min; 38 cycles of 94°C for 35 s, 48°C for 30 s, 72°C for 1 min; final extension at 72°C for 10 min. For the second primer pair (5'-GGA TCG ATG AAG AAC G-3', 5'-GCT TAA ATT CAG CGG-3'; WEEKERS et al. 2001) an identical PCR set up as for ND1 gene fragments was used. PCR products were purified with the SIGMA Gen-Elute PCR DNA Purification Kit. For cycle sequencing the reaction mix included 2.0 µl Ready-Mix (ABI Prism Big Dye ™), 1.0 µl primer (10 pM/µl), 1–6 µl DNA, 1–6 µl ddH_2O. PCR conditions were 94°C for 2 min, 25 cycles of 92°C for 15 s, 50°C for 15 s, 60°C for 2.5 min, 8 cycles of 93°C for 20 s, 60°C for 15 s. The cycle sequencing products were precipitated with ethanol prior to automated sequencing (Applied Biosystems 377 DNA sequencer).

2.3. Alignment

ClustalX (THOMPSON et al. 1997) was used to align sequences using the default parameters. The ends of the sequences contained the primer sequences and therefore were truncated. Sequence alignments were adjusted visually using Bioedit 5.0.9 (HALL 1999). Hyper-variable parts of ITS2 resulted in ambiguous alignment and therefore were excluded. Substitutional saturation was analysed by plotting the Ti/Tv-ratio between taxa. Inhomogeneity of base composition among taxa was checked using the Chi-Square-Homogeneity test as implemented in PAUP*4.0b10 (SWOFFORD 2002).

2.4. Tree construction

We performed maximum parsimony (MP) reconstructions on the morphological and the two molecular data sets separately and on a combined molecular data set. Heuristic searches using random addition of taxa with 1000 replications and TBR branch swapping on all starting trees were performed. For maximum likelihood (ML) reconstructions a substitution model was fitted separately to the ND1 and ITS2 data sets using the programs PAUP and MODELTEST (v.3.06) (POSADA & CRANDALL 1998). The likelihood ratio test routine was applied to search for optimal models in both data sets. Robustness of reconstructions was tested both with bootstrapping and calculation of Bremer support values in MP. For the morphological data set only Bremer support values were calculated. We used quartet puzzling support values in ML reconstructions, obtained from the maximum number of possible quartets. MP was performed on both morphological and molecular data sets whereas ML was only applied to the molecular data set.

Tab. 2. Morphological character codings used in phylogenetic analysis.

Male and female genitalia

1. Cornu slender, much longer than middle part of spermatheca (0) (Figs. 3, 4); slender, approximately of same length (1) (Figs. 4, 7); wide, of same length (2) (Fig. 6); cornu and middle part not distinguishable (3).
2. Nodulus of spermatheca large and cylindrical (0) (Fig. 7); large and spherical (1) (Figs. 3, 4); slightly produced (2) (Figs. 5, 6); nodulus and middle part not distinguishable (3).
3. Bursa sclerites weakly sclerotized and hardly recognizable (0); strongly sclerotized (1) (Figs. 8, 9).
4. If bursa sclerites strongly sclerotized: two pairs of different shape (0) (Figs. 8a,b); only one pair (1) (Fig. 9).
5. Median lobe of aedeagus compressed dorso-ventrally in the middle (0) (Fig. 10); round in cross-section (1) (Figs. 11, 12).
6. Median lobe not incised apically (0) (Figs. 10–12); incised (1) (Fig. 13).
7. Median lobe in dorsal view with cylindrical apical part (0) (Fig. 13); slightly narrowed in the apical part (1); strongly narrowed in the apical part (apex less than half of width at base) (2) (Fig. 10–12).
8. Median lobe at apex widened (0); parallel-sided (1) (Figs. 10, 12); conical (2) (Fig. 13).
9. Tectum of median lobe much longer than broad at base (0) (Figs. 10–12); much shorter than broad (1) (Fig. 13).
10. Tectum not incised (0) (Figs. 10, 11); incised (1) (Fig. 12).
11. Tectum short (0) (Figs. 10–12); tectum almost reaching apex of median lobe (1) (Fig. 13).
12. Insertion of tegmen approximately in middle of aedeagus (0) (Fig. 10); in basal third (1) (Fig. 11); in apical third (2) (Fig. 13).
13. Endophallic spiculae present (0) (Figs. 10–12); absent (1) (Fig. 13).
14. Endophallic spiculae entirely symmetrically arranged (0) (Figs. 10, 11); partly asymmetrical (1) (Fig. 12).
15. Endophallic spiculae only one type (0); two types (1) (Figs. 11, 12); three types (2) (Fig. 10).
16. Some endophallic spiculae with accessory spines (0) (Fig. 13); all without any accessory spines (1) (Figs. 11, 12).

External morphology

17. Pronotum approximately rectangular (0) (Figs. 16, 19–21); significantly trapezoidal (1) (Figs. 17, 22).
18. Posterior angles of pronotum rounded (0) (Figs. 16, 17); pointed (1).
19. Pronotum without transverse depression (0); with transverse depression (1).
20. Elytra entirely yellow or brownish (0) (Figs. 19, 20, 22); yellow and black (1); yellow, black, and red (2) (Fig. 21); red (3); red and yellow (4); red and black (5); metallic green or blue (6).
21. Elytra unicolor (0) (Figs. 19, 20, 22); anterior half black, posterior half red (1); large simple spots (2) (Fig. 21); black margins (3); transverse bands (4); spots and bands (5).
22. Palpi yellow or yellowish-brown (0); dark-brown or black (1).
23. Antennomeres 4–8 yellow or red (0); black or brown (1).
24. Antennomeres of ± same coloration (0); basal 2–4 antennomeres with different coloration than others (1).
25. Length of elytron to width of both elytra: < 0.60 (0) (Fig. 20); –0.65 (1); –0.70 (2) (Fig. 21); –0.75 (3) (Fig. 19); > 0.75 (4).
26. Length to anterior width of mesosternum: < 0.80 (0); –0.90 (1); –1.00 (2) (Fig. 18); –1.10 (3); > 1.10 (4).
27. Maximal width (seen from frontal) of eye to minimal distance between eyes: < 0.40 (0); –0.50 (1); –0.60 (2); –0.70 (3); > 0.70 (4).
28. Length of antennomere 2 to 3: < 0.50 (0); –0.60 (1) (Fig. 15); –0.70 (2); –0.80 (3); –0.90 (4); –1.00 (5); > 1.00 (6) (Fig. 14).
29. Length of antennomere 3 to 4: < 0.40 (0) (Fig. 14); –0.50 (1); –0.60 (2); –0.70 (3) (Fig. 15); –0.80 (4); –0.90 (5); > 0.90 (6).
30. Midline length to maximal width of pronotum: < 0.45 (0); –0.50 (1); –0.55 (2); –0.60 (3) (Figs. 17, 19, 22); –0.65 (4); > 0.65 (5) (Figs. 20, 21).
31. Total length: < 4.0 mm (0); –5.0 mm (1); –6.0 mm (2); –7.0 mm (3); > 7.0 mm (4).
32. Length of basi-metatarsus to length of metatibia: < 0.40 (0); –0.45 (1); –0.50 (2) (Figs. 19, 20); < 0.55 (3) (Fig. 22).
33. Sexual dimorphism of elytra absent (0) (Figs. 19, 20, 22); as small postscutellar extrusions in males (1); as broad, bulged extrusions in males (2) (Fig. 20).
34. Sexual dimorphism of head absent (0); as shallow depression at frons in males (1).
35. Sexual dimorphism of antennae absent (0); with broad, bulged antennomeres three to five in males (1).

3. Results

3.1. Morphology-based trees

With our species sample we found that most of the genera examined (*Afrocandezea*, *Afrocrania*, *Barombiella*) form distinct clades. *Monolepta* is split in two groups, where *M. advena* + *M. duplicata* are separated from all other *Monolepta* species, which also form a distinct group, named *Monolepta* s.str. in the following (Fig. 1). *Monolepta advena* + *M. duplicata* can easily be distinguished from *Monolepta* s.str. by strongly elongated third antennomeres, slenderer pronotum and elytra, and distinct differences in genitalic characters. These species will be separated together with some others originally described in *Monolepta* and *Candezea* under a new genus name (Th. Wagner in prep.).

Monolepta s.str. shows three autapomorphies: it possesses two pairs of strongly sclerotized bursa sclerites (character 4 (0); Fig. 8) (Tabs. 2, 3), three types of endophallic spiculae are present (15 (2); Fig. 10), and some endophallic spiculae have accessory spines (16 (0); Fig. 10). Spermathecal noduli are large and spherical (2 (1); Fig. 3), the tegmen is inserted in the middle of the aedeagus (12 (0); Fig. 10), endophallic spiculae are symmetrically arranged (14 (0)), and the second and third antennomeres are approximately of the same length (28 (4/5/6); Fig. 14), whereas the third antennomere is less than half as long as the fourth (29 (0/1)). *Monolepta* s.str. appears as a well supported monophyletic group, but the morphological data set is insufficient for a phylogenetic differentiation within the genus, since all 12 true *Monolepta* species studied form one polytomous clade.

On the supra-generic level, *Afrocrania* + *Afrocandezea* + *Pseudocrania* form one distinct clade. They share many similarities, e.g. form and length of basal antennomeres, the size and form of the pronotum, and the shape of the median lobe (but not endophallic armatures), bursa sclerites and spermatheca. These taxa share the presence of (only) one pair of strongly sclerotized bursa sclerites (4 (1); Fig. 9), insertion of the tegmen in the basal third (12 (2); Fig. 11), and a single type of mostly symmetrically arranged endophallic spiculae (15 (3)). *Afrocandezea* can be distinguished from *Afrocrania* by an incised tectum (10 (1); Fig. 12a), partly asymmetrically arranged endophallic spiculae (14 (1); Fig. 12), broad elytra (25 (2)) and broad pronotum (30 (2); Fig. 19).

Barombiella species cluster with *Galerudolphia tenuicornis*, a species traditionally assigned to the sectio "Scleritides". The latter has only a moderately elongated basi-metatarsus, but shares several characters with *Barombiella* like an apically incised median lobe (6 (1); Fig. 13a), and insertion of the tegmen in the apical third (12 (2); Fig. 13b), conditions which are both presumably convergently developed to the clade *Monolepta advena* + *M. duplicata*, while the significantly trapezoidal pronotum (17 (1); Figs. 17, 22) is an exclusive character of the clade *Barombiella* + *Galerudolphia*.

Also apart from *Galerudolphia*, our morphological analysis does not confirm monophyly of "Monoleptites", as the genera from this group are placed in a large basal polytomy together with taxa from other tribes of Galerucinae.

3.2. Molecular sequences and their variability

The entire nuclear ITS2 with portions of flanking regions of the 5.8S and 28S rRNA genes was sequenced for 24 species. The length of sequenced fragments varied between 544 and 560 bp for all *Monolepta* specimens and altogether between 519 (*Pseudocrania semifulva*) and 709 bp (*Parasbecesta ruwensorica*). The 3' portion of 5.8S was 114 bp while the 5'portion of 28S was 26 bp for all taxa. The length of ITS2 varied from 379 to 569 bp. The conserved flanking regions (5.8S and 28S) were identified by comparing sequences of studied *Timarcha* leaf beetles (Chysomelinae; GÓMEZ-ZURITA et al. 2000). Alignment of the entire sequenced fragment resulted in a 885 character containing matrix, including indels and hypervariable portions. In the analysis 203 alignment-ambiguous and 355 uninformative positions were excluded, 327 parsimony-informative sites were considered. ITS2 contained 25% variable positions when gaps were treated as missing information and 37% with gaps treated as fifth state. No significant differences are found between taxa in terms of base composition ($N = 26$, $\chi^2 = 21.60$ (df = 90), P = 1.00). The average A-T-content is 62.7% (Tab. 4) and ranges from 44,3% (*Linaeidea nubila)* to 74,6% (*Parasbecesta ruwensorica*). ITS2 shows a mean Ti-Tv-ratio of 0.97. The average p-distance is 4.15%. Within the *Monolepta* clade, an average p-distance of 0.24% occurs (after exclusion of *Monolepta advena* + *Monolepta duplicata* and *Monolepta chiron*).

Furthermore, a mitochondrial fragment (533 to 536 bp), composed of a 378 bp ND1 and an approximately 162 bp $tRNA^{leu}$ segment, was sequenced from 22 species. The ND1 analysis was based on 146 parsimony-informative sites, and contained 28% variable positions. Seventeen alignment-ambiguous positions and gap-holding positions in the tRNA segment out of total 535 alignment sites were excluded and further

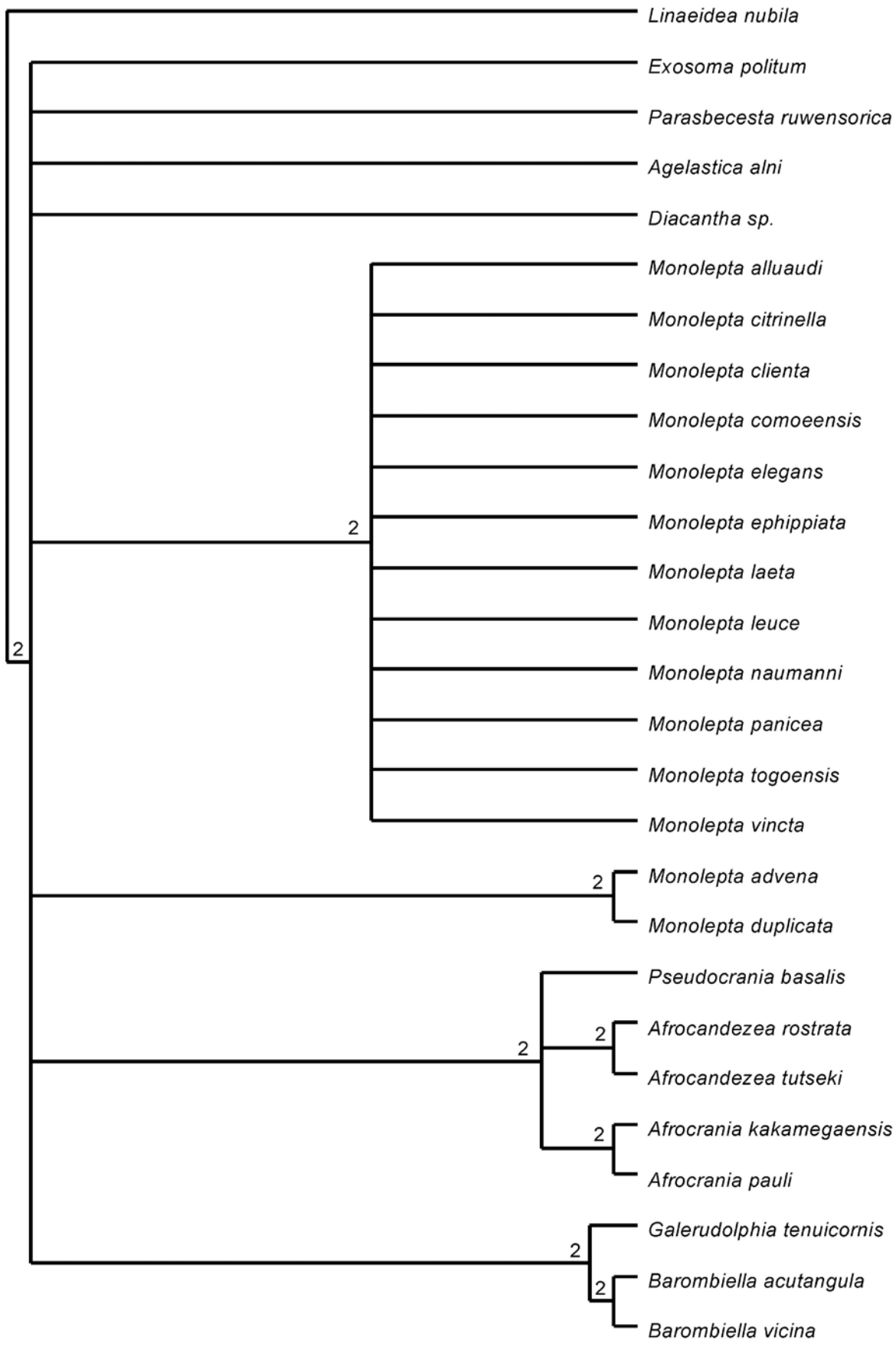


Fig. 1. Strict consensus tree for morphological data resulting from 680 most parsimonious trees obtained with heuristic search after 1000 sequence addition replicates (TL = 163 steps, CI = 0.50, RI = 0.68). From total 35 included characters 33 were parsimony-informative. Bremer support values are indicated at nodes.

Tab. 3. Morphological character matrix.

character	*Monolepta alluaudi*	*Monolepta citrinella*	*Monolepta clienta*	*Monolepta comoeensis*	*Monolepta elegans*	*Monolepta ephippiata*	*Monolepta laeta*	*Monolepta leuce*	*Monolepta naumanni*	*Monolepta paniceа*	*Monolepta togoensis*	*Monolepta vincta*	*Monolepta advena*	*Monolepta duplicata*	*Pseudocrania basalis*	*Afrocandezea rostrata*	*Afrocandezea tutseki*	*Afrocrania kakamegaensis*	*Afrocrania pauli*	*Exosoma politum*	*Galerudolphia tenuicornis*	*Barombiella acutangula*	*Barombiella vicina*	*Parasbecesta ruwensorica*	*Agelastica alni*	*Diacantha* sp.	*Linaeida nubila*
1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	1	1	2	1	1	1	2	1	0	3
2	1	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	2	2	3	0	0	0	2	1	2	3
3	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	-	-	1	1	1	1	1	-	-	-	-	-	-	-	-
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	0	0
7	1	2	1	2	2	2	2	2	1	1	1	1	0	0	2	2	2	2	2	1	0	0	0	1	0	0	0
8	2	2	1	0	1	2	1	1	1	1	1	1	2	2	0	1	1	1	2	0	2	2	2	2	1	2	1
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1	1	1	0	2	2	2	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	-	-	-	-	0	0	-
15	2	2	2	2	2	2	2	2	2	2	2	2	0	0	1	1	1	1	1	0	-	-	-	-	0	0	-
16	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	-	-	-	-	1	1	-
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0
20	3	0	2	3	2	1	4	4	2	5	2	1	2	1	5	3	0	0	0	0	0	0	1	6	6	2	6
21	0	0	2	0	2	5	2	2	4	1	3	4	2	4	1	0	0	0	0	0	0	0	4	0	0	4	0
22	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	1	0	1
23	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1
25	2	2	2	2	1	2	3	2	3	2	2	2	0	0	2	2	3	0	0	1	0	4	2	3	4	2	3
26	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	0	1	0	0	4	0	3	0
27	3	4	3	3	3	3	3	3	3	4	3	3	3	3	0	0	0	0	0	0	1	2	0	0	2	0	0
28	6	5	4	6	4	5	5	5	6	5	5	5	2	2	2	1	1	2	2	2	3	1	1	3	3	0	1
29	0	1	1	0	1	0	1	1	0	0	0	1	3	3	3	3	3	3	3	5	3	5	4	4	3	5	6
30	3	4	4	3	5	4	4	4	3	4	3	4	5	5	5	2	3	5	5	5	3	2	2	5	2	3	0
31	2	1	2	2	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	1	0	1	2	4	4	3
32	2	2	2	2	3	2	2	3	2	3	2	3	2	2	1	2	2	2	3	0	1	3	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	1	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

372 sites were uninformative. The sequenced fragment showed an A-T-content of 86.4% (Tab. 4), which did not change after exclusion of the tRNA segment. The ND1 gene had a remarkably low C-content (2.2%). The Ti-Tv-ratio was 0.64 and the exclusion of the tRNA segment made hardly any difference; transversions remained more frequent than transitions. Base composition does not differ significantly among species ($N = 22$, $\chi^2 = 52.40$ (df = 81), P = 0.99). The average p-distance in ND1 + tRNA sequences was

Tab. 4. Average base composition [%] of sequenced ITS2 and ND1 genes. *N* = number of specimens sequenced.

Gene	A	C	G	T
ND1 (*N* = 22)	50.64	2.24	11.35	35.77
ITS2 (*N* = 24)	29.83	19.93	17.35	32.89

4.7% within the *Monolepta* clade (excluding *M. leuce*, as well as *M. advena*, *M. duplicata*), and 11.6% among all taxa. As tree reconstruction results show, ND1 + tRNA is particularly suitable for resolving relationships among closely related taxa.

3.3. Molecular-based trees

MP analyses. In the phylogenetic analyses of ITS2 sequences, *Monolepta* s.str. excluding *M. chiron*, *Monolepta advena* + *M. duplicata*, *Afrocandezea*, *Afrocrania*, and *Barombiella vicina* + *Galerudolphia* are highly supported by bootstrap values of 100% each (Bremer support values ≥ 9). Within *Monolepta* s.str., *M. ephippiata* and *M. vincta* as well as *M. citrinella* and *M. naumanni* cluster in subgroups with support values of 95% and 83%, respectively. *Monolepta chiron* is placed within a monophyletic group of *Galerudolphia* and *Barombiella* species (bootstrap value 81%).

The tree based on the ND1 data set showed much lower resolution in the basal branching pattern than that from ITS2 data set, but it resolved phylogenetic relationships between closely related species. Both bootstrap values (≥ 99) and Bremer support values (≥ 8) in the apical clades are as high as those obtained in the ITS2 analysis. In the consensus tree obtained with a heuristic search, *Monolepta* s.str. excluding *M. chiron* is sister-group to the clade *Monolepta advena* + *M. duplicata*. Within *Monolepta* s.str., *M. leuce*, *M. elegans*, and *M. laeta* form a well supported subgroup (bootstrap value = 100). The analysis was repeated without the tRNA fragment. Bootstrap values became generally worse.

In the combination of both molecular data sets *Linaeidea nubila* was again used as outgroup (Fig. 2). Only the analysis with gaps treated as fifth character state is shown for taxa where both ND1 and ITS2 sequences were available. 410 characters were parsimony-informative in the combined analysis. *Monolepta* s.str., *Monolepta advena* + *M. duplicata*, and *Afrocandezea* emerged as monophyletic taxa with high bootstrap support (100). Within *Monolepta* s.str. an apical clade consisting of *M. leuce*, *M. elegans*, and *M. laeta* forms a well supported monophylum. On the suprageneric level *Afrocrania* + *Pseudocrania* + *Afrocandezea* is a well supported clade (Fig. 2). *Monolepta chiron* is outside *Monolepta* s.str. and the *Barombiella* appeared polyphyletic.

ML analyses. The evolutionary model best describing the present ITS2 data according to the likelihood-ratio-test was the K80 + Γ model (Ti-Tv-ratio = 1.35; α = 0.25; I = 0). In general, phylogenetic trees generated with the ML method were topologically similar to the MP trees. Therefore, ML results are not shown. However, in ML analyses non-monophyletic *Afrocandezea, Galerudolphia tenuicornis* and *Monolepta chiron* are allied with the remaining *Afrocandezea* + *Afrocrania* complex, including *Pseudocrania semifulva*, which clusters with *Barombiella vicina*. A sister-group relationship of *Monolepta advena* + *M. duplicata* and *Monolepta* s.str. gets low support. Resolution within the genus *Monolepta* is similar to that in MP analyses.

The suggested model after performing the likelihood-ratio-test for the ND1 data set is the GTR + I + Γ model, which took variable base frequencies and different rates among the six pairs of substitution into account: α = 0.67; I = 0.34. Rate matrix parameters estimated on the neighbour-joining tree were: R(a) [A-C] = 0.03, R(b) [A-G] = 26.37, R(c) [A-T] = 4.24, R(d) [C-G] = 4.01, R(e) [C-T] = 2.81, R(f) [G-T] = 1.00. Support values and resolution are low. However, the monophyly of *Monolepta* s.str., of the clade *Monolepta advena* + *M. duplicata*, and of *Afrocandezea* could be confirmed. Unlike former analyses, the sister-group of *Monolepta* consisted of *Afrocandezea*, *Afrocrania*, and also *Pseudocrania* but without significant support.

4. Discussion

4.1. Molecular variation

The A-T share in Galerucinae analysed in this study varied from 53–75% compared to 44% in *Linaeidea nubila*. A 66.9% A-T content obtained from *Diabrotica* (Galerucinae) by Clark et. al. (2001) lies within this range. *Timarcha* (Chrysomelinae) possess an A-T

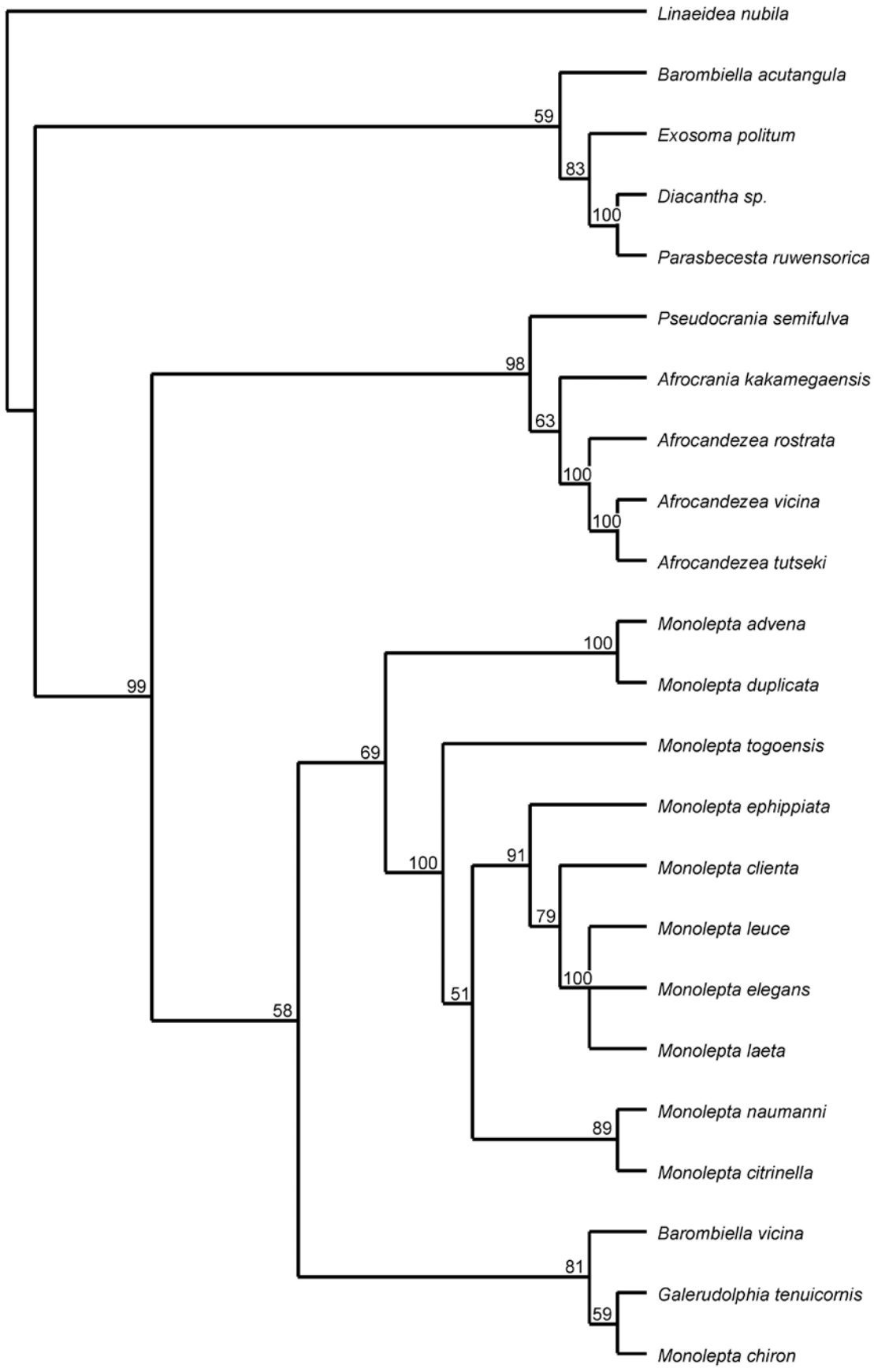


Fig. 2. Strict consensus tree from parsimony analyses for combined ND1 and ITS2 sequence-sets. Bootstrap support values > 50% of 1000 replicates are indicated at nodes. Only taxa with both sequences available were included. TL = 1167 steps, CI = 0.58, RI = 0.65 were recovered from four trees with heuristic search including 410 parsimony-informative sites. Gaps were treated as fifth state.

share of 53.3% (Gómez-Zurita et. al. 2000), Byturidae have an A-T content of 43.6% (Malloch et al. 2001). Also in other insect groups the A-T share varies greatly, e.g. in Diptera from 45% in *Anopheles* (Paskewitz et al. 1993) to 82% in *Drosophila* (Tautz et al. 1988; Schlötterer et al. 1994). ITS2 in our data shows a mean Ti-Tv-ratio of 0.97. The Ti-Tv-ratio is expected to decrease with increasing sequence distance because transversions erase the record of the more frequent transitions (Holmquist 1983). Schlötterer et al. (1994) even found a Ti-Tv-ratio of approximately 0.5 in *Drosophila*. The sequence length variation between 519 and 709 bp is due to insertions and deletions in different evolutionary lineages and high portions of variable simple sequence repeats (SSRs) along the sequence (Gómez-Zurita et al. 2000). This leads to alignment-ambiguous sites, which were excluded in tree reconstruction analyses.

Alignment of protein-coding ND1 genes was straightforward and conformed by the three-base codon reading frame. ND1 genes show 2.4 times more transitions and 4.5 times more transversions among ingroup taxa than ITS2. This possibly led to the poor resolution of early splitting events within the ND1 cladograms. Analyses of the 5' third of ND1 genes in Lepidoptera (Pashley & Ke 1992) showed a 70–76% share of transversions on total substitutions and a high level of homoplasy was found. Observations made on Galerucinae were similar to those results.

The mitochondrial ND1 gene fragment and the nuclear ITS2 gene appear as suitable markers to analyse the phylogeny within the studied Galerucinae, but ND1 only resolves young splitting events. Generally, tree topologies obtained from ML and MP are largely congruent.

4.2. Phylogenetic relationship of *Monolepta* and other Galerucinae

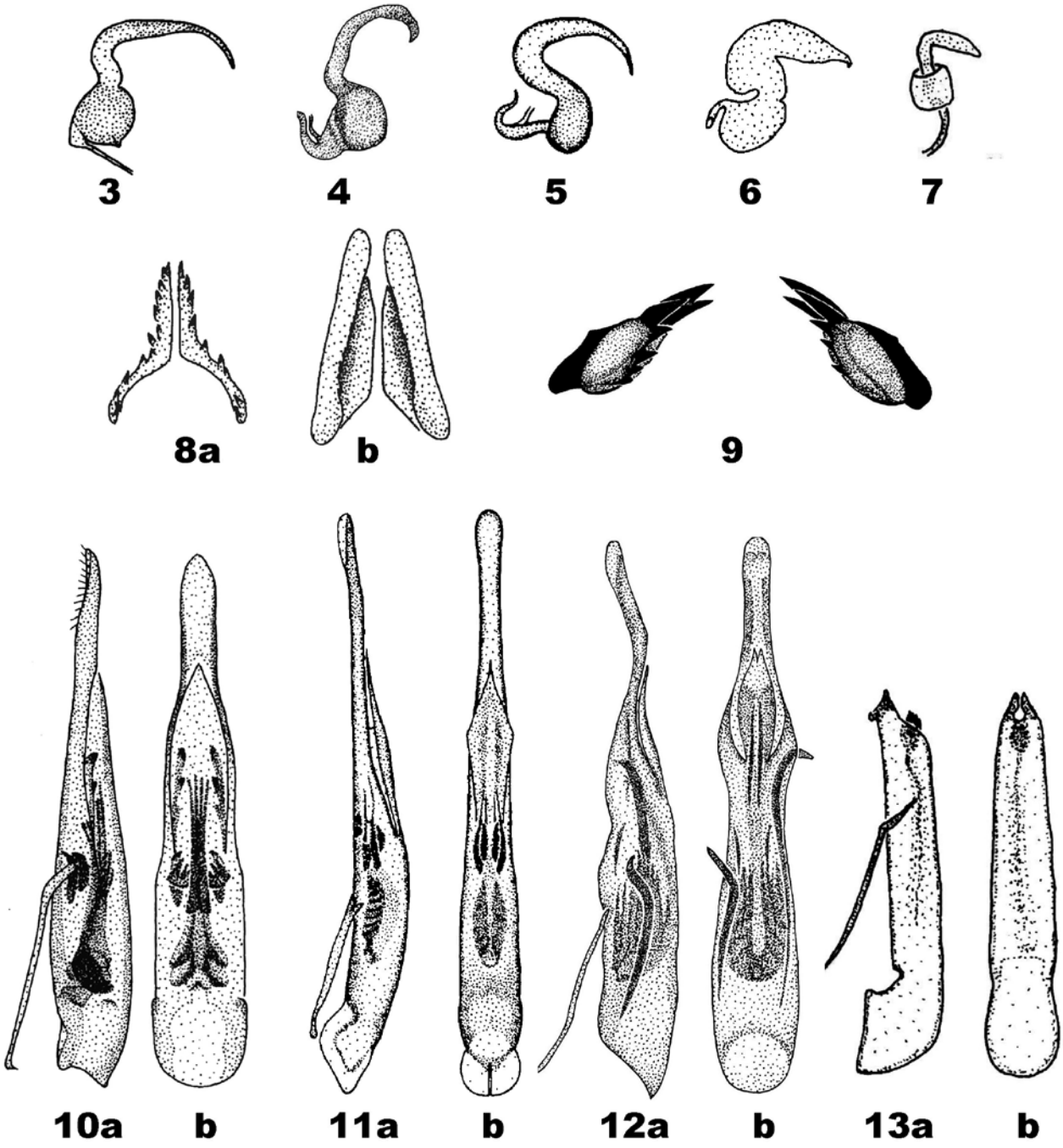
All three data sets gave valuable results on the phylogenetic relationships of *Monolepta* and closely related taxa. The number of included morphological characters in this study was less than a tenth of all parsimony-informative molecular characters. Nevertheless, the tree was similar to those based on molecular data, i.e. the results from the morphological data set is supported by the single and the combined molecular data sets (and vice versa). We conclude from this congruence that the molecular and morphological characters used are well suited to resolve relationships within the studied Galerucinae, in particular on genus level. The molecular data yield much additional resolution (compare Figs. 1 and 2). The extended application of these molecular markers promises a great potential for future phylogenetic analysis within Galerucinae.

Relationships between *Monolepta* s.str. species are only resolved by the molecular analyses. Highly supported is a close relationship between *M. citrinella* + *M. naumanni* and a clade consisting of *M. deleta*, *M. elegans*, and *M. leuce* in both, the ND1 and the ITS2 tree. This underlines the close relationship between these taxa, which share many morphological characters, like the general colour pattern, body form and size, as well as shape and endophallic armature of the median lobe (Wagner 2007b).

Only *Afrocrania* + *Pseudocrania* + *Afrocandezea* appears as a well supported monophyletic suprageneric taxon in all trees, while most other taxa on "genus level" are not differentiated in the morphological analyses. Only the molecular tree supported a sister-group relationship of *Monolepta* s.str. and the *Monolepta advena* + *M. duplicata* clade. These two taxa together appear as sister-group to *Galerudolphia tenuicornis* plus at least one of the two *Barombiella* species and *Monolepta chiron* in both molecular trees, but support of this clade is weak.

Most instructive, and important for the taxonomic revision, are the results on genus level. The analysed species of *Monolepta* s.str. (excluding *M. chiron*), *Afrocandezea*, *Afrocrania*, and the clade *M. advena* + *M. duplicata*, form highly supported monophyletic groups in the molecular, and with less support, in the morphological analyses. The results are very helpful for a modern phylogenetic allocation of species to genera. A genus is a categorial rank that is hardly to be defined objectively and not a monophyletic group in itself, but should be a monophyletic group. Taxonomic revisions of the above mentioned taxa benefit from phylogenetic evidence.

The only significant incongruence between the molecular and morphological tree can be found in the position of the two species of *Barombiella*. They form a distinct clade with a sister-group relationship to *Galerudolphia tenuicornis* in the morphological tree, while in both molecular trees only *Barombiella vicina* + *Galerudolphia tenuicornis* are supported as a monophyletic group. *Barombiella acutangula* appears as more closely related to *Diacantha* + *Parasbecesta* (+ *Exosoma*), species which are currently all placed outside the "Monoleptites" (Wilcox 1971, 1973; Seeno & Wilcox 1982). Several species originally described in *Barombiella* do not show the characteristic metallic coloration of the type species, *Barombiella violacea* (Jacoby, 1903), but many differences particularly in genitalic characters (cf. Wagner 2003a). This also includes *B. acutangula* and *B. vicina*. With its traditional composition *Barombiella* (Wilcox 1973) was surely a non-monophyletic group, and most metallic coloured



Genital morphology of Afrotropical Galerucinae

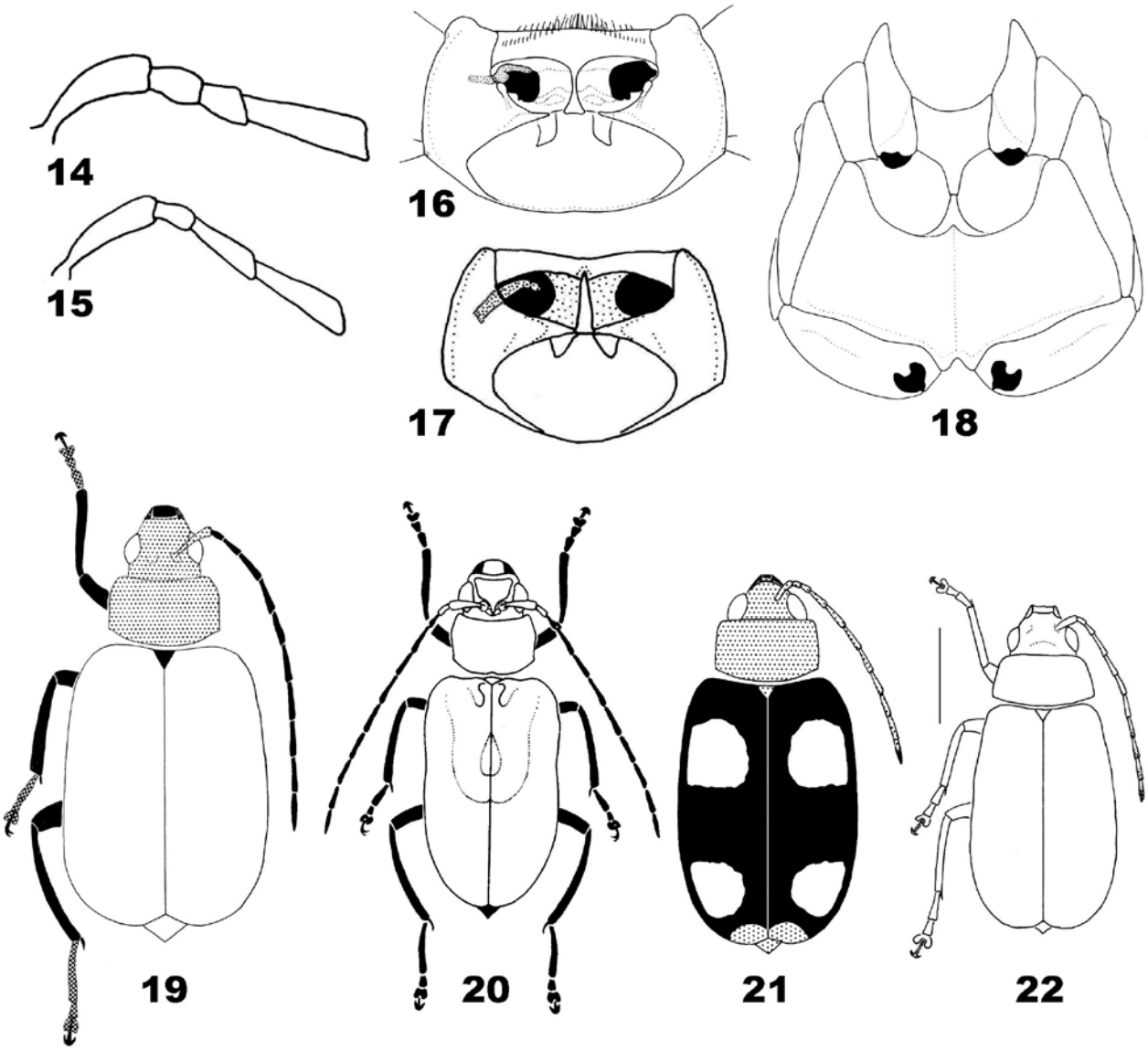
Figs. 3–7. Spermathecae. **3**: *Monolepta elegans* (original). **4**: *Afrocandezea tutseki* (WAGNER & SCHERZ 2002). **5**: *Afrocrania kakamegaenesis* (MIDDELHAUVE & WAGNER 2001). **6**: *Monolepta duplicata* (original). **7**: *Galerudolphia pallida* (BOLZ & WAGNER 2005).

Figs. 8, 9. Bursa sclerites. **8**: *Monolepta elegans* (a. dorsal, b. ventral; WAGNER 2007b). **9**: *Afrocandezea tutseki* (WAGNER & SCHERZ 2002).

Figs. 10–13. Aedeagus from lateral (a) and dorsal (b). **10**: *Monolepta elegans* (original). **11**: *Afrocrania kakamegaenesis* (MIDDELHAUVE & WAGNER 2001). **12**: *Afrocandezea tutseki* (WAGNER & SCHERZ 2002). **13**: *Galerudolphia pallida* (BOLZ & WAGNER 2005).

species have been recently transferred to *Bonesioides* (FREUND & WAGNER 2003). Since *Barombiella violacea* has many distinct characters, it is phylogenetically so isolated that it is left as the only species in the genus, i.e. *Barombiella* is now monotypic (WAGNER & FREUND 2003). Consequently, all other species originally described as *Barombiella* await transferation to other groups, and a revision on these taxa is in preparation.

There is no doubt that at least some species of *Barombiella* are closely related to *Galerudolphia*, as it is expressed in the clade based on morphological characters, and highly supported by both molecular



External morphology of Afrotropical Galerucinae

Figs. 14, 15. Basal four antennomeres. **14**: *Monolepta comoeensis* (Wagner 2000). **15**: *Afrocandezea tutseki* (Wagner & Scherz 2002).

Figs. 16, 17. Prothorax, ventral. **16**: *Monolepta comoeensis* (original). **17**: *Galerudolphia pallida* (Bolz & Wagner 2005).

Fig. 18. Meso- and metathorax of *Afrocandezea rostrata* (original).

Figs. 19–22. Habitus. **19**: *Afrocandezea tutseki* (Wagner & Scherz 2002). **20**: *Afrocrania kakamegaenesis* (original). **21**: *Monolepta elegans* (original). **22**: *Galerudolphia pallida* (Bolz & Wagner 2005).

trees. Actually, there are many similarities in body shape, coloration and genitalic characters of both sexes between these taxa. However, in *Barombiella acutangula*, these similarities are presumably homoplastic.

Furthermore, the data give some information on the traditional allocation of suprageneric groups like the "sectiones" in the last catalogue on the group (Wilcox 1971, 1973). *Galerudolphia* has been placed in the "Scelidites" and lacks the elongated basi-metatarsus, the crucial character of the "Monoleptites". Since the morphological and molecular data clearly underline the close relationship of the short-legged *Galerudolphia* (taxonomic revision: Bolz & Wagner 2005) and at least one *Barombiella* species with extraordinarily long legs, and on the other side, another long-legged *Barombiella* species seems to be closely related to *Diacantha* and *Parasbecesta*, which are both very short-legged, this character seems to be homoplastic (cf. Wagner 2004). Therefore, the polyphyly of the "Monoleptites" (Wilcox 1973; Seeno & Wilcox 1982) is highly probable on base of both morphological and molecular data, despite a comparatively small number of included species.

5. Acknowledgements

Thanks to Viola Clausnitzer and Carsten Mody for collecting and providing some alcohol material. We are grateful to Cort L. Anderson, University of Idaho, and the staff from the ZFMK molecular lab. Financial support was provided by the Deutsche Forschungsgemeinschaft (WA 1393/3–1).

6. References

BOLZ, H. & TH. WAGNER 2005. Revision of *Galerudolphia* Hincks, 1949 (Coleoptera: Chrysomelidae). – Insect Systematics and Evolution **35**: 361–400.

CLARK, T.L., L.J. MEINKE & J.E. FOSTER 2001. Molecular phylogeny of *Diabrotica* beetles (Coleoptera: Chrysomelidae) inferred from analysis of combined mitochondrial and nuclear DNA sequences. – Insect Molecular Biology **10**: 303–314.

DEPAQUIT, J., H. FERTE, N. LEGER, R. KILLICK-KENDRICK, J.A. RIOUX, M. KILLICK-KENDRICK, H.A. HANAFI & S. GOBERT 2000. Molecular systematics of the phlebotomine sandflies of the subgenus *Paraphlebotomus* (Diptera, Psychodidae, *Phlebotomus*) based on ITS2 rDNA sequences. Hypotheses of dispersion and speciation. – Insect Molecular Biology **9**: 293–300.

FREUND, W. & TH. WAGNER 2003. Revision of *Bonesioides* Laboissière, 1925 (Coleoptera; Chrysomelidae; Galerucinae) from continental Africa. – Journal of Natural History **37**: 1915–1976.

FURTH, D.G. 1990. The metatibial extensor and flexor tendons in Coleoptera. – Systematic Entomology **15**: 443–448.

FURTH, D.G. & K. SUZUKI 1994. Character correlation studies of problematic genera of Alticinae in relation to Galerucinae (Coleoptera: Chrysomelidae). Pp. 116–135 *in*: D.G. FURTH (ed.), Proceedings of the Third International Symposium on the Chrysomelidae, Beijing 1992. – Backhuys Leiden.

GÓMEZ-ZURITA, J., C. JUAN & E. PETITPIERRE 2000. Sequence, secondary structure and phylogenetic analyses of the ribosomal internal transcribed spacer 2 (ITS2) in the *Timarcha* leaf beetles (Coleoptera: Chrysomelidae). – Insect Molecular Biology **9**: 591–604.

GUSTINICICH, S., G. MANFIOLETTI, G. DEL DAL, C. SCHNEIDER & P. CANINCI 1991. A fast method for high quality genomic DNA extraction from whole human blood. – Biotechniques **11**: 298–302.

HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nuclear Acids Symposium Series **41**: 95–98.

HASENKAMP, R. & TH. WAGNER 2000. Revision of *Afromaculepta* gen. n., a monophyletic group of Afrotropical galerucinae leaf beetles (Coleoptera: Chrysomelidae). – Insect Systematics and Evolution **31**: 3–26.

HOLMQUIST, R. 1983. Transitions and transversions in evolutionary descent: an approach to understanding. – Journal of Molecular Evolution **19**: 134–144.

KIM, S.J., K.M. KJER & C.N. DUCKETT 2003. Comparison between molecular and morphological-based phylogenies of galerucine/alticine leaf beetles (Coleoptera: Chrysomelidae). – Insect Systematics and Evolution **34**: 53–64.

KONSTANTINOV, A.S. 1994. Comparative morphology and some evolutionary trends in flea-beetles (Alticinae). Pp. 383–391 *in*: P.H. JOLIVET, M.L. COX & E. PETITPIERRE (eds.), Novel Aspects of the Biology of the Chrysomelidae. – Kluwer Academic Publishers; Dordrecht, Boston, London.

LINGAFELTER, S.W. & A.S. KONSTANTINOV 2000. The monophyly and relative rank of alticinae and galerucinae leaf beetles: A cladistic analysis using adult morphological characters (Coleoptera: Chrysomelidae). – Entomologica Scandinavica **30**: 397–416.

MALLOCH, G., B. FENTON & M.A. GOODRICH 2001. Phylogeny of raspberry beetles and other Byturidae (Coleoptera). – Insect Molecular Biology **10**: 281–291.

MIDDELHAUVE, J. & TH. WAGNER 2001. Revision of *Afrocrania* (Coleoptera: Chrysomelidae, Galerucinae). Part I: Species in which the males have head cavities or extended elytral extrusions. – European Journal of Entomology **98**: 511–531.

PASHLEY, D.P. & L.D. KE 1992. Sequence evolution in the mitochondrial ribosomal and ND1 genes in Lepidoptera: implications for phylogenetic analyses. – Molecular Biology and Evolution **9**: 1061–1075.

PASKEWITZ, S.M., D.M. WESSON & F.H. COLLINS 1993. The internal transcribed spacers of ribosomal DNA in five members of the *Anopheles gambiae* species complex. – Insect Molecular Biology **2**: 247–257.

POSADA, D. & K.A. CRANDALL 1998. Modeltest: testing the model of DNA substitution. – Bioinformatics **14**: 817– 818.

REID, C.A.M. 1995. A cladistic analysis of subfamilial relationships in the Chrysomelidae sensu lato (Chrysomeloidea). Pp. 559–631 *in*: J. PAKALUK & S.A. SLIPINSKI (eds.), Biology, phylogeny, and classification of Coleoptera: Papers celebrating the 80th birthday of Roy A. Crowson. – Muzeum i Instytut Zoologii PAN, Warszawa.

ROKAS, A., J.A.A. NYLANDER, F. RONQUIST & G.N. STONE 2002. A maximum likelihood analysis of eight phylogenetic markers in gallwasps (Hymenoptera: Cynipidae); implications for insect phylogenetic studies. – Molecular Phylogeny and Evolution **22**: 206–219.

SCHLÖTTERER, C., M.T. HAUSER, A. VON HAESELER & D. TAUTZ 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. – Molecular Biology and Evolution **11**: 513–522.

SCHERZ, X. & WAGNER, TH. 2007. Revision of *Afrocandezea* Wagner & Scherz, 2002 from tropical Africa (Coleoptera: Chrysomelidae, Galerucinae). – Entomologische Zeitschrift **117**: 161–183.

SCHMITZ, J. & TH. WAGNER 2001. *Afromegalepta* gen. nov. from tropical Africa (Coleoptera: Chrysomelidae, Galerucinae). – Entomologische Zeitschrift **111**: 283–286.

SEENO, T.N. & J.A. WILCOX 1982. Leaf beetle genera (Coleoptera: Chrysomelidae). – Entomography **1**: 1–221.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU & P. FLOOK 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. – Annals of the Entomological Society of America **87**: 651–701.

Stapel, H. & Th. Wagner 2000. Revision of *Monoleptocrania* Laboissière, 1940 (Coleoptera, Chrysomelidae, Galerucinae). – Mitteilungen des Internationalen Entomologischen Vereins **25**: 137–145.

Stapel, H. & Th. Wagner 2001. *Afrotizea* gen. n. from tropical Africa (Coleoptera, Chrysomelidae, Galerucinae). – Beiträge zur Entomologie **51**: 365–373.

Swofford, D.L. 2002. PAUP* Phylogenetic Analysis Using Parsimony (* and other methods). – Sinauer, Sunderland, MA.

Tamura, K. 1992. The rate and pattern of nucleotide substitution in *Drosophila* mitochondrial DNA. – Molecular Biology and Evolution **9**: 814–825.

Tautz, D., J.M. Hancock, D.A. Webb, C. Tautz & G.A. Dover 1988. Complete sequences of the rRNA genes of *Drosophila melanogaster*. – Molecular Biology and Evolution **5**: 366–376.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin & D.G. Higgins 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. – Nuclear Acids Research **24**: 4876–4882.

Vogler, A.P. & R. DeSalle 1993. Phylogeographic patterns in coastal North American tiger beetles (*Cicindela dorsalis* Say) inferred from mitochondrial DNA sequences. – Evolution **47**: 1192–1202.

Wagner, Th. 2000. Revision of Afrotropical *Monolepta* species (Coleoptera, Chrysomelidae, Galerucinae). Part I: Species with red and black coloured elytra, pronotum and head, with description of new species. – Entomologische Zeitschrift **110**: 226–237.

Wagner, Th. 2001a. New *Monolepta*-species (Coleoptera, Chrysomelidae, Galerucinae) from Central and Southern Africa. – Entomologische Blätter **96**: 199–210.

Wagner, Th. 2001b. Biogeographical and evolutionary aspects of Afrotropical *Monolepta*, *Afromaculepta* and *Bonesioides* (Coleoptera: Chrysomelidae, Galerucinae). – Cimbebasia **17**: 237–244.

Wagner, Th. 2001c. Revision of Afrotropical *Monolepta* Chevrolat, 1837 (Coleoptera: Chrysomelidae, Galerucinae) Part II: Species with red elytra, pronotum and head, with descriptions of new species. – Bonner Zoologische Beiträge **50**: 49–65.

Wagner, Th. 2002. Revision of Afrotropical *Monolepta* species (Coleoptera, Chrysomelidae, Galerucinae) Part III: Species with red elytra and yellow prothorax, including descriptions of new species. – Deutsche Entomologische Zeitschrift **49**: 27–45.

Wagner, Th. 2003a. Present status of a taxonomic revision of Afrotropical *Monolepta* and related groups (Galerucinae). Pp. 133–146 *in*: D.G. Furth (ed.), Special Topics in Leaf Beetle Biology. Proc. 5th Int. Symp. on the Chrysomelidae. – Pensoft, Sofia, Moscow.

Wagner, Th. 2003b. Revision of Afrotropical *Monolepta* Chevrolat, 1837 (Coleoptera, Chrysomelidae, Galerucinae). – Part IV: Species with red head and thorax (including pronotum) and black elytra or black elytra with red apex, with description of new species. – Annales Sciences Zoologiques, Miscellanea **49**: 37–89.

Wagner, Th. 2004. Phylogeny of Afrotropical *Monolepta* and related taxa (Galerucinae). Pp. 75–84 *in*: P.H. Jolivet, J.A. Santiago-Blay & M. Schmitt (eds.), New Contributions to the Biology of Chrysomelidae. – Academic Publishing, The Hague.

Wagner, Th. 2005. Revision of the *vincta* species-group of *Monolepta* Chevrolat, 1837 from Africa, Arabia and the Near East (Coleoptera, Chrysomelidae, Galerucinae). – Bonner Zoologische Beiträge **52**: 255–282.

Wagner, Th. 2007a. Revision of *Afrocrania* (Coleoptera: Chrysomelidae: Galerucinae) Part II: Species in which the males lack head cavities or extended elytral extrusions. – European Journal of Entomology **104**: 801–814.

Wagner, Th. 2007b. Revision of Afrotropical *Monolepta* Chevrolat, 1837 – VI: Species with reddish or black cross-like elytral pattern (Coleoptera: Chrysomelidae, Galerucinae). – Journal of Afrotropical Zoology **3**: 83–152.

Wagner, Th. 2007c. *Monolepta* Chevrolat, 1837, the most speciose galerucine taxon: redescription of the type species *Monolepta bioculata* (Fabricius, 1781) and key to related genera from (Chrysomelidae, Coleoptera). – Journal of Natural History **41**(1): 81–100.

Wagner, Th. & W. Freund 2003. Revision of *Barombiella violacea* (Jacoby, 1984) (Coleoptera: Chrysomelidae, Galerucinae). – Entomologische Zeitschrift **113**: 258–262.

Wagner, Th. & A. Kurtscheid 2005. Revision of *Candezea* Chapuis, 1879 (Coleoptera, Chrysomelidae, Galerucinae) from continental Africa. – Journal of Natural History **39**: 2591–2641.

Wagner, Th. & X. Scherz 2002. *Afrocandezea* gen. nov. from tropical Africa (Coleoptera: Chrysomelidae, Galerucinae). – Entomologische Zeitschrift **112**: 357–362.

Weekers, P.H.H., J.F. De Jonckheere & H.J. Dumont 2001. Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representatives of the genus *Calopteryx* (Insecta: Odonata) of the west Mediterranean and adjacent west European zone. – Molecular Biology and Evolution **20**: 89–99.

Weller, S.J., D.P. Pashley, J.A. Martin & J.L. Constable 1994. Phylogeny of noctuoid moths and the utility of combining independent nuclear and mitochondrial genes. – Systematic Biology **43**: 194–211.

White, T.J., T. Bruns, S. Lee & J.W. Taylor 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 *in*: M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (eds.), PCR Protocols: a Guide to Methods and Applications. – Academic Press, New York.

Wilcox, J.A. 1973. Chrysomelidae: Galerucinae: Luperini: Luperina. Pp. 433–664 *in*: W. Junk (ed.), Coleopterorum Catalogus Suppl. 78. – Junk, 's-Gravenhage.