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Molecular phylogeny of Megacephalina Horn, 1910 tiger beetles (Coleoptera: Cicindelidae)

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Abstract

The pantropical subtribe Megacephalina represented by more than 100 species is the most diverse of the basal cicindelid groups. Today, most taxonomists recognize eight genera within the subtribe. This is in contrast to Horn who, back in 1910, conceded only two genus-level taxa: the monospecific Aniara and Megacephala sensu Horn (a genus which united the seven other taxa). In the present study we provide a molecular phylogeny of Megacephalina based on the nuclear 18S and the mitochondrial 16S and cytochrome oxidase III genes. The dataset includes 60 specimens of more than 40 mostly South American and Australian taxa. Three cicindelid species of derived lineages were used as outgroups. The resulting phylogenetic trees are basically in agreement with the current classification system. Megacephala and Grammognatha are placed basal in the dendrogram. Pseudotetracha and Australicapitona form a monophyletic Australian clade. Phaeoxantha, Tetracha and Aniara also form a monophyletic group. The position of Metriocheila remains uncertain. The most striking deviation from the traditional classification is the well-supported placement of Aniara within Tetracha, rendering the latter a paraphyletic taxon. Several monophyletic subgeneric species groups are observed in Pseudotetracha, Phaeoxantha and Tetracha/Aniara. Within the latter the monophyletic sobrina, carolina and brasilienis clades together represent a monophyletic group. Additionally, habitat types were assigned to the taxa and mapped on the phylogenetic tree. The basal African species inhabit non-flooded uplands. The Australian species moved to inland and/or coastal salt plains. The American groups were most likely first confined to river margins and then colonized secondarily and independently non-flooded uplands and/or coastal habitats.

Keywords: Aniara, Australia, cicindelid, habitat, Megacephala, monophyletic group, phylogenetic tree, South America

Introduction

Recent studies on the phylogenetic relationships within the tiger beetle subfamily (Cicindelidae) identified the subtribe Megacephalina Horn, 1910 with well over 100 species as the most diverse group within the basal clades of cicindelids (Vogler & Pearson, 1996; Vogler & Barraclough, 1998; Pearson & Vogler, 2001; Galian et al., 2002). Megacephalina is a pantropical taxon with the highest species diversity in South America, Australia and Africa. However, the relationships within Megacephalina are still controversial. Over the last 150 years several subgroups at the genus or subgenus level were established by a variety of authors (see Huber, 1994 for a chronological synopsis). In 1910, Walther Horn reunited these taxa within the genus Megacephala except for the monospecific genus Aniara which thus represented the sister taxon to Megacephala sensu Horn. Today,
there are eight genera recognized within the subtribe Megacephalina according to Wiesner's Checklist of Tiger Beetles of the World (1992; update in preparation) and this is supported by many taxonomists (Figure 1a).

Horn (1910) proposed a detailed evolutionary history of Megacephala sensu Horn based on biogeographical considerations and morphological characters. He considered Africa the geographical origin of Megacephala and accordingly conceived the African Megacephala as the taxon's most ancestral lineage. In his scenario Horn (1910) assumed two major migration routes across postulated intercontinental land bridges. Accordingly, one Megacephala lineage had moved towards Europe giving rise to Grammognatha euphratica in the Mediterranean region. Further north there is a taxon known from Baltic amber, which was initially described as "Megacephala carolina", a contemporary North American species (but see Röschmann, 1999).

According to Horn's (1910) scenario this "northern" Megacephala lineage moved westward and colonized as "Tetracha" eastern North America. Tetracha subsequently spread south forming Tetracha suturalis, which Horn considered ancestral to all neotropical Tetracha species. The second Megacephala lineage moved directly from Africa to Southern America forming Tetracha germaini. At this point the lineage split into two branches, which both made it eventually to Australia. One branch gave rise to the neotropical Phaeoxantha that further developed into the neotropical Metriocheila and the Australian Australicapitona. The other Megacephala branch developed into the Australian Pseudotetracha.

One has to keep in mind that Horn developed the scenario well before the idea of plate tectonics were put forward, and his ideas as summarized in Figure 2 are therefore outdated. Pearson & Vogler (2001) explicitly state that the distribution of Megacephala could only be understood in view of the theory of plate tectonics.

Figure 1. (a) Currently used genus-level classification of the subtribe Megacephalina following Huber (1994, but extended and modified). The geographical distribution of the genera and the number of species (in parentheses) is given according to Wiesner’s Checklist of the Tiger Beetles of the World (1992; update in preparation). (b) Genus-level relationships according to the present study.
continental drift. They refer specifically to the break-up of the ancient Gondwana megacontinent which sequentially disconnected the southern continents. Nevertheless, some systematic implications of Horn (1910) are still valid and are addressed here.

In the present study we provide a molecular phylogeny of Megacephalina that is based on the nuclear 18S, the mitochondrial 16S and cytochrome oxidase III (coxIII) genes. The dataset includes more than 40 taxa and we address questions relating to the monophyly of Megacephalina sensu Horn, 1910 and currently recognized genera within the subtribe. Furthermore, the obtained data set allows the testing of several hypotheses that are implicit to the scenario put forward by Horn (1910):

1. The African Megacephala represents the most ancestral clade within Megacephalina sensu Horn.
2. Grammognatha is the sister taxon of Tetracha.
3. The American Tetracha is a monophyletic taxon.
4. Tetracha carolina is ancestral and the neotropical Tetracha represent the most derived species within Tetracha.
5. Phaeoxantha is a paraphyletic taxon with Australicapitona and Metriocheila included within the clade.
6. Phaeoxantha/Australicapitona/Metriocheila is the sister taxon of Pseudotetraclacheta.
7. Neither Tetracha and Phaeoxantha nor Pseudotetraclacheta and Australicapitona are sister taxa.

Finally, we mapped the habitat type of the respective taxa on the molecular phylogeny: Tetracha and Phaeoxantha species, for example, colonized mostly wetland habitats whereas Megacephala species did not. Within Tetracha there are also some terra firme upland species, and we address the question whether or not these adaptations are monophyletic and whether the observed habitat preference is derived or ancestral.

Materials and methods

Taxon sampling

The species included in this study are listed in Table I. Several species of the eight genera of Megacephalina (Figure 1a) were included in the analyses if available. For Phaeoxantha, Tetracha and Pseudotetraclacheta, many species were selected in order to cover the biogeographical range of these subgenera. For some species and subspecies, specimens from different localities were collected. In total, 60 specimens of Megacephalina representing nine Australian, three African/Eurasian, and 31 American species or subspecies were analyzed. Three cicindelid species, Neocollyris sp., Odontocheila confusa and Pentacoma discrepans (that are only distantly related to the subtribe Megacephalina), were used as outgroups. The 18S, 16S and coxIII sequences of these outgroup species were retrieved from GenBank (accession numbers are listed in Table I). The habitat types of Megacephalina species were assigned according to Pearson et al. (1999), Sumlin (1997), McCairns et al. (1997) and Zerm et al. (2001).

PCR amplification and DNA sequencing

DNA was extracted following the protocol of the Puregene kit (Gentra Systems, Minneapolis, MN, USA). Sections of three genes were subsequently amplified by PCR, i.e. the nuclear 18S gene (primers: 5’-TTGTCTCAAAGATTAAGCCATGC-3’ and 5’-CCGTTACAACCATGGTAGGCG-3’), the mitochondrial 16S gene (primers: 5’-CCGATTCTAAAGATTAGCACTGC-3’ and 5’-CGCTTGATCGGATGTCGCCC-3’), and the mitochondrial coxIII gene (primers: 5’-CTTCTTATTATCTTATTCTATCTG-3’ and 5’-TACCTGATCCTTACTTGTTCC-3’). Primers were designed specifically for the purpose of this study on the basis of sequence alignments of publicly available sequences, targeting highly conserved regions within the genes. The PCR protocols differed slightly between species and can be provided on request. The obtained PCR products were purified following the protocol of the QIAquick PCR purification kit (Qiagen, Hilden, Germany), and subsequently sequenced on an ABI310 automatic sequencer (Applied Biosystems, Foster City, CA, USA) using the BigDye chemistry (Applied Biosystems). ClustalX (Thompson et al., 1997) was used for establishing multiple sequence alignments.
Table I. Tiger beetle specimens used in this study.

<table>
<thead>
<tr>
<th>Species name</th>
<th>Collecting site</th>
<th>Collecting date</th>
<th>GenBank numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus <em>Aniara</em> Hope 1938</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 <em>Aniara seculatris</em> (Fabricius, 1801) Brazil, Amazonas, near Manaus: Reserva Ducke</td>
<td>10/82</td>
<td>DQ152074 DQ152015 DQ152133</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Metriotrecha</em> Thomson 1857</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 <em>Metriotrecha nigricollis</em> (Reiche, 1842) Bolivia, Carrasco, Santa Cruz, Rio Blanquito</td>
<td>12/02</td>
<td>DQ152075 DQ152016 DQ152133</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Phaeoxantha</em> Chaudoir 1850</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 <em>Phaeoxantha aequinoctialis aequinoctialis</em> (Dejean, 1825) Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Pacencia</td>
<td>10/97</td>
<td>DQ152076 DQ152017 DQ152135</td>
<td></td>
</tr>
<tr>
<td>4 <em>Phaeoxantha aequinoctialis bifasciata</em> (Brullé, 1837) Bolivia, Carrasco, Rio Cesarsama</td>
<td>12/02</td>
<td>DQ152077 DQ152018 DQ152136</td>
<td></td>
</tr>
<tr>
<td>5 <em>Phaeoxantha cruciata</em> (Brullé, 1837) Paraguay, Dep. Concepcion, San Carlos, Rio Apa</td>
<td>6/98</td>
<td>DQ152078 DQ152019 DQ152137</td>
<td></td>
</tr>
<tr>
<td>6 <em>Phaeoxantha klugii</em> (Chaudoir, 1850) Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Marchantaria</td>
<td>3/02</td>
<td>DQ152080 DQ152021 DQ152139</td>
<td></td>
</tr>
<tr>
<td>7 <em>Phaeoxantha limata</em> (Perry, 1830) Bolivia, Cordillera, Santa Cruz, Rio Seco</td>
<td>10/02</td>
<td>DQ152081 DQ152022 DQ152140</td>
<td></td>
</tr>
<tr>
<td>8 <em>Phaeoxantha lindemannae</em> (Mandl, 1964) Brazil, Amazonas, near Manaus: Rio Negro, Praia Grande</td>
<td>10/97</td>
<td>DQ152082 DQ152023 DQ152141</td>
<td></td>
</tr>
<tr>
<td>9 <em>Phaeoxantha theus</em> (Brulle´, 1837) Bolivia, Cordillera, Santa Cruz, Rio Surutu</td>
<td>9/98</td>
<td>DQ152083 DQ152024 DQ152142</td>
<td></td>
</tr>
<tr>
<td>10 <em>Phaeoxantha theus</em> (Brulle´, 1837) Bolivian, Ent. Province Hola</td>
<td>6/99</td>
<td>DQ152084 DQ152025 DQ152143</td>
<td></td>
</tr>
<tr>
<td>11 <em>Phaeoxantha theus</em> (Brulle´, 1837) Botswana, Francistown</td>
<td>12/01</td>
<td>DQ152085 DQ152026 DQ152144</td>
<td></td>
</tr>
<tr>
<td>12 <em>Phaeoxantha theus</em> (Brulle´, 1837) Australia, Queensland, 2 km E, 5 km N Highway 1, off Aims Road (SE Townsville)</td>
<td>3/02</td>
<td>DQ152086 DQ152027 DQ152145</td>
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</tr>
<tr>
<td>Genus <em>Microtecta</em> Chaudoir 1850</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13 <em>Phaeoxantha theus</em> (Brulle´, 1837) Australia, W.A., L. Lefroy (SE Kalgoorlie)</td>
<td>4/03</td>
<td>DQ152087 DQ152028 DQ152146</td>
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<tr>
<td>14 <em>Phaeoxantha theus</em> (Brulle´, 1837) Australia, W.A., L. Moore, 24 km SE Menzies</td>
<td>4/03</td>
<td>DQ152088 DQ152029 DQ152147</td>
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<td>Genus <em>Grammognatha</em> Motschulsky 1850</td>
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<td></td>
</tr>
<tr>
<td>15 <em>Grammognatha euphratica</em> (Latreille &amp; Dejean, 1822) Israel, North Coastal Plain, Atlit</td>
<td>5/02</td>
<td>DQ152089 DQ152030 DQ152148</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Megacephala</em> Latreille 1902</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16 <em>Megacephala regalis citernii</em> (Horn, 1912) Kenya, Eastern Province Hola</td>
<td>5/01</td>
<td>DQ152090 DQ152031</td>
<td></td>
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<tr>
<td>17 <em>Megacephala regalis regalis</em> (Boheman, 1848) Botswana, Francistown</td>
<td>12/01</td>
<td>DQ152091 DQ152032</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Australicapitona</em> Sumlin, 1992</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>18 <em>Australicapitona australasiae</em> (Hope, 1841) Australia, Queensland, 2 km E, 5 km N Highway 1, off Aims Road (SE Townsville)</td>
<td>11/02</td>
<td>DQ152092 DQ152033 DQ152149</td>
<td></td>
</tr>
<tr>
<td>Genus <em>Pseudotetracha</em> Fleutiaux, 1894</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>19 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Argentina, Arroyo Urquiza, Dep. Colon, Prov. Entre Rios</td>
<td>4/03</td>
<td>DQ152093 DQ152034 DQ152150</td>
<td></td>
</tr>
<tr>
<td>20 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., L. Lefroy (SE Kalgoorlie)</td>
<td>4/03</td>
<td>DQ152094 DQ152035 DQ152151</td>
<td></td>
</tr>
<tr>
<td>21 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., Lake Ballard (24 km NE Menzies)</td>
<td>4/03</td>
<td>DQ152095 DQ152036 DQ152152</td>
<td></td>
</tr>
<tr>
<td>22 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Argentina, Arroyo Urquiza, Dep. Colon, Prov. Entre Rios</td>
<td>4/03</td>
<td>DQ152096 DQ152037 DQ152153</td>
<td></td>
</tr>
<tr>
<td>23 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., L. Auld-Wapet Rd.</td>
<td>4/03</td>
<td>DQ152097 DQ152038 DQ152154</td>
<td></td>
</tr>
<tr>
<td>24 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., Lake Ballard (24 km NE Menzies)</td>
<td>4/03</td>
<td>DQ152098 DQ152039 DQ152155</td>
<td></td>
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<tr>
<td>25 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., L. Barlee (W Menzies)</td>
<td>4/03</td>
<td>DQ152099 DQ152040 DQ152156</td>
<td></td>
</tr>
<tr>
<td>26 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., L. Noonie (S Sandstone)</td>
<td>4/03</td>
<td>DQ152100 DQ152041 DQ152157</td>
<td></td>
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<tr>
<td>Genus <em>Tetracha</em> Hope 1838</td>
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</tr>
<tr>
<td>27 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Australia, W.A., Iron Stone Lagoon</td>
<td>3/03</td>
<td>DQ152101 DQ152042 DQ152158</td>
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</tr>
<tr>
<td>28 <em>Pseudotetracha affinis brevisulcata</em> (Fleutiaux, 1895) Argentina, Arroyo Urquiza, Dep. Colon, Prov. Entre Rios</td>
<td>2/02</td>
<td>DQ152102 DQ152043 DQ152159</td>
<td></td>
</tr>
</tbody>
</table>
alignments. Aligning the sequences of all three marker genes was straightforward and congruent with previously established alignments that can be retrieved from websites such as http://www.bio.ic.ac.uk/research/tigerb/benzpaper.htm by A. Vogler, Imperial College, London, UK.
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Phylogenetic analyses

Neighbor-joining and Maximum Parsimony analyses were performed with MEGA version 2.1 (Kumar et al., 2001). Bayesian inference reconstructions were conducted with MrBayes 3.04b (Huelsenbeck & Ronquist, 2001). MrModeltest (Nylander, 2002), a simplified version of Modeltest 3.06 (Posada & Crandall, 1998), was used to find optimal models for use with the probabilistic methods. The preferred model was always a GTR model with a gamma distributed rate heterogeneity and a certain proportion of invariant sites. Bayesian inference analyses were performed under 4000000 generations and four Metropolis-coupled Markov chains, taking samples every 100 generations. The initial 20% of the trees (8000) were discarded as burn-in. From the resulting 30000 trees a posteriori probabilities for individual clades were assessed based on their observed frequencies.

Results

Analysis of molecular data

Complete sequence data sets of the nuclear 18S, mitochondrial 16S and coxIII genes were obtained for 58 specimens. For the specimens from Megacephala regalis regalis and Australicapitona australasiae only the sequences of the 16S and 18S genes could be determined. The alignment of all three genes was straightforward and in total 1145 bp long (primers excluded); 359 bp relate to the 18S gene, 364 bp to the 16S gene and 422 bp to the coxIII gene. All sequences were deposited in GenBank. The accession numbers are listed in Table I.

Phylogenetic relationships in the subtribe Megacephalina Horn, 1910

The obtained alignment was subsequently used for phylogenetic analyses. First, MrModeltest (Nylander, 2002) was used to estimate optimal substitution model for the data set. The preferred model for the three genes separately as well as the combined dataset was always a GTR model with a gamma distributed rate heterogeneity and a certain proportion of invariant sites. This GTR model was then applied for the subsequent Bayesian inference reconstruction. The resulting tree is given in Figure 3. All currently recognized genera of the subtribus Megacephalina with the exception of Aniara and Tetracha form monophyletic groups in the dendrogram.

The phylogenetic relationships of M. regalis regalis and A. australasiae were only deduced with a reduced data set consisting of the 16S and 18S sequences and are therefore not depicted in Figure 1. M. regalis regalis grouped together with the second Megacephala taxon, M. regalis cisternii. A. australasiae which is the only Australicapitona species in this study forms its own clade close to Pseudotetracha.

However, some clades are only supported by low posterior probabilities after Bayesian inference and low bootstrap support in Neighbor-joining and Maximum Parsimony analyses, respectively. Nevertheless, the Neighbor-joining and Maximum Parsimony analyses yielded, with one exception, basically the same tree topologies (not shown). The South American Metriocheila species M. nigricollis was positioned very basal among the outgroup species. Otherwise, only minor differences in the grouping of species within the genera were observed.

With respect to the currently recognized genera of the subtribus Megacephalina, the results of the phylogenetic analyses can be summarized as follows:

- **Aniara** Hope, 1938: the only Aniara species, *A. sepulcralis*, is nested within the clade of all Tetracha species which makes *Megacephala sensu* Horn a paraphyletic taxon.

- **Metriocheila** Thomson, 1857: the only Metriocheila species, *M. nigricollis* forms its own clade with affinity to the Tetracha/Aniara and the Phaeoxantha clade (but see above for the different position of this clade in the Neighbor-joining and Maximum Parsimony analyses).

- **Phaeoxantha** Chaudoir, 1850: the Phaeoxantha species form a monophyletic group that is, however, only supported by low posterior probability. It comprises three distinct clades consisting of (1) *aequinotialis-cruciata*, (2) *limata* and (3) *klugii-lindemanniae-twimmer*.

- **Grammognatha** Motschulsky, 1850: the only Grammognatha species, *G. euphratica*, is found basal in the tree.

- **Megacephala** Latreille, 1902: the African *M. regalis regalis* and *M. regalis cisternii* cluster together with some affinity to the Australian Pseudotetracha clade.

Figure 3. Phylogenetic reconstruction by Bayesian inference of the combined data set consisting of coxIII, 16S and 18S sequences. Posterior probabilities are indicated at the respective nodes. Specimen running codes from Table I are given in brackets as well as the habitat type of the respective taxa (*r*, margins of rivers and lake shores, floodplains; *c*, coastal habitats; *s*, inland salt flats; *t*, non-flooded uplands). Roman numbers indicate well-supported subgeneric groups (see text). Arrows indicate the occurrence of four teeth on the right mandible within Tetracha. P., Phaeoxantha; Ps., Pseudotetracha.
Phylogeny of Megacephalina

Tetracha

Aniara

Tetracha

Phaeoxantha

Metriocheila

Pseudotetraicha

Megacephala

Grammognatha

T. sobrina sobrina (53, c)
T. sobrina (54, c)
T. spixi (58, r)
T. sobrina sommeri (55, r)
T. sobrina punctata (51, r)
T. sobrina punctata (52, r)
T. carolina chilenis (38, r/c/t)
T. floridana (42, c)
T. nicaraguensis (45, r)
T. carolina carolina (36, c/r/s)
T. carolina carolina (37, c/r/s)
T. carolina (35, c/r/s)
T. brasiliensis (30, r)
T. brasiliensis (33, r)
T. brasiliensis (31, r)
T. brasiliensis (32, r)
T. virginica (60, c/r/t)
A. sepulchralis (1, t)
T. campisi (34, t)
T. fuliginosa (44, r/t)
T. affinis brevisculata (28, t)
T. affinis brevisculata (29, t)
T. pilospennis (47, r)
T. pilospennis (48, r)
T. pilospennis (49, r)
T. pilospennis (46, r)
T. pseudodistinguenda (50, r)
T. distinguenda (40, r)
T. distinguenda (41, r)
T. spinosa (57, r)
T. fulgida (43, r)
T. thomasoniana (59, r)
T. sparsippunctata (56, r)
P. aequinoctialis bifasciata (5, r)
P. aequinoctialis aequinoctialis (4, r)
P. aequinoctialis aequinoctialis (3, r)
P. cruciata (6, r)
P. cruciata (7, r)
P. limata (11, r)
P. limata (12, r)
P. klugii (8, r)
P. linderi (13, r)
P. klugii (9, r)
P. wimmeri (14, r)
P. klugii (10, r)
M. nigricollis (2, r)
Ps. helmaia (22, s)
Ps. ion (23, s)
Ps. oleadoris (25, s)
Ps. blackburni (19, s)
Ps. whelani (27, s)
Ps. mendacia (24, s)
Ps. pulchra (26, s)
Ps. corpulentia (20, s)
Ps. cuprascens (21, s)
M. regalis citerii (16, t)
G. euphratica (15, t)
O. confusa
P. discrepans
Neocollyris sp.
• *Pseudotetracha* Fleutiaux, 1894: the Australian *Pseudotetracha* species forms a monophyletic clade that is supported by high posterior probability. Several well-supported species groups occur within the *Pseudotetracha* clade.

• *Australicapitona* Sumlin, 1992: the genus is only represented by one species, *A. australasiae*. The positioning of the *Australicapitona* clade close to *Pseudotetracha* is not based on a complete data set, but only on the analyses of the 16S and 18S sequences.

• *Tetracha* Hope, 1838: the nesting of *A. sepulcralis* within the clade makes the genus *Tetracha* paraphyletic. Four monophyletic species groups were found in *Tetracha*: (1) the *sobrina* clade consisting of *T. sobrina*, *T. spixi* and *T. carolina chilensis*, (2) the *carolina* clade consisting of *T. carolina*, *T. floridana* and *T. nicaraguensis*, (3) the *brasiliensis* clade consisting of *T. brasiliensis* and *T. chacoensis*, and (4) the *fulgida* clade consisting of *T. pilosipennis*, *T. distinguenda*, *T. pseudodistinguenda*, *T. thomsoniana*, *T. sparsim punctata*, *T. fulgida* and *T. spinosa*. Some other species such as *T. camposi*, *T. affinis*, *T. fuliginosa* and *T. virginica* did not form well-supported clades with other *Tetracha* species.

**Discussion**

The currently used genus-level classification of the subtribe Megacephalina follows that of Huber (1994) with some extensions/modifications (see Figure 1a), and consists of eight genera. The phylogenetic relationships of the Megacephalina species based on the mitochondrial 16S, coxIII and the nuclear 18S genes (Figure 3) are in good agreement with this classification system.

As expected, *M. regalis citernii* and *G. euphratica*, i.e. the species that represent the African genera *Megacephala* and *Grammognatha*, are found at basal positions in the tree. Our current study also provides strong evidence for the monophyly of an Australian clade comprising *Pseudotetracha* and *Australicapitona*, as well as an American clade comprising *Aniara*, *Tetracha*, *Metrychoeila* and *Phaeoxantha*.

The most striking deviation from the traditional classification is certainly the placement of *Aniara* within *Tetracha*, making the latter a paraphyletic taxon. It can be noted that this finding was observed in all analyses, even when the three markers were analyzed separately. This is consistent with the results of a study on the molecular phylogeny of Cicindelidae at higher taxonomic levels (based on full-length 18s rRNA data) by Galian et al. (2002). The authors found that *Aniara* was the sister taxon of one of two *Megacephala* s.l. species. Thus, in this study *Megacephala* s.l. was paraphyletic as well. The puzzling placement of *Aniara* within a derived branch of *Tetracha* is not corroborated by morphological, ecological or ethological characters.

Within *Tetracha* several monophyletic species groups were observed. These groups are congruent with morphological considerations: the *sobrina* clade as well as the *carolina* and *brasiliensis* clades are grouped together by morphologists for having four instead of three teeth on the right mandible, although this has never been documented in the taxonomic literature. A right mandible with four teeth is apparently the plesiomorphic state within *Tetracha/Aniara*. The right mandible of *T. virginica* also has four teeth but with a different morphology. One may therefore believe that it makes sense that *T. virginica* does not cluster together with the species of the *sobrina*, *carolina* and *brasiliensis* clades and that the four teeth on the right mandible of *T. virginica* are considered an independent development. However, this congruence may just be incidental; a proper analytical interpretation of comprehensive morphological datasets would be required to test the hypothesis.

The grouping of several species within *Pseudotetracha*, however, is not consistent with the literature. Based on morphological characters Sumlin (1997) describes *P. blackburni*, *P. corpulentia*, *P. mendacia* and *P. cuprascens* (as well as another species not included in the present study) as a species complex. However, it is a polyphyletic group in our analyses. Sumlin (1997) also considers *P. ion* the closest relative of *P. wheelani* which is also not confirmed in the molecular phylogeny. McCairns et al. (1997) described *P. helmsi* (along with some other species not considered here) as closely related to *P. oleodorsa* which is also not corroborated by our data.

According to the nuclear 18S, the mitochondrial 16S and the coxIII sequences two subspecies may be considered true species: *Tetracha carolina chilensis* does not group with the other *T. carolina* subspecies but is found close to the *sobrina* clade of *Tetracha*. A similar situation was observed in the genus *Phaeoxantha*: *P. aequinoctialis bifasciata* does not group with *P. aequinoctialis aequinoctialis*. Both *T. carolina chilensis* as well as *P. aequinoctialis bifasciata* should thus be treated as true separated species.

With the exception of the basal position of the African *Megacephala* and *Grammognatha* species, all hypotheses implicit to the evolutionary scenario of *Megacephala* by Horn (1910) are falsified by the present results. It is very unlikely that the incorporation of *Tetracha germaniie* in this study, a species central for the evolution of *Phaeoxantha* and *Pseudotetracha* in Horn’s scenario, would have altered substantially the observed tree topology. The present results, in particular the monophyly of
both the American and Australian clades, strongly suggest that the early evolution of Megacephalina took place during the break-up of the ancient Gondwana continent. A future classification of Megacephalina based on the present results should reflect these supragenus-level relationships (Figure 1b) as well as the subgeneric clades in Tetracha, Phaeoxantha and Pseudotetracha (Figure 3).

Although the resolution of our data set is limited, at the intraspecific level two interesting phylogeographic patterns emerge. Within the klugii clade of Phaeoxantha, specimens 14 and 9 (Table I; Figure 3) were collected in eastern Bolivia and specimens 8 and 13 near Manaus (Brazil). Both areas belong to the Amazon basin. Specimen 10 was collected at the Rio Paraguay close to the Pantanal basin which is part of a different river system. Having this in mind, the grouping of the specimens in the klugii clade reflects their biogeographical distribution. This implies, however, that either all sampled specimens of P. klugii, P. lindemannyae and P. simmeri represent the same or, alternatively, different species. When kept in the laboratory, adults of P. klugii and P. lindemannyae readily copulate with each other (M. Zerm, personal observation).

The second instance relates to Tetracha pilosipennis. Specimens from Bolivia, Argentina and Paraguay form one group that is separated from the Ecuadorian specimen (D). This tree topology also reflects the biogeography. However, future studies need to address such intraspecific phylogeographic patterns in more detail, the sampling for this study does not allow for more detailed conclusions.

The habitat preferences of the Megacephalina taxa under study were also mapped on to the phylogenetic tree presented in Figure 3. For this purpose we considered four different habitat types, i.e. (1) margins of rivers and lake shores, and floodplains, (2) coastal habitats, (3) inland salt plains, and (4) non-flooded uplands. The basal African species inhabit non-flooded uplands, the Australian species, and to a lesser extent some American Tetracha species, Aniara gutierrezi and to a lesser extent some Phaeoxantha species colonized secondarily and independently non-flooded uplands and/or coastal habitats.

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