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ORIGINAL ARTICLE

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 *Metrocheila* 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 *sobrino*, *carolina* and *brasiliensis* 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

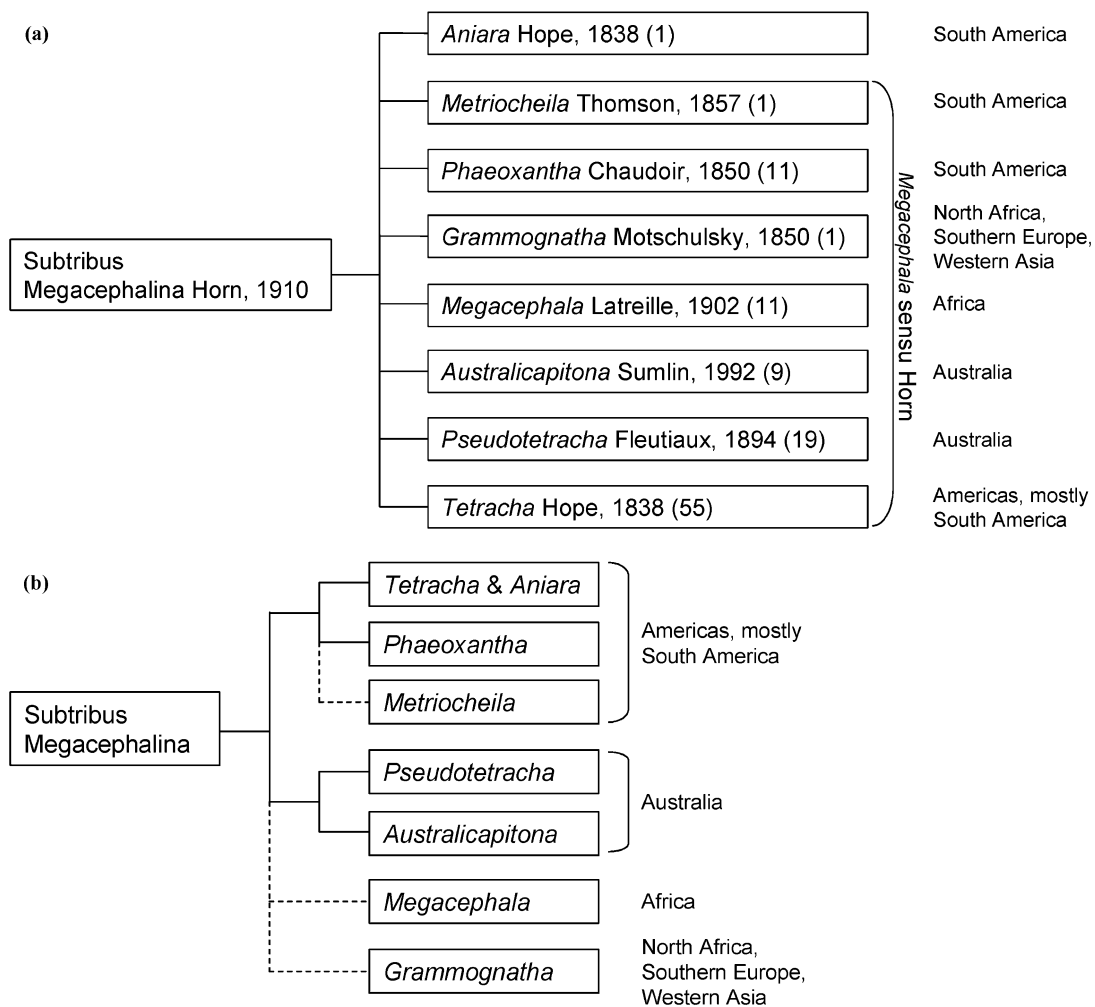


Figure 1. (a) Currently used genus-level classification of the subtribe Megacephalina following Huber (1994, but extended and modified). The geographical distribution of the genera and the number of species (in parentheses) is given according to Wiesner's *Checklist of the Tiger Beetles of the World* (1992; update in preparation). (b) Genus-level relationships according to the present study.

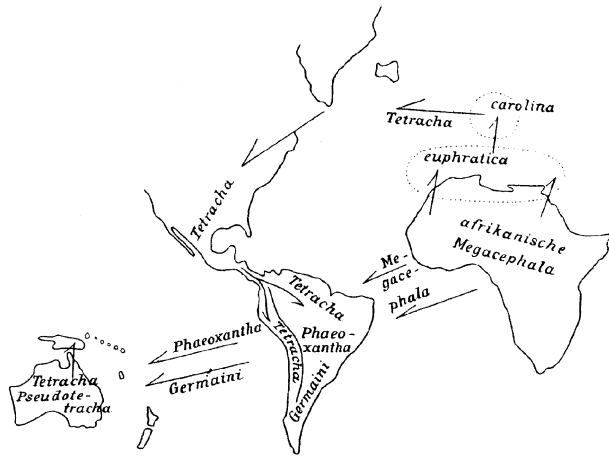


Figure 2. Horn's (1910) biogeographical scenario of the evolution of *Megacephala sensu* Horn with hypothetical intercontinental dispersal routes.

continental drift. They refer specifically to the breakup 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 *Megacephala 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 *Pseudotetracha*.
7. Neither *Tetracha* and *Phaeoxantha* nor *Pseudotetracha* 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 *Pseudotetracha*, 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 *Pentacomia 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'-CCGAGTATTTTGACTGTGC-3' and 5'-TAATCCAA-CATCGAGGTCGCAA-3') and the mitochondrial coxIII gene (primers: 5'-CTGTAGAAYTWGGR-AGAAGTTGRCC-3'' and 5'-TACTATATAAAT-TATCTACCTCATC-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

Table I. Tiger beetle specimens used in this study.

| Species name | Collecting site | Collecting date | GenBank numbers | | |
|---|--|-----------------|-----------------|----------|----------|
| | | | 18S | 16S | coxIII |
| Genus <i>Aniara</i> Hope, 1938 | | | | | |
| 1 <i>Aniara sepulcralis</i> (Fabricius, 1801) | Brazil, Amazonas, near Manaus: Reserva Ducke | 10/82 | DQ152074 | DQ152015 | DQ152133 |
| Genus <i>Metrocheila</i> Thomson, 1857 | | | | | |
| 2 <i>Metrocheila nigricollis</i> (Reiche, 1842) | Bolivia, Carrasco, Santa Cruz, Río Blanquito | 12/02 | DQ152075 | DQ152016 | DQ15213 |
| Genus <i>Phaeoxantha</i> Chaudoir, 1850 | | | | | |
| 3 <i>Phaeoxantha aequinoctialis aequinoctialis</i> (Dejean, 1825) | Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Paciencia | 10/97 | DQ152076 | DQ152017 | DQ152135 |
| 4 | Bolivia, Carrasco, Río Cesarsama | 12/02 | DQ152077 | DQ152018 | DQ152136 |
| 5 <i>Phaeoxantha aequinoctialis bifasciata</i> (Brullé, 1837) | Brazil, Amazonas, near Manaus: Rio Negro, Praia Grande | 6/98 | DQ152078 | DQ152019 | DQ152137 |
| 6 <i>Phaeoxantha cruciata</i> (Brullé, 1837) | Paraguay, Dep. Concepcion, San Carlos, Río Apa | 10/02 | DQ152079 | DQ152020 | DQ152138 |
| 7 | Bolivia, Cordillera, Santa Cruz, Río Seco | 3/02 | DQ152080 | DQ152021 | DQ152139 |
| 8 <i>Phaeoxantha klugii</i> (Chaudoir, 1850) | Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Marchantaria | 10/97 | DQ152081 | DQ152022 | DQ152140 |
| 9 | Bolivia, Ichilo, Santa Cruz, Río Surutu | 10/02 | DQ152082 | DQ152023 | DQ152141 |
| 10 | Brazil, Mato Grosso, Pantanal, Rio Paraguay, Praia Carne Seca, Cacaes | 9/98 | DQ152083 | DQ152024 | DQ152142 |
| 11 <i>Phaeoxantha limata</i> (Perty, 1830) | Brazil, Mato Grosso, Santo Andre, Rio Cuiaba: Praia Volta Grande | 6/99 | DQ152084 | DQ152025 | DQ152143 |
| 12 | Brazil, Amazonas, near Manaus: Sitio Bom Sossego | 10/82 | DQ152085 | DQ152026 | DQ152144 |
| 13 <i>Phaeoxantha lindemanna</i> (Mandl, 1964) | Brazil, Amazonas, near Manaus: Rio Negro, Praia Grande | 3/98 | DQ152086 | DQ152027 | DQ152145 |
| 14 <i>Phaeoxantha wimmeri</i> (Mandl, 1958) | Bolivia, Carrasco, Cochambamba, Río Cesarsama | 12/02 | DQ152087 | DQ152028 | DQ152146 |
| Genus <i>Grammognatha</i> Motschulsky, 1850 | | | | | |
| 15 <i>Grammognatha euphratica</i> (Latreille & Dejean, 1822) | Israel, North Coastal Plain, Atlit | 5/02 | DQ152088 | DQ152029 | DQ152147 |
| Genus <i>Megacephala</i> Latreille, 1902 | | | | | |
| 16 <i>Megacephala regalis citernii</i> (Horn, 1912) | Kenya, Eastern Province Hola | 5/01 | DQ152089 | DQ152030 | DQ152148 |
| 17 <i>Megacephala regalis regalis</i> (Boheman, 1848) | Botswana, Francistown | 12/01 | DQ152090 | DQ152031 | |
| Genus <i>Australicapitona</i> Sumlin, 1992 | | | | | |
| 18 <i>Australicapitona australasiae</i> (Hope, 1841) | Australia, Queensland, 2 km E, 5 km N Highway 1, off Aims Road (SE Townsville) | 11/02 | DQ152091 | DQ152032 | |
| Genus <i>Pseudotetracha</i> Fleutiaux, 1894 | | | | | |
| 19 <i>Pseudotetracha blackburni</i> (Fleutiaux, 1895) | Australia, W.A., L. Lefroy (SE Kalgoorlie) | 4/03 | DQ152092 | DQ152033 | DQ152149 |
| 20 <i>Pseudotetracha corpulenta</i> (Horn, 1907) | Australia, N.T., 33 km E Curtin Spgs | 3/03 | DQ152093 | DQ152034 | DQ152150 |
| 21 <i>Pseudotetracha cuprascens</i> (Sumlin, 1997) | Australia, W.A., L. Moore, 24 km SE Payne Find | 4/03 | DQ152094 | DQ152035 | DQ152151 |
| 22 <i>Pseudotetracha helmsi</i> (Blackburn, 1892) | Australia, W.A., Lake Annean (S Meekathara) | 4/03 | DQ152095 | DQ152036 | DQ152152 |
| 23 <i>Pseudotetracha ion</i> (Sumlin, 1997) | Australia, W.A., L. Auld-Wapet Rd. | 4/03 | DQ152096 | DQ152037 | DQ152153 |
| 24 <i>Pseudotetracha mendacia</i> (Sumlin, 1997) | Australia, W.A., Lake Ballard (24 km NE Menzies) | 4/03 | DQ152097 | DQ152038 | DQ152154 |
| 25 <i>Pseudotetracha oleadora</i> (Sumlin, 1992) | Australia, W.A., L. Barlee (W Menzies) | 4/03 | DQ152098 | DQ152039 | DQ152155 |
| 26 <i>Pseudotetracha pulchra</i> (Brown, 1869) | Australia, W.A., L. Noondie (S Sandstone) | 4/03 | DQ152099 | DQ152040 | DQ152156 |
| 27 <i>Pseudotetracha whelani</i> (Sumlin, 1992) | Australia, S.A., Iron Stone Lagoon | 3/03 | DQ152100 | DQ152041 | DQ152157 |
| Genus <i>Tetracha</i> Hope, 1838 | | | | | |
| 28 <i>Tetracha affinis brevisulcata</i> (Horn, 1907) | Argentina, Arroyo Urquiza, Dep. Colón, Prov. Entre Ríos | 2/02 | DQ152101 | DQ152042 | DQ152158 |
| 29 | Paraguay, Dep. Boquerón, La Patria | 11/02 | DQ152102 | DQ152043 | DQ152159 |

Table 1. Continued.

| Species name | Collecting site | Collecting date | GenBank numbers | | |
|--|---|-----------------|-----------------|---------------------|----------|
| | | | 18S | 16S | coxIII |
| 30 <i>Tetracha brasiliensis</i> (Kirby, 1818) | Brazil, Mato Grosso, Pantanal, Pirizal: Baia de Coqueiro | 8/00 | DQ152103 | DQ152044 | DQ152160 |
| 31 | Brazil, Mato Grosso, Pantanal, Pirizal: Fazenda Retiro Novo | 6/99 | DQ152104 | DQ152045 | DQ152161 |
| 32 | Paraguay, Dep. Concepcion, San Carlos, Rio Apa | 10/02 | DQ152105 | DQ152046 | DQ152162 |
| 33 | Argentina, Arroyo Urquiza, Dep. Colón, Prov. Entre Ríos | 2/03 | DQ152106 | DQ152047 | DQ152163 |
| 34 <i>Tetracha camposi</i> (Horn, 1900) | Ecuador, Manabi, Crucita | 2/01 | DQ152107 | DQ152048 | DQ152164 |
| 35 <i>Tetracha carolina</i> (Linné, 1766) | USA, GenBank | | L76821 | L42918 ^a | |
| 36 <i>Tetracha carolina carolina</i> (Linné, 1766) | USA, Texas | 11/02 | DQ152108 | DQ152049 | DQ152165 |
| 37 | Mexico, Baja California | 11/02 | DQ152109 | DQ152050 | DQ152166 |
| 38 <i>Tetracha carolina chilensis</i> (Laporte, 1834) | Ecuador, Manabi, Crucita | 2/01 | DQ152110 | DQ152051 | DQ152167 |
| 39 <i>Tetracha chacoensis</i> (Sawada & Wiesner, 1997) | Paraguay, Dep. Boquerón, La Patria | 5/03 | DQ152111 | DQ152052 | DQ152168 |
| 40 <i>Tetracha distinguenda</i> (Dejean, 1831) | Paraguay, Dep. Pte. Hayes, Trans Chaco Highway, km 415 | 1/03 | DQ152112 | DQ152053 | DQ152169 |
| 41 | Bolivia, Cordillera, Santa Cruz, Ravelo, Orilla de laguna | 4/03 | DQ152113 | DQ152054 | DQ152170 |
| 42 <i>Tetracha floridana</i> (Leng & Mutchler, 1916) | USA, Florida | 11/02 | DQ152114 | DQ152055 | DQ152171 |
| 43 <i>Tetracha fulgida</i> (Klug, 1834) | Bolivia, Andrés Ibañez, Santa Cruz, Quebrada "Forestal" | 4/02 | DQ152115 | DQ152056 | DQ152172 |
| 44 <i>Tetracha fuliginosa</i> (Bates, 1874) | Nicaragua | 11/02 | DQ152116 | DQ152057 | DQ152173 |
| 45 <i>Tetracha nicaraguensis</i> (Johnson, 1993) | Nicaragua | 11/02 | DQ152117 | DQ152058 | DQ152174 |
| 46 <i>Tetracha pilosipennis</i> (Mandl, 1958) | Ecuador, Pastaza, Puyo, Canelos | 2/01 | DQ152118 | DQ152059 | DQ152175 |
| 47 | Bolivia, Cordillera, Santa Cruz, Camiri orillas río Parapeti | 4/03 | DQ152119 | DQ152060 | DQ152176 |
| 48 | Argentina, Arroyo Urquiza, Dep. Colón, Prov. Entre Ríos | 2/03 | DQ152120 | DQ152061 | DQ152177 |
| 49 | Paraguay, Dep. Concepcion, Santa Sofia | 10/02 | DQ152121 | DQ152062 | DQ152178 |
| 50 <i>Tetracha pseudodistinguenda</i> (Horn, 1905) | Paraguay, Dep. Pte Hayes, Lolita, Yaragui, Rio Verde | 3/03 | DQ152122 | DQ152063 | DQ152179 |
| 51 <i>Tetracha sobrina punctata</i> (Castelnau, 1835) | Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Marchantaria | 11/97 | DQ152123 | DQ152064 | DQ152180 |
| 52 | Brazil, Amazonas, near Manaus: Rio Negro, Praia Grande | 8/98 | DQ152124 | DQ152065 | DQ152181 |
| 53 <i>Tetracha sobrina sobrina</i> (Dejean, 1831) | Venezuela, Margarita Island, Playa Parguito | 2/02 | DQ152125 | DQ152066 | DQ152182 |
| 54 | Puerto Rico | 11/02 | DQ152126 | DQ152067 | DQ152183 |
| 55 <i>Tetracha sobrina sommeri</i> (Chaudoir, 1851) | Brazil, Santo Andre, Rio Cuiaba: Praia Volta Grande | 6/99 | DQ152127 | DQ152068 | DQ152184 |
| 56 <i>Tetracha sparsimpunctata</i> (Mandl, 1961) | Bolivia, O. Santiesteban, Santa Cruz, Río Pirai Puente Eisenhower | 5/03 | DQ152128 | DQ152069 | DQ152185 |
| 57 <i>Tetracha spinosa</i> (Brullé, 1837) | Brazil, Amazonas, near Manaus: Rio Solimoes, Ilha de Paciencia | 11/97 | DQ152129 | DQ152070 | DQ152186 |
| 58 <i>Tetracha spixi</i> (Brullé, 1837) | Bolivia, Florida, Santa Cruz, Río Cruces | 1/03 | DQ152130 | DQ152071 | DQ152187 |
| 59 <i>Tetracha thomsoniana</i> (Horn, 1915) | Bolivia, Florida, Santa Cruz, Pampagrande | 1/03 | DQ152131 | DQ152072 | DQ152188 |
| 60 <i>Tetracha virginica</i> (Linné, 1766) | USA, Kansas | 11/02 | DQ152132 | DQ152073 | DQ152189 |
| Outgroups | | | | | |
| <i>Neocollyris</i> sp. | India, GenBank | | L76822 | AF438905 | AF438930 |
| <i>Odontocheila confusa</i> (Dejean, 1825) | Bolivia, GenBank | | L76824 | AF438903 | AF438928 |
| <i>Pentacomia discrepans</i> (Horn, 1893) | Bolivia, GenBank | | L76833 | AF438902 | AF438927 |

^a<http://www.bio.ic.ac.uk/research/tigerb/benzpaper.htm>

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.

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 4 000 000 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 30 000 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.

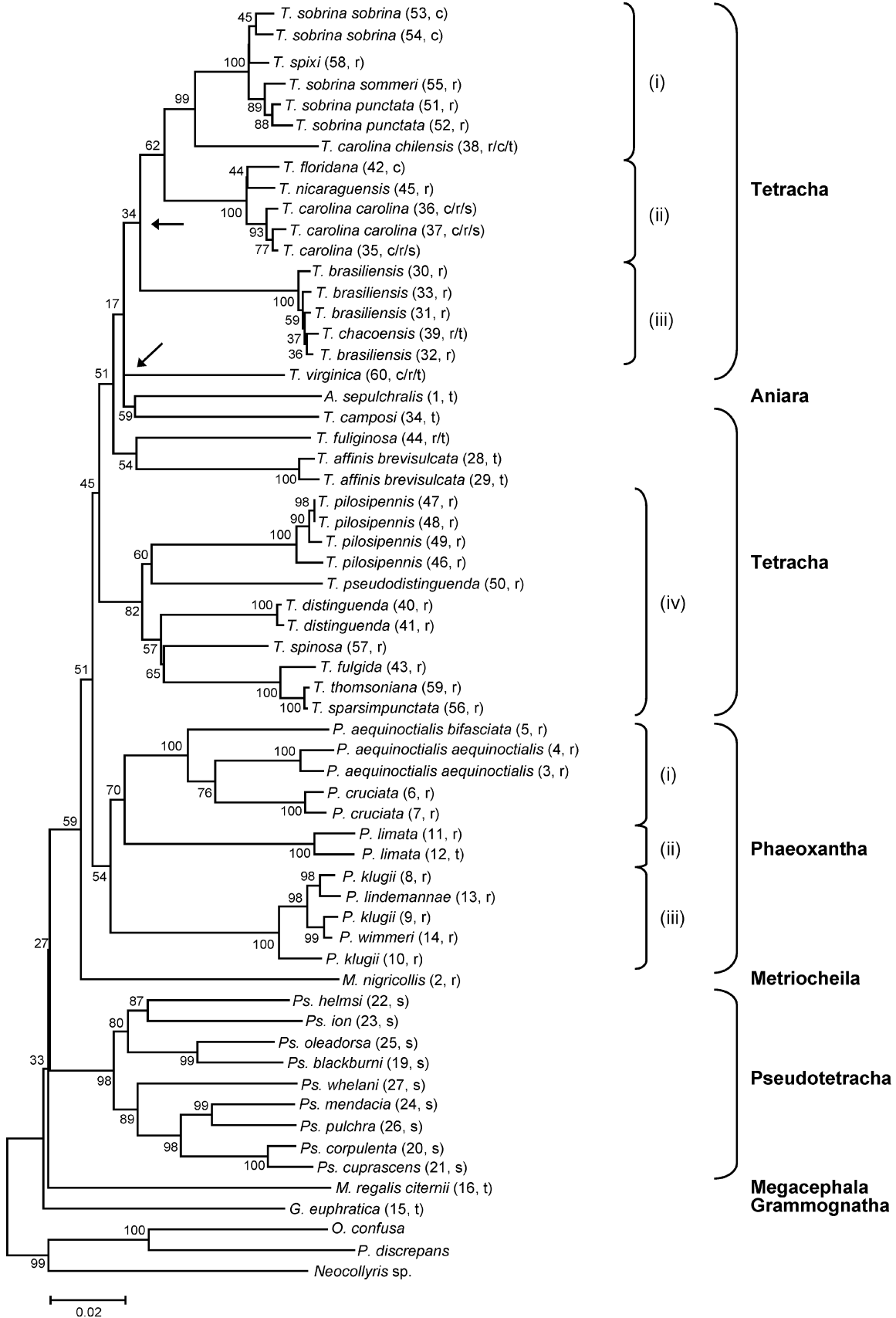
The phylogenetic relationships of *M. regalis regalis* and *A. australasiae* were only deduced with a reduced data set consisting of the 16S and 18S sequences and are therefore not depicted in Figure 1. *M. regalis regalis* grouped together with the second *Megacephala* taxon, *M. regalis cisternii*. *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-lindemanna-wimmeri*.
- *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*.



- *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. sparsimpunctata*, *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*, *Metricheila* 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 three 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. corpulenta*, *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. whelani* 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 germaini* 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. lindemanna*e and *P. wimmeri* represent the same or, alternatively, different species. When kept in the laboratory, adults of *P. klugii* and *P. lindemanna*e 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 have moved to inland and/or coastal salt plains but the American species, however, are mainly confined to river margins. According to this interpretation it is likely that some American *Tetracha* species, *Aniara sepulcralis* and to a lesser extent some *Phaeoxantha* species colonized secondarily and independently non-flooded uplands and/or coastal habitats.

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