Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 32 (2004) 866–880
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae)

Michael Balke,[a,*] Ignacio Ribera,[b] and Alfried P. Vogler[a,c]

[a] *Department of Entomology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*
[b] *Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, José Gutiérrez Abascal 2, 28006 Madrid, Spain*
[c] *Department of Biology, Imperial College at Silwood Park, Ascot SL5 7PY, UK*

Received 24 September 2003; revised 2 March 2004
Available online 10 May 2004

Abstract

Copelatinae is a diverse lineage of diving beetles (Dytiscidae) frequently encountered in wet tropical and subtropical forests, but phylogenetic relationships are very poorly understood. We performed a phylogenetic and biogeographic analysis of this worldwide distributed group based on 50 species including a representative sample of major taxonomic groups and biogeographical regions. DNA sequences were obtained for the mitochondrial genes cytochrome oxidase I, cytochrome *b*, and 16S rRNA, for a total of 1575 aligned nucleotide positions. We found Copelatinae to be monophyletic, placed in a derived position and not sister to all remaining dytiscids, as had been suggested by earlier authors. The largest genus, *Copelatus* with some 460 known species was paraphyletic with respect to the smaller genera *Lacconectus* and *Aglymbus*. Among the major lineages of *Copelatus*, the subgenus *Papuadytes* was consistently recovered as sister to all other species (including *Lacconectus* and *Aglymbus*) with the possible exception of two western Palearctic taxa. We propose that the subgenus *Papuadytes* is removed from *Copelatus* and assigned generic status. Likewise, the two western Palearctic *Copelatus* are removed from this genus, and assigned the available genus name *Liopterus*. Our best phylogenetic hypothesis retrieved Afrotropical and New Guinean plus Australian species of *Copelatus* as monophyletic. Asian species were paraphyletic with respect to a species from Sulawesi which grouped with the species from New Guinea. Asian species were also paraphyletic with respect to Oriental *Lacconectus*, which was grouped with a clade of Neotropical species. Neotropical *Copelatus* form at least two separate lineages. The biogeographical evolution of *Papuadytes* is consistent with the relative age of the landmasses in the Austral region. Basal species are Australian, and successively derived ones are from New Caledonia and New Guinea. One species apparently dispersed from New Caledonia to China. Assuming a molecular clock and using a standard calibration of 2% divergence/MY the origin of Copelatinae is estimated to be between 85 and 95 MY.

1. Introduction

With more than 540 described species (Guéorguiev, 1968; Nilsson, 2001; Nilsson et al., 1996), Copelatinae is one of the most diverse subfamilies of diving beetles (Dytiscidae). They are the most commonly encountered water beetles in wet tropical and subtropical forests, and utilize a wide variety of aquatic habitats ranging from underground waters to bromeliad water tanks, and from the most temporary standing water bodies, such as water caught on fallen palm leaves, to permanent running-water habitats (e.g., Balke, 2004; Balke et al., 2004). They exhibit significant levels of local endemism, particularly among the running-water species. This combination of endemism and high diversity makes Copelatinae a suitable group for the study of historical biogeography and speciation processes.

Many species of Copelatinae still remain to be described. As an example, only 10 species were known from New Caledonia, while estimates after recent fieldwork suggest an actual number of more than 40 (G. Wewalka and M. Balke, unpublished data). The most species rich copelatine genus, *Copelatus*, contains more than 400 described species, and is also the most species rich genus of diving beetles. Despite their species diversity, Copelatinae are morphologically rather

* Corresponding author.
E-mail address: michb@nhm.ac.uk (M. Balke).

1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2004.03.014

homogenous, mainly of oval and dorsoventrally weakly convex body shape and ca. 3–10 mm in length. Many species of *Copelatus* possess conspicuous longitudinal furrows (or striae) on the dorsal side of the elytron of both sexes.

Traditionally the Copelatinae were considered a tribe, Copelatini, within the subfamily Colymbetinae (e.g., Guéorguiev, 1968; Pederzani, 1995; Zimmermann, 1934), although at present they are considered a subfamily (Miller, 2001; Nilsson, 2001). The relationships of Copelatinae within the family Dytiscidae remain contentious. In a phylogenetic analysis of ovipositor characters, Burmeister (1976) found Copelatinae in an isolated phylogenetic position and was unable to identify the closest relatives. Several authors proposed that Copelatinae were the sister to the remaining Dytiscidae (Beutel, 1997; DeMarzo and Nilsson, 1986; Ruhnau and Brancucci, 1984; see Balke, 2004 for a recent review). Beutel (1997) suggested two presumed apomorphies of Dytiscidae excluding Copelatinae: (1) musculus frontobuccalis posterior arranged in two rows of three to four bundles (single pair in Copelatinae) and (2) absence of a crop (= part of digestive tract) (crop present in Copelatinae). Copelatinae were thus characterized merely by plesiomorphies. Miller (2001) suggested a more derived position of Copelatinae within Dytiscidae, as the sister group of Hydrodytinae plus Hydroporinae. In a phylogenetic analysis of Dytiscidae based on 18S rRNA sequences, Ribera et al. (2002a) found Copelatinae as a polyphyletic group and in a basal position among the non-hydroporine dytiscids, but this result was affected by insufficient taxon and character sampling. In a more comprehensive analysis of Dytiscidae phylogeny based on four genes, Copelatinae were retrieved as a monophyletic group (Ribera et al., in press).

The Copelatinae as delimited today include four genera: *Copelatus* Erichson, 1832 (468 spp., including the subgenera *Copelatus* s.str. with 433 spp. and *Papuadytes* Balke, 1998 with 35 spp.), *Lacconectus* Motschulsky, 1855 (43 spp.), *Agaporomorphus* Zimmermann, 1921 (5 spp.), and *Aglymbus* Sharp, 1882 (24 spp.). *Agaporomorphus* was recently redefined by Miller (2001), who removed some of the species previously included in the genus and placed them in a new genus (*Hydrodytes*) in a new subfamily of Dytiscidae (Hydrodytinae), considered to be the sister group of Hydroporinae.

The relationships among genera of Copelatinae, and their respective monophyly, have never been addressed using phylogenetic methods. The large genus *Copelatus* is not defined by specific apomorphies, leaving doubts about its monophyly. Within *Copelatus*, most species have been assigned to the large subgenus *Copelatus* sensu stricto, with several species groups defined according to the number of elytral striae (Sharp, 1882) (Table 1). However, the application of this grouping criterion appears rather imprecise, as the number of striae characterizing a group may either be an exact number, or variable (e.g., 'three to four striae') (Guéorguiev, 1968; Table 1). A further subdivision of *Copelatus* was achieved with the description of the subgenus *Papuadytes*, defined by the presence of a strong, hook-shaped seta ventrally on the male fore tarsus (Balke, 2000). Similarly, to date no specific apomorphies have been defined for *Lacconectus* and *Aglymbus*. In the case of *Agaporomorphus*, the lack of

Table 1
Distribution of extant *Copelatus* species according to species group and major biogeographical region

Species group*	Dorsal stripes	Submarginal stripe	Total Species	AF	AU	NE	NT	PL	OR
abonnenci	22	1	1				1		
capensis	3–4	1	3	3					
chevrolati	7–9	1	10	6	1	1	2		
consors	9–10	—	16	5			11		
duodecimstriatus	6	—	18	8	4		1		5
erichsonii	10	1	124	87	3	3	30		3
haemorrhoidalis	—	—	71	24	20[a]		25	2	
inaequalis	8	—	5				5		
irinus	6	1	100	46	10		3	14	30
longicornis	3–5	—	36	19	3		13	1	
macellus	2	—	4	2			1	1	
nigrolineatus	11–12	—	7		3[b]		2	1	1
pulicarius	5	1	9	4		1	2	1	1
simoni	12 or 13	1 or —	5	1	3[c]		1		
trilobatus	11	1	24	6	7		7		4

AF, Ethiopian; AU, Australian; NE, Nearctic; NT, Neotropical; PL, Palearctic; OR, Oriental.

Data extracted from Nilsson (2001).

Transferred to *Papuadytes*: [a]20 spp., [b]2 spp., [c]1 sp. One species may occur in two regions (i.e., counted twice). *Guignot (1939) and Guéorguiev (1968); bold, representatives studied here.

a bursa copulatrix (part of female genital tract) was suggested as an apomorphy for the genus (Miller, 2001).

Species of Copelatinae are distributed widely in the tropical regions of the world. The subgenus *Copelatus* sensu stricto has a pantropical core range, and there are only a few species in temperate regions. Most species groups as currently suggested have representatives in several major biogeographical regions (Table 1). The subgenus *Papuadytes* was previously considered a New Guinean endemic (Nilsson, 2001), although recent studies revealed the group to have a much wider distribution, including species from Australia, New Caledonia, and Hawaii, which had previously been assigned to the *haemorrhoidalis* group of *Copelatus* sensu stricto (Table 1, M. Balke, unpublished data). A further species of *Papuadytes* was recently discovered in China (Balke and Bergsten, 2003). The genus *Lacconectus* is mostly Oriental (few reach the southern Palearctic) and all species of *Agaporomorphus* are Neotropical. As delimited so far the genus *Aglymbus* has a widely disjunct range: there are 12 Neotropical species, three Ethiopian (incl. Middle East), nine Malagasy, and one species on the Comoro Islands (Nilsson, 2001). The wide range of *Copelatus* s.str, and the widely disjunct ranges on all major continents of representatives of some of the species groups or genera within the subfamily (Table 1) raise the question of their monophyly, and how and when such patterns originated.

To address the relationships and biogeographical origin of Copelatinae we conducted a cladistic analysis based on a wide geographical and taxonomic coverage of the group. We use three fragments of mitochondrial DNA (16S rRNA, cytochrome oxidase 1, and cytochrome *b*) to test the monophyly of Copelatinae and to explore the delimitation of the genera within the subfamily. We discuss the biogeographical origin of the main lineages within Copelatinae and provide an approximate temporal framework for the evolution of the group.

2. Materials and methods

2.1. Taxon sampling

We included representatives of all Copelatinae genera with the exception of *Agaporomorphus*, of which no specimens could be obtained. We aimed to achieve as wide a coverage of the possible lineages within *Copelatus* as possible, and selected species with different number of elytral striae as well as species from different geographical regions. We also tried to obtain species with divergent body size and shape, and deviating male genital morphologies (Table 1). Species of subgenus *Papuadytes* were collected from the entire range of this group, with the exception of Hawaii. For most species included in this analysis it was impossible to provide species names, either because no reliable taxonomic revisions are currently available, or because the species have yet to be described. The numbered operational units we use here reflect a priori identified morphospecies.

Outgroups were selected (Wenzel, 2002) among representatives of other subfamilies of Dytiscidae, including *Hydrodytes* (recently separated from Copelatinae, see above and Miller, 2001), and representatives of the closely related families Amphizoidae and Hygrobiidae. All trees were rooted in Amphizoidae, which is clearly outside Dytiscidae (Miller, 2001; Ribera et al., 2002a,b).

2.2. DNA extraction and sequencing

Beetles were collected into 95–99% ethanol and stored at −20 °C as soon as possible (usually within 3 weeks) (www.waterbeetles.info/molecular.htm). Total DNA was extracted from single beetles using the Qiagen DNeasy tissue kit (Qiagen, Hilden, Germany), removing the abdomen of the beetle before digesting the tissue for 90 min, or by a standard phenol–chloroform extraction. In the Qiagen extractions we used 200 µl buffer as supplied in the kit for the first elution, and 100 µl for a second elution. We keep the DNA from the first elution in the NHM tissue collection at −70 °C. The DNA from the second elution is kept separately at −20 °C as a working stock.

The 3′ ends of the 16S rRNA (16S) and cytochrome oxidase 1 (COI) genes, and a central fragment of cytochrome *b* (Cytb), internal to the pair CB1/CB2 (Simon et al., 1994) were amplified using standard PCR. The oligonucleotide primer pairs used for gene amplification are listed in Table 2. These three mitochondrial genes were chosen to: (1) combine information from different mtDNA regions (Otto et al., 1996), (2) use genes for which extensive datasets for Dytiscidae already exist and which have proven informative in previous studies, and (3) use genes presumably informative at different hierarchical levels (Baraclough et al., 1999; Ribera et al., 2001). PCR temperature regime was: 30 min at 94 °C, 30 min at 47 °C, 60 min at 72 °C (35 cycles), and 10 s at 72 °C. Amplification products were purified with Qiagen Qiaquick PCR purification columns (Qiagen) or Millipore Multiscreen 96-well plates (Millipore, Billerica, MA, USA). Cycle sequencing conditions were: 15 min at 96 °C, 15 min at 50 °C, and 240 min at 60 °C (35 cycles), using the PCR primers. Sequencing reactions were purified by ethanol precipitation (50 µl of 99% ethanol, 2.0 µl of 3 M sodium acetate added to product; centrifuged, washed with 250 µl of 70% ethanol), and electrophoresed on an ABI3700 (Applied Biosystems, Foster City, CA, USA).

Table 2
Oligonucleotide primers used in this study

Gene	Forward	Reverse
Cytochrome oxidase 1	5′-caa cat tta ttt tga ttt ttt gg-3′ (Jerry) (Simon et al., 1994)	5′-ttc att gca cta atc tgc cat att a-3′ (M70) (Lunt et al., 1996)
16S rRNA	5′-cgc ctg ttt atc aaa aac at-3′ (M14, or 16Sar, Simon et al., 1994)	5′-ggt ccc tta cga att tga ata tat cct-3′ (M223, or ND1A, Simon et al., 1994)
Cytochrome *b*	5′-gag gag caa ctg taa tta cta a-3′ (CB3) (Baraclough et al., 1999)	5′-aaa aga aa(ag) tat cat tca ggt tga at-3′ (CB4) (Baraclough et al., 1999)

2.3. Phylogenetic analysis

Sequences were trimmed by removing ambiguously resolved parts of the 3′ and 5′ ends and edited using the Sequencher 4.1 software package (GeneCodes, Ann Arbor, Michigan). As the 16S sequences were length-variable, sensitivity to alignment parameters was tested (Wheeler, 1995). We used the following four alignment procedures: Alignment 1, using the program Clustal W (Thompson et al., 1994) with default values (gap cost 15, extension cost 6.6); Alignment 2, using Clustal W with gap cost 2 and extension cost 1; A1ignment 3, manual with reference to a secondary structure model for beetle 16S rRNA (Buckley et al., 2000); and Alignment 4, the same as Alignment 3 but with positions that could not be aligned unambiguously (i.e., uncertain positional homology statements, Gatesy et al., 1993) excluded. Excluded characters were in positions 16–22, 127–134, 211–213, 241–243, and 328–334 of the aligned matrix (available at www.waterbeetles.info/XXX). The preferred parameter combination was selected according to the maximum congruence among genes, as measured with the incongruence-length difference test, ILD (Farris et al., 1994), and the modified ILD (WILD, Dowton and Austin, 2002; Wheeler and Hayashi, 1998), which represents a normalized ILD allowing for more general comparisons among analyses. Such a congruence based approach is justified under the assumption that only one 'true' phylogeny exists, and thus the lowest level of incongruence should represent the most favorable configuration of the matrix (see, e.g., Meier and Wiegmann, 2002). New sequences have been submitted to GenBank (Accession No. AY334102–AY334272). Additional sequences were taken from Ribera et al. (2002b) (Table 3).

We carried out equally weighted parsimony searches of the aligned sequence data using PAUP* version 4.0b10 (Swofford, 2002) using TBR heuristic searches with 10,000 random addition sequences. Gaps were coded as a fifth character state (Giribet and Wheeler, 1999). The three data sets, Cytb, CO1, and 16S, were analyzed separately and in combination. For the alignment obtained with the set of parameters considered to be optimal we also ran a search with characters reweighted a posteriori according to the rescaled consistency index (RC) (Farris, 1969). Non-parametric bootstrapping, with 10,000 pseudoreplicates and 10 random additions per pseudoreplicate, was used to examine the robustness of nodes (Felsenstein, 1985). Partitioned Bremer support values (PBS) (Baker and DeSalle, 1997) were established searching on constraint trees generated with TreeRot (Sorenson, 1996), using the reweighted characters whenever applicable.

We also used a maximum likelihood approach to test for consistency with the parsimony analyses, and to obtain an estimation of the branch lengths for assessing genetic divergences among Copelatinae. Bayesian analyses were conducted with MrBayes 3.04 (Huelsenbeck and Ronquist, 2001), using a $GTR + I + \Gamma$ model (the optimal model explaining our data as estimated with Modeltest; Posada and Crandall, 1998), allowing the estimations of rate parameters for the three gene partitions independently. We used the default priors (uniform probabilities) starting with random trees, and ran the three heated and one cold Markov chains for 500,000 generations, sampled at intervals of 100 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, and visually determined when the log-likelihood values reached a stable equilibrium. To avoid the risk of the analysis being trapped in local optima, we repeated the procedure twice, beginning with different starting random trees. If the log-likelihood scores were similar, indicating convergence of the two analyses, the trees (once burn-in samples were discarded) were combined in a single majority consensus topology, and the percentage of the nodes were taken as a posteriori probabilities (Huelsenbeck and Ronquist, 2001). As these are posterior probabilities of the clades under the assumed models, we consider values of 95% or greater to be significantly supported (Rannala and Yang, 1996).

To compare the topology of the optimal tree obtained with parsimony and that of the tree obtained by combining the two MrBayes runs we used a Shimodaira–Hasegawa test using the RELL approximation (Shimodaira and Hasegawa, 1999), with 1000 bootstrap replicates, as implemented in PAUP. We used the $GTR + I + \Gamma$ maximum likelihood model with parameter values as estimated by Modeltest for the combined data, as PAUP does not allow the use of different parameter estimations for the partitions in a combined analysis. The Shimodaira–Hasegawa test was preferred to the

Table 3
Taxa studied, geographical origin, and collectors

Species	Our sample ID	Locality	Collector
Aglymbus cf *formosulus* Guignot, 1956	MB 009	Madagascar	Gerecke
Aglymbus cf *elongatus* Kolbe, 1883	MB 015	Madagascar	Gerecke
Aglymbus leprieurii Aube, 1838	MB 307	French Guyana	Balke & Kotrba
Copelatus 008	MB 008	Madagascar	Gerecke
Copelatus oblitus Sharp, 1882	MB 044	Singapore	Balke
Copelatus 051	MB 051	West Papua	Riedel
Copelatus 053	MB 053	West Papua	Riedel
Copelatus 062	MB 062	Republic South Africa	Ribera
Copelatus 069	MB 069	Ivory Coast	Reintjes
Copelatus 109	MB 109	Indonesia: Sulawesi	Stastny
Copelatus 110	MB 110	Indonesia: Sulawesi	Stastny
Copelatus 143	MB 143	Comoro Islands	Kotrba
Copelatus 175	MB 175	Indonesia: Bali	Riedel
Copelatus 178	MB 178	Comoro Islands	Glaw
Copelatus 188	MB 188	Argentina	Beutel
Copelatus 189	MB 189	Argentina	Beutel
Copelatus 206	MB 206	Costa Rica	Balke & Longhorn
Copelatus 211	MB 211	Costa Rica	Balke & Longhorn
Copelatus 212	MB 212	Costa Rica	Balke & Longhorn
Copelatus 222	MB 222	Costa Rica	Balke & Longhorn
Copelatus 224	MB 224	Costa Rica	Balke & Longhorn
Copelatus 270	MB 270	Papua New Guinea	Balke
Copelatus atriceps Sharp, 1882	MB 249	Italy	Bergsten
Copelatus haemorrhoidalis F., 1787	MB 047	Germany	Balke & Kotrba
Papuadytesaubei Montrouzier, 1860	MB 005	New Caledonia	Balke & Wewalka
Papuadytes 001	MB 001	New Caledonia	Balke & Wewalka
Papuadytes 056	MB 056	West New Guinea	Riedel
Papuadytes 059	MB 059	West New Guinea	Riedel
Papuadytes 122	MB 122	New Caledonia	Balke & Wewalka
Papuadytes 128	MB 128	New Caledonia	Balke & Wewalka
Papuadytes 130	MB 130	New Caledonia	Balke & Wewalka
Papuadytes 163	MB 163	New Caledonia	Balke & Wewalka
Papuadytes 166	MB 166	New Caledonia	Balke & Wewalka
Papuadytes 256	MB 256	Papua New Guinea	Balke & Sagata
Papuadytes 257	MB 257	Papua New Guinea	Balke & Sagata
Papuadytes 262	MB 262	Papua New Guinea	Balke & Sagata
Papuadytes 263	MB 263	Papua New Guinea	Balke & Sagata
Papuadytes 265	MB 265	Papua New Guinea	Balke & Sagata
Papuadytes 266	MB 266	Papua New Guinea	Balke & Sagata
Papuadytes 267	MB 267	Papua New Guinea	Balke & Sagata
Papuadytes 269	MB 269	Papua New Guinea	Balke & Sagata
Papuadytes 273	MB 273	Papua New Guinea	Balke & Sagata
Papuadytes ater Sharp, 1882	MB 279	Australia: Western Australia	Hendrich
Papuadytes cf *ferrugineus* Sharp, 1882	MB 104	Australia: South Australia	Balke & Watts
Papuadytes simplex Clark, 1863 (1)	MB 105	Australia: South Australia	Balke & Watts
Papuadytes simplex Clark, 1863 (2)	MB 107	Australia: South Australia	Balke & Watts
Papuadytes shizong Balke and Bergsten, 2003	MB 050	China: Yunnan	Bergsten
Papuadytes IR073	IR 073	West New Guinea	Balke
Outgroups			
Amphizoa insolens LeConte, 1853	IR 142	USA: California	Cognato
Colymbetes paykulli Erichson, 1837	MB 219	Germany	Hendrich
Hydaticus transversalis Pontoppidan, 1763	IR 053	UK	Ribera
Hydrodytes opalinus Zimmermann, 1921	MB 313	France: French Guyana	Balke & Kotrba
Hygrobia hermanni F., 1775	IR 031	Spain	Ribera
Laccophilus poecilus Klug, 1834	IR 026	Spain	Ribera
Laccornellus copelatoides Sharp, 1882	IR 117	Chile	Ribera
Laccornis oblongus Stephens, 1835	IR 055	UK	Bilton
Lancetes nigriceps Erichson, 1834	IR 116	Chile	Ribera
Thermonectus sp.	IR 104	Venezuela	Bilton

more widely used Kishino–Hasegawa test, as our topologies were selected a posteriori (Goldman et al., 2000).

2.4. Rate variation

To estimate branch lengths, sequence variation was fitted by maximum likelihood (ML) on the topology of the preferred parsimony tree. Optimum ML models for the combined data were selected using Modeltest 3.06 (Posada and Crandall, 1998). To estimate relative node ages we fitted ML branch lengths assuming a molecular clock and compared the likelihood to that obtained assuming no clock (Felsenstein, 1981). As the ML ratio was significant (see Section 3), an ultrametric tree was estimated using the non-parametric rate smoothing (NPRS) method of Sanderson (1997), as implemented in TreeEdit v1.0 (Rambaut, A. and Charleston, M.; *evolve.zoo.ox.ac.uk*). An alternative estimation of branch lengths was that obtained with MrBayes. The estimated trees (with branch lengths) of the two runs, once the trees for the 'burn-in' were excluded, were combined and a new set of branch lengths was estimated. To obtain an ultrametric tree the NPRS of Sanderson (1997) was used on the ingroup only. Approximate calibration of absolute time was based on the standard insect molecular clock estimate of 2% divergence/million years (MY) for mtDNA (Brower, 1994), corresponding to a base rate (per branch) of 0.01 substitutions/site/MY.

2.5. Character evolution

Geographical origin and number of elytral striae were coded as multistate characters and character transformations were optimized and visualized using MacClade 4.0 (Maddison and Maddison, 2000).

3. Results

3.1. Sequence variability

All PCR amplifications were successful, with the exception of the 16S fragment of *Aglymbus leprieurii* (Table 3). The length of the protein-coding fragments (Cytb, 343 bp and COI, 736 bp) was not variable (see Table 4 for the number of informative sites per position). The 16S fragments were length-variable, ranging from 481 (*Copelatus* 109 and 110) to 488 bp (*Papuadytes* 056) in Copelatinae, and from 479 (*Laccornellus copelatoides*) to 484 bp (*Hydaticus transversalis* and *Hygrobia hermanni*) among the outgroups. Typical for insect mtDNA (Simon et al., 1994), sequences were AT rich (33% A, 40% T, 13% C, 14% G, mean of 1560 sites; $\chi^2 = 133.66$, $df = 177$, $P = 0.99$). Maximum uncorrected p distance among any two sequences was 0.18, between *Amphizoa insolens* and *Aglymbus* cf. *elongatus*. Maximum p distance within the ingroup was 0.16, between *Copelatus atriceps* and *Copelatus* sp. 270. Minimum p distance was 0.006, between *Papuadytes* sp. 273 and *Papuadytes* sp. 267 (two New Guinean species), corresponding to a difference of 9 bp.

3.2. Phylogeny of Copelatinae

Among the combined datasets with different 16S alignment parameters the alignment by eye (Alignment 3) provided the shortest trees with the lowest ILD and WILD (Table 5). Tree topologies using all 16S characters and those obtained after excluding ambiguously aligned regions (i.e., Alignment 4) were mostly congruent, with lower bootstrap values obtained for the latter analysis. We thus selected Alignment 3 with full 16S sequences for further analyses.

Table 4
Number of parsimony informative and constant characters in first, second, and third positions of CO1 and CytB (inf., informative; totl, total)

	CytB				CO1			
	First	Second	Third	1 + 2 + 3	First	Second	Third	1 + 2 + 3
Total	114	114	115	343	245	245	246	736
Informative	38	14	110	162	60	15	219	294
% inf of total	33	12	96	/	24	6	89	/

Table 5
Treelengths using partitions separately, combined, and under different alignment parameters and the ILD/WILD

Alig.[a]	16S	COI	Cytb	Sum length partitions	Combined analysis	N trees	ILD	WILD
1	1267	2594	1560	5421	5583	7	162	0.03
2	1583	2594	1560	5737	6070	14	333	0.05
3	1018	2594	1560	5172	5301	14	129	0.02

Alig., 16S alignment used; ILD, incongruence-length difference test (Farris et al., 1994; ILD = length combined − sum length individual partitions); WILD, Wheeler's ILD (Wheeler and Hayashi, 1998; WILD = ILD/length combined).

[a] 1, 2, 3: Alignments, 1, gap cost 15, gap extension 6.6; 2, gap cost 2, gap extension 1; 3, manual.

In the strict consensus of the 14 shortest trees (length 5301, CI = 0.27, RI = 0.44), Copelatinae were monophyletic but weakly supported (<50% bootstrap). Within Copelatinae, *Papuadytes* was monophyletic and well supported (bootstrap 75%, Bremer support value 10), but *Copelatus* s.str. formed an unresolved polytomy, including *Aglymbus* and *Lacconectus*. The two Malagasy *Aglymbus* species always formed a strongly supported monophyletic group (Fig. 1, Clade A), sister to *Copelatus haemorrhoidalis* and *C. atriceps* (Clade B), the only extant western Palearctic species of Copelatinae. *Lacconectus* was monophyletic and sister

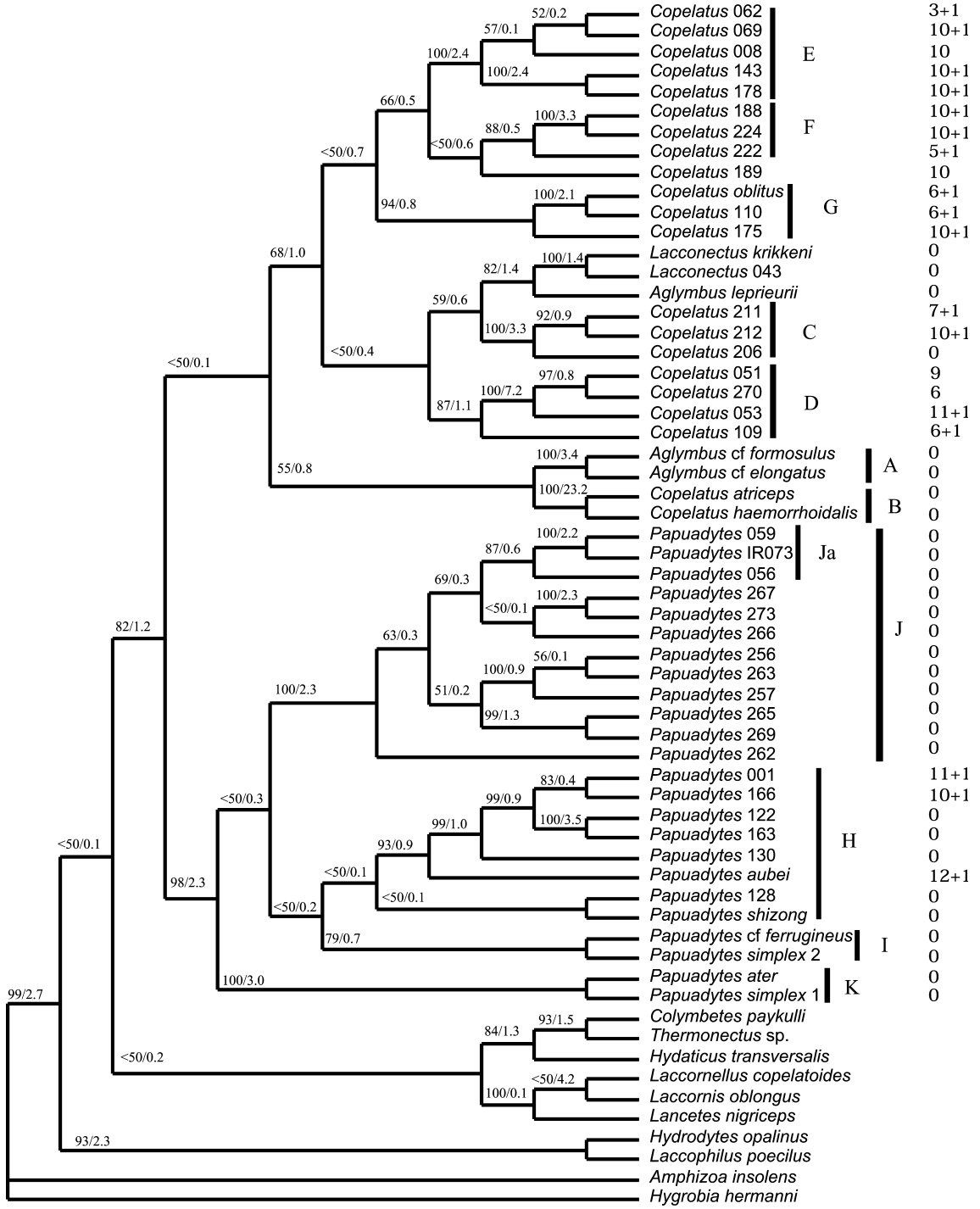

Fig. 1. The single most parsimonious tree obtained from Alignment 3 (gap cost 15, extension cost 6.66, manually modified) of the combined dataset, after reweighting the characters according to the rescaled consistency index. Above branches, bootstrap values (only >50% shown)/combined Bremer support values. Bremer support values were computed based on the reweighted characters. (A–K) clades discussed in the text. Numbers on the right refer to the number of elytral striae (0, no striae; $X + 1$, X dorsal and one submarginal striae).

to the South American *Aglymbus leprieurii*, and both sister to a lineage of Neotropical species of *Copelatus*. Other species of *Copelatus* s.str. were assigned to five clades, labelled C–G in Fig. 1, which are congruent with major biogeographical regions (see below).

A secondary search after the characters were reweighted a posteriori according to the rescaled consistency index (RC) resulted in a single most parsimonious tree (CI = 0.23, RI = 0.44, equal weight cost 5304) (Figs. 1 and 2). The reweighting increased the congruence among partitions, as measured with the correlation among PBS values (Table 6). In the equally weighted tree none of the pairwise correlations between partitions were significant (as measured with the PBS), while in the reweighted tree the only non-significant correlation is that between Cytb and 16S (Table 6).

Table 6
Correlation among the partitioned Bremer support values in the equally weighted and in the reweighted trees

	16S	CytB	CO1	Total BS
16S	1	n.s.	0.40	0.62
CytB	n.s.	1	0.27	0.43
CO1	n.s.	n.s.	1	0.95
Total BS	0.46	0.48	0.87	1

Below diagonal, correlations in the equally weighted tree; above diagonal, correlations in the tree reweighted a posteriori according to the rescaled consistency index. Total BS, total (combined) Bremer support value.

The ingroup topology of the reweighted tree was essentially identical to that of the consensus of the 14 trees found in the equally weighted analysis. The support for the monophyly of Copelatinae was increased to a

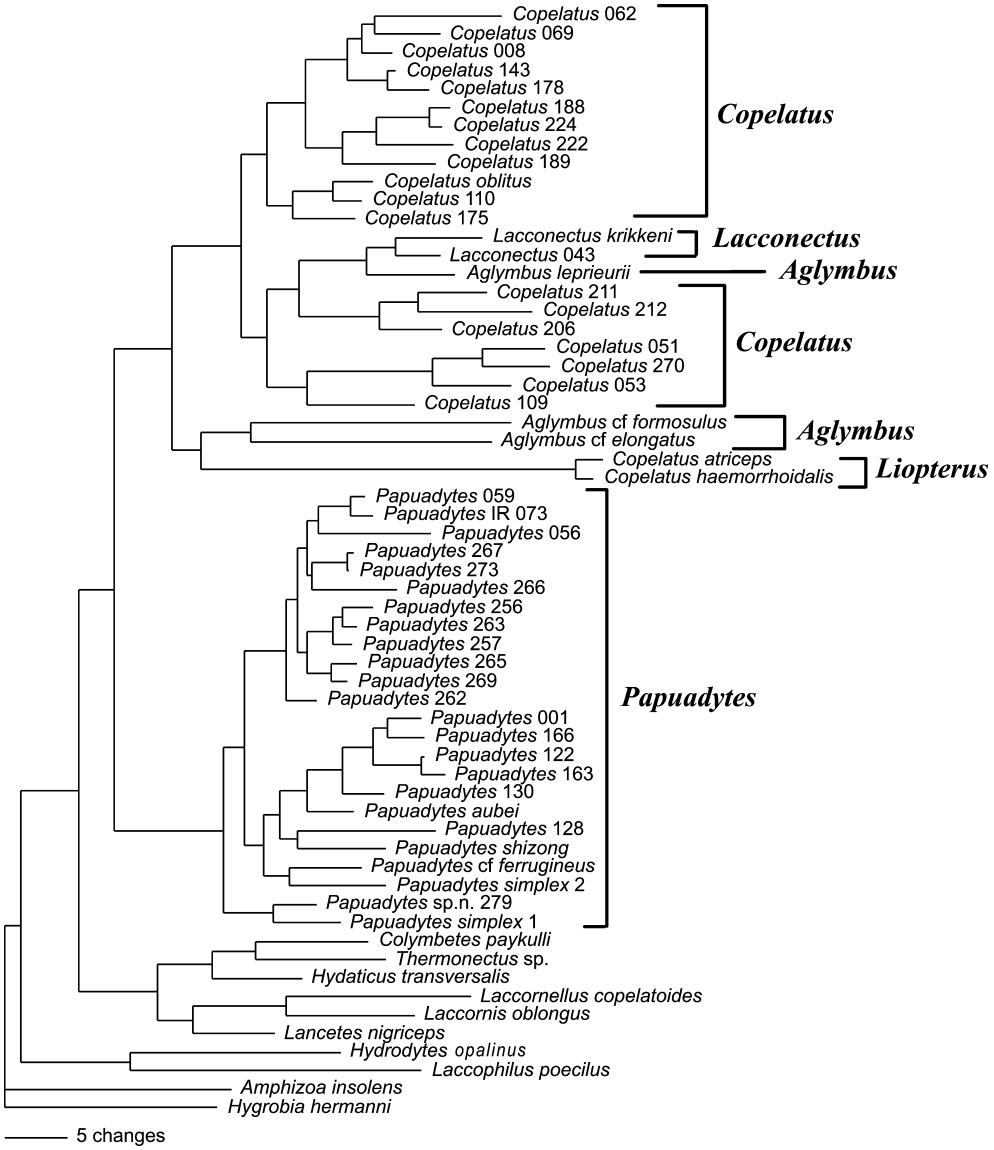

Fig. 2. Phylogram of the single most parsimonious tree obtained with Alignment 3 and character reweighting. Branch lengths refer to parsimony reconstructed characters with equally weighted characters.

bootstrap value of 82%, with a Bremer Support of 1.2 (using reweighted character values). Within Copelatinae there were two main clades, *Papuadytes* and the remaining *Copelatus* plus *Aglymbus* and *Lacconectus*. Within the latter, the western Palearctic species and the Malagasy *Aglymbus* were sister to *Aglymbus* and *Lacconectus*, grouped with other *Copelatus* species in a large clade with relatively low support (68% bootstrap) (Fig. 2).

Within *Papuadytes* clades consistently retrieved were labelled H–K in Fig. 1: Clade H included the New Caledonian species plus the Chinese *Papuadytes shizong*; Clade J represented all New Guinean species; and Clades I and K contain Australian taxa. While the monophyly of the New Guinean clade was highly supported (bootstrap 100%; Bremer 2.3), the New Caledonian species formed a weakly supported lineage including the Chinese *P. shizong*. The four Australian species of *Papuadytes* were paraphyletic with respect to the New Caledonian and New Guinean clades.

Aglymbus leprieurii, without 16S sequence, was excluded from one combined analysis to test the sensitivity to missing data. The search resulted in two most parsimonious trees with a length of 5199 steps. The topology of the strict consensus of these two trees was very similar to that found including all taxa, with the only difference of the placement of the Australian *Papuadytes* Clade I (Fig. 1), sister to the New Guinean species in the analysis excluding *A. leprieurii* but sister to the New Caledonian species on the tree including all taxa.

In the two independent runs of MrBayes the Markov chains reached stability after ca. 30,000 generations (i.e., 300 data points), although we discarded 50,000 (i.e., 500 data points) as a conservative estimate. The two runs reached very similar maximum likelihood values: −22795.83 and −22798.54, respectively (arithmetic mean of the estimated marginal likelihood of −22802.87 and −22805.45). The two majority rule consensus trees, showing the posterior probabilities of each node, are almost identical in the two runs both in topology and node posterior probabilities. The only differences were in some nodes with posterior probabilities of less than 70% within the *Copelatus* s.str. clade, which were unresolved in one of the trees, and in the internal topology of some of the outgroups nodes. The results of both runs were thus combined in a single tree (Fig. 3). The main difference with respect to the parsimony tree was the basal position of the *Copelatus haemorrhoidalis* plus *C. atriceps* clade, which was sister to the remaining Copelatinae instead of sister to *Copelatus* s.str. as in the parsimony tree. *Papuadytes* and *Copelatus* s.str. (the latter including *Lacconectus* and *Aglymbus* but excluding the two European species *C. haemorrhoidalis* and *C. atriceps*) were recovered as monophyletic, with high posterior probabilities (Fig. 3). Within each of the clades, the main lineages were identical to those obtained with parsimony, except for the position of *A. leprieurii*, which forms a monophyletic clade with the other two species of *Aglymbus* (sister to *Lacconectus* in the parsimony tree). Some of the relationships among species groups were however different, although involving nodes with relatively low support (less than 75% posterior probabilities).

The comparison of the topology of the optimal (reweighted) tree obtained with parsimony and that of the tree obtained by combining the two MrBayes runs using a Shimodaira–Hasegawa test revealed significant incongruence ($p = 0.013$), probably reflecting the different position of the *C. haemorrhoidalis*/*C. atriceps* clade.

3.3. Rate of divergence

The optimal model for the combined dataset as estimated by Modeltest is a complex GTR + I + Γ, with a proportion of invariate sites of 0.46 and a Gamma shape parameter of 0.40. The ingroup sequences had significantly unequal rates, as measured with a likelihood ratio between the scores enforcing vs. not enforcing a molecular clock (−lnML non-enforcing a clock = 18227.75;−lnML enforcing a clock 18280.95; χ^2 with 48 degrees of freedom $p \ll 0.001$). We therefore applied NPRS (Sanderson, 1997) to estimate an ultrametric tree (Fig. 4). The same method was applied to the tree obtained with MrBayes. Branch lengths of the common branches between these two ultrametric trees were highly correlated ($r^2 = 0.89$, $n = 65$, $p \ll 0.001$; Fig. 5). The slope of the regression is not significantly different from 1 (1.09 ± 0.1, 95% confidence interval) and the intercept from 0 (0.001 ± 0.02), indicating that the branch lengths of the two trees can be considered to be equivalent.

Using the standard insect molecular clock of 2% divergence/MY, the origin of the Copelatinae radiation was estimated at 85–95 MY ago, based on the Bayesian and ML branch lengths fitted onto the preferred parsimony topology, respectively. The start of the radiations of *Papuadytes* on New Caledonia were estimated at 47–55 MY, and in New Guinea around 30 MY (Figs. 4 and 5).

4. Discussion

4.1. Phylogeny and classification of Copelatinae

We found Copelatinae monophyletic under all analyses, with fairly strong support in the parsimony a posteriori reweighted tree and in the Bayesian probability analyses. Copelatinae was not recovered as the sister to all other Dytiscidae, as suggested by some authors based on morphological evidence (see Balke, 2004). We also did not find it sister to Hydroporinae or part of Colymbetinae, as suggested by Miller (2001) and Sharp (1882), respectively, but to a poorly supported

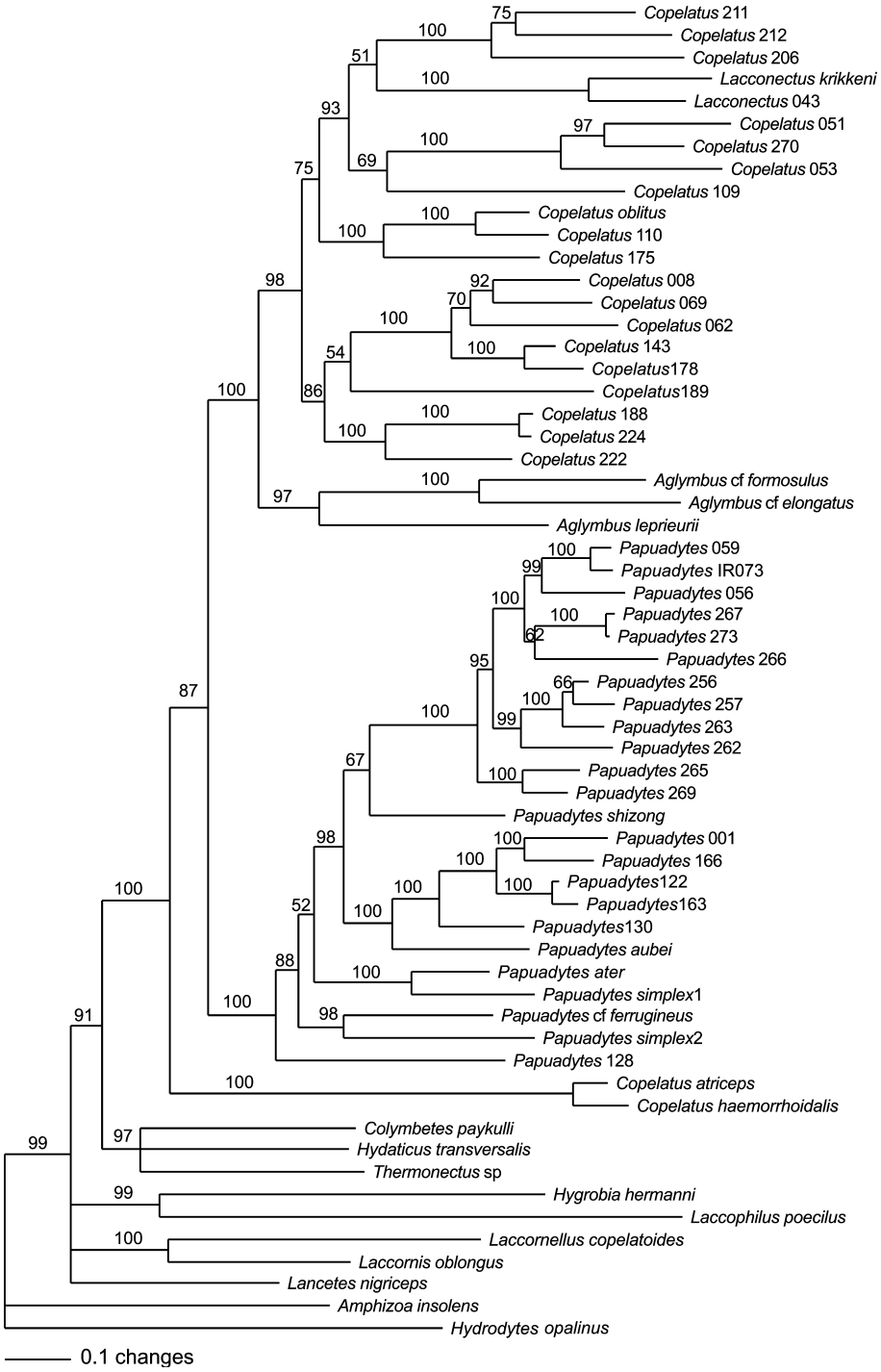

Fig. 3. The 50% majority rule consensus of the majority consensus rule of the trees obtained with the combined two runs of MrBayes (with the 9000 burn-in trees discarded). Number above nodes are posterior probabilities. Branch lengths are MrBayes estimates.

clade containing Colymbetinae, Hydroporinae, Dytiscinae, and Lancetinae.

The most salient finding of our analyses was the deep split within what has been considered the genus *Copelatus*, which we found to consist of two well defined clades: *Papuadytes* and the remaining species, the latter with or without the inclusion of the two western Palearctic taxa. The level of separation of these two species was surprising, and they may even represent the sister group of the rest of the Copelatinae. The monophyly of *Papuadytes* and its deep separation from other *Copelatus* sensu lato is strongly supported in all analyses. The group is readily characterized by a morphological apomorphy, the presence of a strong, distinctly hook-shaped seta on the anteroventral angle of the fourth male foretarsal segment (plesiomorphic state: thin seta

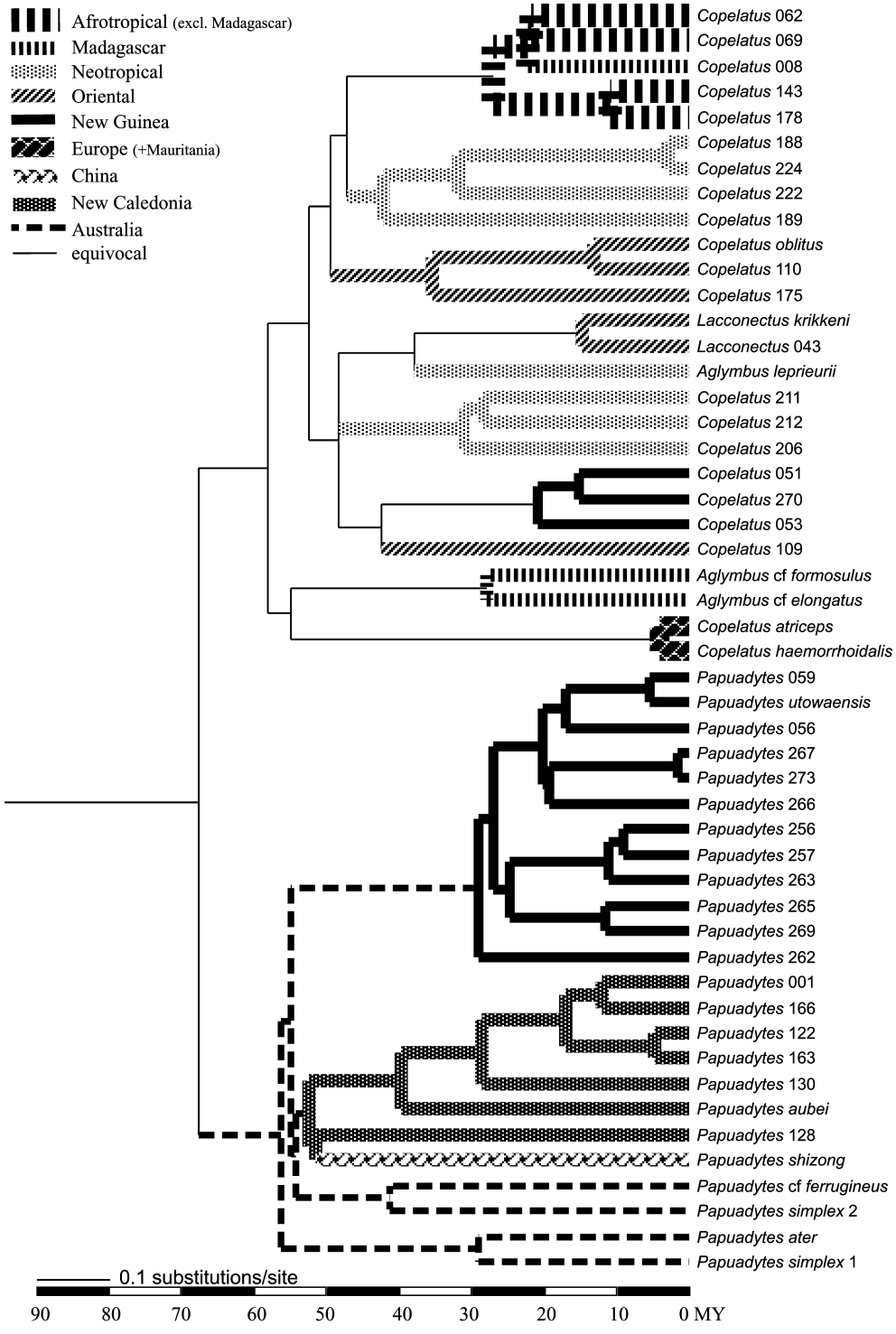

Fig. 4. Ultrametric tree obtained with NPRS based on ML branch lengths and the tree topology of Fig. 1. The time scale was calibrated using a standard rate 2% divergence/MY (Brower, 1994). Ancestral geographical distributions were optimized with MacClade, using the most parsimonious non-ambiguous reconstruction.

present) (Balke, 1998, 2000). *Papuadytes* has so far been considered a subgenus of *Copelatus*, but given with the results reported here we assign it generic status, as *Papuadytes* Balke, 1998 *new status*, genus of Dytiscidae with type species *Copelatus rivulus* Balke, 1998.

The analysis also revealed that the smaller copelatine genera are consistently subordinated within *Copelatus* sensu lato, in particular *Lacconectus*. However, the position of *Lacconectus* is unstable and weakly supported in all analyses. Because of this uncertainty, and the

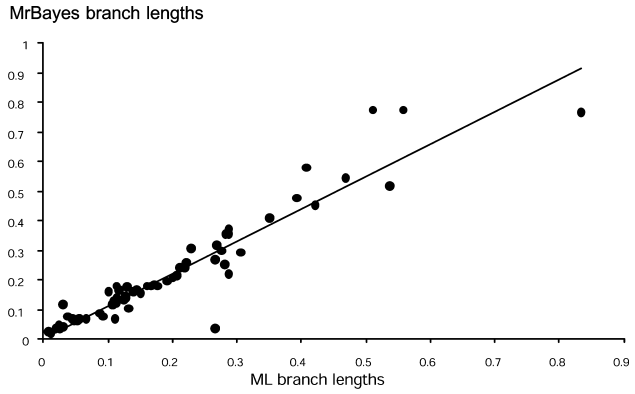

Fig. 5. Relationship between the length of the branches estimated with the NPRS applied to the tree obtained with ML (GTR + I + G model) and MrBayes (see Section 2). Only equivalent branches are included in the comparison. Equation of the regression line: length Bayesian probability = 1.09 (ML length), $r^2 = 0.89$, $n = 65$.

numerous nomenclatorial changes that would be required (because of species homonymies) we do not formally synonymize *Lacconectus* with *Copelatus* at this stage. *Lacconectus* was divided into species groups by Brancucci (1986), who revised the genus and provided a phylogenetic analysis based on morphology. The method applied was typological and it remains unclear how polarity decisions were derived, leaving the outcome rather preliminary. However, the monophyly of this group may be suggested by the very similar design of the male genital morphology (Brancucci, 1986).

Likewise, the position and monophyly of *Aglymbus* (the South American and Malagasy lineages) remain a contentious issue. In the parsimony analysis, all nodes leading to *Aglymbus* species have relatively low support and the genus is polyphyletic. In the Bayesian analyses it is a well supported-monophyletic group sister to *Copelatus* (including *Lacconectus* but excluding the European species). *Aglymbus* was traditionally characterized by the lack of metacoxal lines, which are impressions on both metacoxae running from the metacoxal process in cranial direction towards the metaventrite (Miller, 2001). This character is also present in *Lacconectus* (Brancucci, 1986; Sharp, 1882) and some *Copelatus* (M. Balke, unpublished observations; see also Miller, 1997). As noted by Brancucci (1986), the generic separation of *Lacconectus* and *Aglymbus* based on morphology is not very well founded: in general it appears that all the Copelatinae with reduced metacoxal lines occurring in the Oriental region were assigned to the genus *Lacconectus*, while species with reduced metacoxal lines from the rest of the world were assigned to *Aglymbus*. The Malagasy *Aglymbus* are well characterized morphologically, and were suggested to form a monophyletic group of uncertain position (Balke, 2000). An apomorphy for this group of beetles is the presence of a very stout, straight, spine-like seta on the anteroventral angle of the fourth male foretarsal segment (plesiomorphic state: thin seta present). At least nine such species are known (Wewalka, 1982) and denser sampling in this clade would help to resolve its phylogenetic position. A sister-group relation of Malagasy *Aglymbus* with *Papuadytes* was suggested based on presence of an thickened seta on the male protarsus (plesiomorphic state: thin seta present; derived states seta (1) very stout, spine-like and (2) distinctly hook shaped) (Balke, 2000). Our results do not support this hypothesis, although the weakly supported basal nodes of Copelatinae require further investigation.

The only two western Palearctic species of Copelatinae, *C. haemorrhoidalis* and *C. atriceps*, form a monophyletic, rather isolated lineage. They have traditionally been included in the "*C. haemorrhoidalis* group" together with all species without elytral striae (Nilsson, 2001). This is, however, the plesiomorphic character state for the whole subfamily (see below), and this group included species of *Papuadytes* and of *Copelatus* of different lineages (e.g., *C. laccophilinus* Sharp, 1882 which belongs to the same morphological group as *Copelatus* sp. 206 studied here). The name *Liopterus* Dejean, 1833, currently considered to be a synonym of *Copelatus*, was created for what is today known as *C. haemorrhoidalis* (Nilsson, 2001; see also Balfour-Browne, 1939). It was considered to be a subgenus of *Copelatus* by some authors (e.g., Guignot, 1931). We propose to resurrect *Liopterus* Dejean, 1833 ***new status*** as a valid genus name of Dytiscidae, for *L. haemorrhoidalis* and *L. atriceps*, with *Dytiscus haemorrhoidalis* as type species.

We did not recover *Copelatus* sensu stricto as delimited by Nilsson (2001) in any of the analyses. According to Miller (2001) the genus *Copelatus* (including *Liopterus* as defined here) is characterized by two apomorphic features, a strongly sclerotized bursa copulatrix and aciculate striae on the metacoxa. However, the metacoxal striae are also present in *Papuadytes* and *Aglymbus*, and thus the status of this character remains to be explored.

Copelatus (including *Liopterus* and some species of what is now *Papuadytes*) was traditionally divided into 15 species groups according to the number of elytral striae (Table 1; Miller and Balke, 2003; described a 16th, fossil group) (Nilsson, 2001; see Balfour-Browne, 1939, 1950 for a taxonomic review). The elytron may be completely smooth, i.e., devoid of any such line (as in most *Lacconectus* and *Papuadytes*); with only irregularly spaced short longitudinal cuts ("strioles") (as in some *Papuadytes* and *Liopterus*, but also *Lacconectus* and *Aglymbus*); or bear up to 23 striae, classified according to their relative position in dorsal and submarginal (Guéorguiev, 1968; Nilsson, 2001; Sharp, 1882). The possible homology of striae and strioles remains to be clarified. According to our preferred phylogenetic hypothesis, groups defined by striae are not monophyletic

(Fig. 1). There are at least three independent origins (and one loss) of the elytral striae in *Copelatus*, plus at least one in *Papuadytes*. The ancestral condition of the largest clade with elytral striae (within *Copelatus*) as reconstructed in our phylogeny is 10 dorsal plus one submarginal (10 + 1), and the general tendency seems to be toward a reduction of their number (although with some increases, e.g., *C. abonnenci* Guignot, 1939 and related, undescribed species from South America, with up to 22 + 1 striae). However, the number of striae seems to be highly labile, as seen in, e.g., Clade C, which includes three closely related *Copelatus* species from Costa Rica with respectively, 0, 7 + 1, and 10 + 1 striae. The latter configuration is also found in three unrelated clades of *Copelatus*, as well as in *Papuadytes aubei* (Fig. 1). Clearly, changes in strial characters do not conform with the molecular tree. However, other morphological traits are consistent. For example, Clade C can be defined by the median lobe of the aedeagus, which is long, thin, and with the inner margin saw like. In Clade G the median lobe is short and stout, and the distal parties broad and club shaped. The median and lateral lobes in all species of Clade Ja (from West New Guinea) exhibit both a peculiar shape different from all other Copelatinae (M. Balke, unpublished observations). Hence, genitalic characters seem to further support the molecular tree, and the notion that the striae are highly homoplastic.

4.2. Biogeography and historical origin

The pantropical distribution of Copelatinae raises the question of the geographical origin of the group, specifically whether its wide distribution on the southern continents would be explained by an ancient Gondwanian origin, with vicariance events due to continental breakup explaining the present distribution of major lineages. Our hypotheses of copelatine relationships did not allow for an unambiguous reconstruction of their biogeographical origin (Fig. 1). However, when a standard insect molecular clock was applied, the origin of Copelatinae was estimated to between 85 and 95 MY ago (Fig. 4). The origin of the two main clades within Copelatinae, and the basal radiation within each of them, was dated to between 60 and 75 MY. The main continental masses had already been isolated from each other for ca. 60 MY (Smith et al., 1994). Although this conclusion depends on the molecular clock calibration based on a rate estimate from external sources, which may not be applicable to Copelatinae, the suggestion is that whatever its geographical origin the basal lineages are not the product of vicariant events related to the breakdown of Gondwana. There are a few known species of fossil *Copelatus* of recent age (Miocene) which might be useful for a direct calibration (Miller and Balke, 2003; Nilsson, 2001) but it was not possible to place them in our phylogenetic tree. Two further species have been described recently from specimens preserved in Eocene Baltic and Dominican amber (Miller and Balke, 2003), estimated to be, respectively, 45–50 and 30 MY old (Wichard and Weitschat, 1996). The species from Baltic amber (*C. aphroditae* Balke, 2003) had 19 dorsal elytral striae, a number not present in any of the known extant species. However, if this species is assumed to be descended from the common ancestor of the extant *Copelatus* with elytral striae (clades C–G in Fig. 1), its estimated maximum age of 55 MY based on the standard insect mtDNA clock is within the expected range. The species from Dominican amber (*C. predaveterus* Miller, 2003; with 11 + 1 striae) has external morphological resemblance to *Copelatus* sp. 211, from Costa Rica. If this is taken as evidence of phylogenetic relationship—which is perhaps not justified considering the general morphological homogeneity of the species of *Copelatus*—this would place the origin of the clade (*C. predaveterus* plus *Copelatus* sp. 211) at no more than 30 MY, which again is consistent with both mtDNA calibrated trees.

Accepting the mtDNA calibration and the topology of the tree, the distribution of major extant lineages must have been affected by inter-continental dispersal, as taxa found in major biogeographical realms have diverse phylogenetic affinities. For example, the Oriental and Neotropical species are divided in several lineages with apparently independent origins. Similarly, among the Oriental species the two *Copelatus* from Sulawesi have a disparate phylogenetic origin: *Copelatus* sp. 109 groups with species from New Guinea, while *Copelatus* sp. 110 groups with species from Southeast Asia. Sulawesi lies in the center of "Wallacea," and is well known among biogeographers for its intermediate position between Asia and Australia, where the faunas of these two biogeographical regions mix (Hall and Holloway, 1998; Wallace, 1860). Only the *Copelatus* plus *Lacconectus* from the Ethiopian plus Malagasy (= Afrotropical) species flocks are monophyletic, indicating less exchange with other regions.

Rare dispersal events also appear to explain the biogeographical patterns in *Papuadytes*. The phylogenetic relationships within *Papuadytes* are consistent with an Australian origin, with independent colonization of New Guinea and New Caledonia (Fig. 4). The New Guinean species are monophyletic, as already suggested by Balke (2000) based on morphology. The New Caledonian species are paraphyletic with respect to the Chinese *P. shizong*, a surprising affinity but plausible from morphological evidence. Thus, New Caledonia was likely colonized from Australia only once, and the distribution of the isolated *P. shizong* can be explained by long distance dispersal from the Australasian region, or the extinction of related lineages in the intermediate regions.

In conclusion, our results imply that the origin of the main lineages within Copelatinae involved occasional

long range dispersal among continental masses. It is also clear that the sampling density has to be increased to gain a more detailed picture of the biogeographical evolution of the group, especially in the newly redefined genus *Copelatus*.

Papuadytes, which make up ca. 90% of Austro-Pacific species of Copelatinae, may have radiated in this region since at least 60 million years. Their spatial evolution apparently reflects the geological genesis of the region, with the youngest clades occurring in the youngest landmasses (New Guinea). Time calibrated molecular phylogenies will allow the organisms themselves to inform us about the temporal dimension of their evolution, thus providing a tool to discriminate between vicariance and dispersal (De Jong, 2003; Holloway, 2003). We suggest that the study of this group (specifically adding slowly evolving nuclear genes for more accurate age estimations, Whitfield, 2002) could significantly improve our understanding of the puzzling geological and faunal evolution in this megadiverse region, a challenge ever since Wallace (1860) and his contemporaries studied this question.

Acknowledgments

We thank the following friends and colleagues for help in the lab, field and/or contributing specimens. Our projects cannot possibly be successful without their help: F. Glaw and M. Kotrba (München, Germany); J. Bergsten (Umeå, Sweden); D. Bilton (Plymouth, UK); Kelly Miller (Provo, USA); A. Herrera (INBio, San José, Costa Rica); S. Longhorn (London, UK); C.H.S. Watts (Adelaide, Australia); G. Wewalka (Wien, Austria); J. Stastny (Liberec, Czech Republic); C. Poellabauer and family (Noumea, New Caledonia); A. Riedel (here today, gone tomorrow); M.A. Jäch (Wien, Austria); K. Sagata and the WCS (Goroka, PNG); C.M. Yang (Singapore); and Joep Moonen and family (French Guyana). Thanks to Dr. Michael Monaghan and Dr. Jesus Gomez-Zurita (NHM, London) for critically reading the manuscript. This work was supported by the Linnean Society of London and a Marie Curie Postdoctoral Fellowship under Contract No. HPMF-CT-2001-01365 of the European Commission to M.B., and travel was supported by the Deutsche Forschungsgemeinschaft (DFG) (BA 2152/1-1), and the European Union Large Scale Facility (Sysresource) at the Natural History Museum (IHP-MCFI-99-1).

References

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. Syst. Biol. 46, 654–673.

Balfour-Browne, J., 1939. On *Copelatus* Er. and *Leiopterus* Steph. (Col. Dytiscidae) with descriptions of new species. Trans. R. Ent. Soc. Lond. 88 (2), 57–88.

Balfour-Browne, F., 1950. British Water Beetles. vol. 1, Ray Society, London.

Balke, M., 1998. Revision of New Guinea *Copelatus* Erichson, 1832 (Insecta: Coleoptera: Dytiscidae): the running water species, Part I. Ann. Naturhist. Mus. Wien B 100, 301–341.

Balke, M., 2000. Die Schwimmkäfer Neu Guineas. Artenreichtum, Phylogenie, Biogeographie und Lebensweise (Coleoptera: Dytiscidae). Dissertation.de, Berlin.

Balke, M., 2004. Dytiscidae. in: Beutel, R.G., Leschen, R. (Eds.), Handbook of Zoology. Coleoptera. DeGruyter, Berlin, pp. 000–000 (in press).

Balke, M., Bergsten, J., 2003. Dytiscidae I.: *Papuadytes shizong* sp.n. from Yünnan (China), the first member of the subgenus *Papuadytes* Balke found west of the Wallace Line (Coleoptera). In: Jäch, M.A., Ji, L. (Eds.), Water Beetles of China, vol. 3. Zoologisch-Botanische Gesellschaft in Österreich und Wiener Coleopterologenverein, Wien, pp. 89–94.

Balke, M., Watts, C.H.S., Cooper, S., Humphreys, W.F., Vogler, A.P., 2004. A highly modified stygobiont diving beetle of the genus *Copelatus* (Coleoptera, Dytiscidae): taxonomy and cladistic analysis based on mtDNA sequences. Syst. Entomol. 29, 59–67.

Baraclough, T.G., Hogan, J.E., Vogler, A.P., 1999. Testing whether ecological factors promote cladogenesis in a group of tiger beetles (Coleoptera: Cicindelidae). Proc. R. Soc. Lond. B 266, 1061–1067.

Beutel, R.G., 1997. Über Phylogenese und Evolution der Coleoptera (Insecta). Abh. naturwiss. Ver. Hamburg (NF) 31, 1–164.

Brancucci, M., 1986. Revision of the genus *Lacconectus* Motschulsky (Coleoptera, Dytiscidae). Ent. Bas. 11, 81–202.

Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA 91, 6491–6495.

Buckley, T.R., Simon, C.M., Flook, P.K., Misof, B., 2000. Secondary structure and alignment of domains IV and V of the insect mitochondrial large subunit rRNA gene. Insect Mol. Biol. 9 (6), 565–580.

Burmeister, E.G., 1976. Der Ovipositor der Hydradephaga (Coleoptera) und seine phylogenetische Bedeutung unter besonderer Berücksichtigung der Dytiscidae. Zoomorphologie 85, 165–257.

De Jong, R., 2003. Are there butterflies with Gondwanan ancestry in the Australian region? Inv. Syst. 17, 143–156.

DeMarzo, L., Nilsson, A.N., 1986. Morphological variation and fine structure of some head structures in larvae of Dytiscidae (Coleoptera). Ent. Bas. 11, 29–42.

Dowton, M., Austin, A.D., 2002. Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behaviour of the incongruence length difference test in mixed-model analyses. Syst. Biol. 51 (1), 19–31.

Farris, J.S., 1969. A successive approximations approach to character weighting. Syst. Zool. 18, 374–385.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. Cladistics 10, 315–320.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gatesy, J., DeSalle, R., Wheeler, W., 1993. Alignment-ambiguous nucleotide sites and the exclusion of systematic data. Mol. Phylogenet. Evol. 2, 152–157.

Giribet, G., Wheeler, W.C., 1999. On gaps. Mol. Phylogenet. Evol. 13, 132–143.

Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652–670.

Guéorguiev, V.B., 1968. Essai de classification des Coleopteres Dytiscidae. 1. Tribus Copelatini (Colymbetinae).. Izvestija na Zoologitjeskija Institut s Musei Sofia 28, 5–45.

Guignot, F., 1931-1933b: Les Hydrocanthares de France. Toulouse: Les Frères Douladoure, 1057.

Guignot, F., 1939. Nouveaux *Copelatus* de l'Amérique du Sud et des Antilles (Col., Dytiscidae). Bull. Soc. Ent. Fr. 43 (1938), 245–250.

Hall, R., Holloway, J.D. (Eds.), 1998. Biogeography and Geological Evolution of SE Asia. Backhuys, Leiden.

Holloway, J.D., 2003. Biological images of geological history: through a glass darkly or brightly face to face? J. Biogeogr. 30, 165–179.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Lunt, D.H., Zhang, D.X., Szymura, J.M., Hewitt, G.M., 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primes for phylogenetic studies. Insect Mol. Biol. 5, 153–165.

Maddison, W.P., Maddison, D.R., 2000. MacClade, Version 4.0.. Sinauer, Sunderland, MA.

Meier, R., Wiegmann, B.M., 2002. A phylogenetic analysis of Coelopidae (Diptera) based on morphological and DNA sequence data. Mol. Phylogenet. Evol. 25, 393–407.

Miller, K.B., 1997. Reassignment of *Agabus eminens* Kirsch to *Aglymbus* Sharp (Coleoptera: Dytiscidae) and redescription of the species. Coleopt. Bull. 51, 285–288.

Miller, K.B., 2001. On the phylogeny of the Dytiscidae (Insecta: Coleoptera) with emphasis on the morphology of the female reproductive system. Ins. Syst. Evol. 32, 45–92.

Miller, K.B., Balke, M., 2003. The unusual occurence of aquatic beetles in amber, *Copelatus aphroditae* Balke, sp.n. and *C. predaveterus* Miller, sp.n. (Coleoptera: Dytiscidae: Copelatinae). Proc. Ent. Soc. Wash. 105, 809–815.

Nilsson, A.N., 2001. Dytiscidae. World Catalogue Insects 3, 1–395.

Nilsson, A.N., Bilardo, A., Rocchi, S., 1996. A check list of *Copelatus* Erichson, 1832 species (Coleoptera: Dytiscidae), from Afrotropical and Madagascan regions with a review of published penis drawings. Trop. Zool. 9, 11–48.

Otto, S., Cummings, M., Wakeley, J., 1996. Inferring phylogenies from DNA sequence data: the effects of sampling. In: Harvey, P.H. et al. (Eds.), New Uses for New Phylogenies. Oxford University Press, Oxford, pp. 103–115.

Pederzani, F., 1995. Keys to the identification of the genera and subgenera of adult Dytiscidae (sensu lato) of the world (Coleoptera: Dytiscidae).—Atti dell'Accademia Roveretana degli Agiati 244 (1994), Ser. VII, IV B, pp. 5–83.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43, 304–311.

Ribera, I., Hernando, C., Aguilera, P., 2001. *Agabus alexandrae* n.sp. from Morocco, with a molecular phylogeny of the western Mediterranean species of the *A. guttatus* group (Coleoptera: Dytiscidae). Ins. Syst. Evol. 32, 253–262.

Ribera, I., Hogan, J.H., Vogler, A.P., 2002a. Phylogeny of hydradephagan water beetles inferred from 18S rDNA sequences. Mol. Phylogenet. Evol. 23, 43–62.

Ribera, I., Beutel, R.G., Balke, M., Vogler, A.P., 2002b. Discovery of Aspidytidae, a new family of aquatic Coleoptera. Proc. R. Soc. Lond. B 269, 2351–2356.

Ribera, I., Balke, M., Vogler, A.P., 2004. A phylogenetic analysis of the beetle family Dytischidae based on mt and nDNA sequence data, in press.

Ruhnau, S., Brancucci, M., 1984. Studies on the genus *Lancetes*. 2. Analysis of its phylogenetic position using preimaginal characters (Coleoptera, Dytiscidae). Ent. Bas. 9, 80–107.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Sharp, D., 1882. On aquatic carnivorous Coleoptera or Dytiscidae. Sci. Trans. R. Dubl. Soc. 2, 179–1003.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Simon, C., Frati, F., Beckenbach, A.T., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Ent. Soc. Am. 87, 651–701.

Smith, A.G., Smith, D.G., Funnell, B.M., 1994. Atlas of Mesozoic and Cenozoic Coast Lines. Cambridge University Press, Cambridge.

Sorenson, M.D., 1996. TreeRot. University of Michigan, Ann Arbor.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis using Parsimony (* and other methods), Version 4.0b10.. Sinauer Associates, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Wallace, A.R., 1860. On the zoological geography of the Malay Archipelago (communicated by Charles Darwin to the LSL meeting of 3 Nov. 1859). J. Proc. Linn. Soc. Lond. 4, 172–184.

Wenzel, J.W., 2002. Phylogenetic analysis: the basic method. In: DeSalle, R., Giribet, G., Wheeler, W. (Eds.), Techniques in Molecular Systematic and Evolution. Birkhäuser, Basel, etc., pp. 4–30.

Wewalka, G., 1982. Neue Copelatini aus Madagascar und Afrika (Dytiscidae, Col.). Koleopt. Rdsch. 56, 127–133.

Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.

Wheeler, W.C., Hayashi, C.Y., 1998. The phylogeny of the extant chelicerate orders. Cladistics 14, 173–192.

Whitfield, J.B., 2002. Estimating the age of the polydnavirus/braconid wasp symbiosis. Proc. Natl. Acad. Sci. USA 99, 7508–7513.

Wichard, W., Weitschat, W., 1996. Wasserinsekten im Bernstein Eine paläobiologische Studie. Entomologische Mitteilungen aus dem Löbbecke Museum + Aquazoo 4, 1–122.

Zimmermann, A., 1934. Monographie der paläarktischen Dytiscidae V. Colymbetinae (1. Teil). Koleopt. Rdsch. 20, 138–214.