ACTA ENTOMOLOGICA MUSEI NATIONALIS PRAGAE
Published 15.xii.2010 Volume 50(2), pp. 437–443 ISSN 0374-1036

Copelatus sibelaemontis sp. nov. (Coleoptera: Dytiscidae) from the Moluccas with generic assignment based on morphology and DNA sequence data

Jiří HÁJEK[1)], Lars HENDRICH[2)], Oliver HAWLITSCHEK[2)] & Michael BALKE[2, 3)]

[1)] Department of Entomology, National Museum, Kunratice 1, CZ-148 00 Praha 4, Czech Republic; e-mail: jiri_hajek@nm.cz

[2)] Zoologische Staatssammlung, Münchhausenstrasse 21, D-81247 München, Germany; e-mail: coleoptera-zsm@zsm.mwn.de

[3)] GeoBioCenter, Ludwig-Maximilians-University, Richard-Wagner-Strasse 10, D-80333 München, Germany

Abstract. We describe *Copelatus sibelaemontis* sp. nov. from Bacan and Morotai Islands, Moluccas (Maluku), Indonesia. This is the only known Indonesian *Copelatus* Erichson, 1832 with smooth elytra. It has been assigned to *Copelatus* based on morphology and DNA sequence data.

Keywords. Dytiscidae, *Copelatus*, description, new species, DNA sequences, Moluccas, Bacan, Morotai, Oriental Region

Introduction

The diving beetle genus *Copelatus* Erichson, 1832, has a mainly pantropical distribution and is very speciose. The genus currently contains more than 400 described species (Nilsson 2001, Balke et al. 2004) and at least many dozens of others wait for description. Most species of *Copelatus* are characterised by longitudinal elytral striae, whose number has been used to group the species into species groups (Sharp 1882). Although this character bears only limited evidence for phylogeny (Balke et al. 2004), the species groups delimited by number and position of elytral striae are frequently used as a tool for better orientation within the genus (Zimmermann 1919, 1934; Guignot 1961; Guéorguiev 1968; Nilsson et al. 1997).

A very peculiar group is known as the *Copelatus hydroporoides* (or *haemorrhoidalis*, in earlier studies) species group. Their elytra are smooth, at most with some strioles. After recent transfer of two Western Palaearctic species to the genus *Liopterus* Dejean, 1833, and Australian, New Guinean and Oceanian species to the genus *Exocelina* Broun, 1886

(= *Papuadytes* Balke, 1998) (Balke et al. 2004, Nilsson 2007), the *C. hydroporoides* species group comprises 51 Afrotropical and Neotropical species (Nilsson 2001). No species of the *C. hydroporoides* group has yet been found in the Oriental Region, including Indonesia as far east as New Guinea.

Here we report the discovery of a smooth Copelatinae species in the Moluccas, which we assign to *Copelatus* based on morphological characters and DNA sequence data.

Material and methods

Life Science Identifier (LSID) of this paper. urn:lsid:zoobank.org:pub:31C6048B-2BF7-4616-9E1D-F455566D687D.

DNA extraction and amplification. We extracted DNA from individual specimens using the Qiagen DNA DNeasy Tissue Kit (Qiagen, Hilden, Germany). We amplified the 3'-end of the mitochondrial cytochrome c oxidase 1 gene (COI or *cox1*), using the primers Jerry (F, 5'-CAA CAT TTA TTT TGA TTT TTT GG-3') and Pat (R, 5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') (Simon et al. 1994). We chose this gene because it has proven informative in previous studies, and large datasets for dytiscid beetles already exist (e.g. Balke et al. 2004).

PCR conditions were: initial denaturation 180 s at 94°C followed by 40 cycles: denaturation 30 s at 94°C, primer annealing for 30 s at 47°C, extension for 60 s at 72°C. PCR products were purified by ethanol precipitation (25 µl of 99% ethanol, 2.5 µl of 3M sodium acetate added to 25 µl PCR product; 20 min centrifugation at 3500 rpm, discard liquid, wash pellet with 25 µl of 70% ethanol, discard liquid, dry pellet at 40°C).

Cycle sequencing (CS) conditions were: 15 s at 96°C, 15 s at 50°C, and 240 s at 60°C (35 cycles). CS products were purified using the CleanSeq Kit (Agencourt Bioscience Corporation, Beverly, MA, USA) (32 µl of 85% ethanol and 2.5 µl of Clean Seq added to 5 µl of CS product; 15 min in magnetic plate, then washed with 100 µl of 85% ethanol in magnetic plate, rediluted in 70 µl of water).

The products were then run on an ABI3730 sequencer (Applied Biosystems, Foster City, CA, USA) at the sequencing unit of the Ludwig-Maximilians-University, Munich. Sequences were edited with Sequencher 4.8 software (GeneCodes Corp.) and aligned using the MUSCLE software (Edgar 2004), available at CIPRES Portal v2.0 (Cyberinfrastructure for Phylogenetic Research, http://www.phylo.org/), and subsequently checked for alignment errors with BioEdit (Hall 1999). New sequences were submitted to GenBank (accession numbers FR667637–667639).

Analyses of DNA sequence data. We inferred phylogenetic relationships using the maximum likelihood and parsimony criteria. For the maximum likelihood analysis we used the software GARLI (Zwickl 2006), as available at CIPRES Portal v2.0, using the default options and the GTR+I+G model, also for 100 bootstrap replications (Felsenstein 1985). The main goal of these analyses was to confirm the suspected position of the species in the genus *Copelatus*.

Using *cox1* data only, *Copelatus* does not form a monophyletic group relative to *Exocelina*. However, the latter always forms a well-delineated clade; therefore, our analyses are adequate to test whether the new species belongs to *Exocelina* or not – if not, it is a *Copelatus*. Consequently, we used our unpublished Copelatinae *cox1* database which at present contains 200 specimens from SE Asia and the Australian Region. Many of those are listed in Balke et al. (2004, 2007). Since then we have added species mainly from New Guinea and Indonesia. These data will be published elsewhere.

Morphological observations. The material studied was examined under an Olympus SZX12 stereoscopic microscope. The genitalia were studied in dry condition. The habitus photograph was taken with a Leica Photar 1:2 / 25 on bellows attached to a Nikon D700 camera, an image stack was produced with the Cognisys StackShot system (www.cognisys-inc.com) and combined with Helicon Focus software (www.heliconsoft.com). Exact label data are cited for the material. A forward slash (/) separates different lines and a double slash (//) different labels of data. Additional remarks are found in square brackets.

Additional abbreviations used in descriptions are: TL – total length, a single measurement of length from front of head to apex of elytra; TL-h – total length minus head length, length of body from anterior margin of pronotum to apex of elytra; TW – maximum width of body measured at right angles to TL.

Collections. The specimens included in this study are deposited in the following institutional collections:

NHMW Naturhistorisches Museum, Wien, Austria (Manfred A. Jäch);
NMPC Národní muzeum, Praha, Czech Republic (Jiří Hájek);
SMNS Staatliches Museum für Naturkunde, Stuttgart, Germany (Wolfgang Schawaller);
ZSMC Zoologische Staatssammlung, München, Germany (Michael Balke).

Results

The analysis of 827 base pairs of *cox1* suggests that the examined Moluccan Copelatinae belong to the genus *Copelatus*. For the older specimens from Morotai, which were stored dry in a collection for more than 10 years, we only managed to sequence 367 bp of *cox1*. The Morotai specimens consistently group with the specimen from Bacan; thus we can exclude contamination. All Moluccan specimens group outside *Exocelina*, with a group of mostly unidentified *Copelatus* species from the Australian Region and Oceania. The bootstrap value for this clade was 77. The genus *Exocelina* was supported with bootstrap value of 99.

The specimens from Morotai and Bacan Island differ genetically by 4.36–4.63 % uncorrected *cox1* p-distance. In the 367 aligned *cox1* base pairs for the three sequenced individuals, we recognize 14 diagnostic characters.

The shape of the median lobe, similar to some undescribed New Guinea *Copelatus* with striate elytra, as well as the lack of a hook-like seta on the male protarsomere 4 confirm placement in *Copelatus* despite the overall similarity to *Exocelina* species.

Copelatus sibelaemontis sp. nov.

(Figs. 1–4)

WWW site on wikispecies. http://species.wikimedia.org/wiki/Copelatus_sibelaemontis
Life Science Identifier. urn:lsid:zoobank.org:act:49FC9D3B-4AC7-434E-BC7C-69D5322151D5

Type locality. Indonesia: Northern Maluku, Bacan Island, ca. 5 km SE of Makian village, SE slopes of Mount Sibela, 500–750 m a.s.l. (approximate position from GoogleEarth: 0°44.388′S 127°33.984′E).
Type material. Holotype: ♂ (NMPC): 'INDONESIA, N Moluccas / Bacan Isl., 500-750 m / SE slopes of Mt. Sibela / 5 km SE of Makian vill. / S. Jákl leg., 2.-12.V.2008 [printed] // HOLOTYPE / COPELATUS / sibelaemontis sp. nov. / J. Hájek et al. des. 2010 [red label, printed]'. Paratypes: 8 ♂♂ 4 ♀♀, same label data as holotype (NHMW, NMPC, SMNS, ZSMC). Each paratype is provided with the respective red printed label. One male paratype (ZSMC) bears a green label with M. Balke's DNA extraction number MB 3456.
Additional material examined. 1 ♂ 1 ♀, 'MALUKU:Is.Morotai / W Daruba, Raja / 18.XI.1999, 50-100m / leg. A.RIEDEL' (SMNS, ZSMC). The specimens bear green labels with M. Balke's DNA extraction numbers, MB 3151 (♂) and MB 3152 (♀).

Description. Body oblong-oval, broadest in basal third of elytra, moderately convex. Head relatively broad; clypeus rounded. Pronotum broadest between posterior angles, lateral margins moderately curved. Base of elytra as broad as pronotal base; lateral margins of elytra moderately curved (Fig. 1).

Measurements. Bacan: TL: 4.6–5.0 mm (holotype 4.7 mm), TL-h: 4.1–4.5 mm (holotype 4.3 mm), TW: 2.2–2.4 mm (holotype 2.3 mm). Morotai: TL: 4.6 mm, Tl-h: 4.1–4.2 mm, TW: 2.2–2.3 mm.

Colouration. Body colour dark brown, head in front of eyes, sides of pronotum, appendages, and basal transverse band on elytra ferrugineous. Beetle rather shiny.

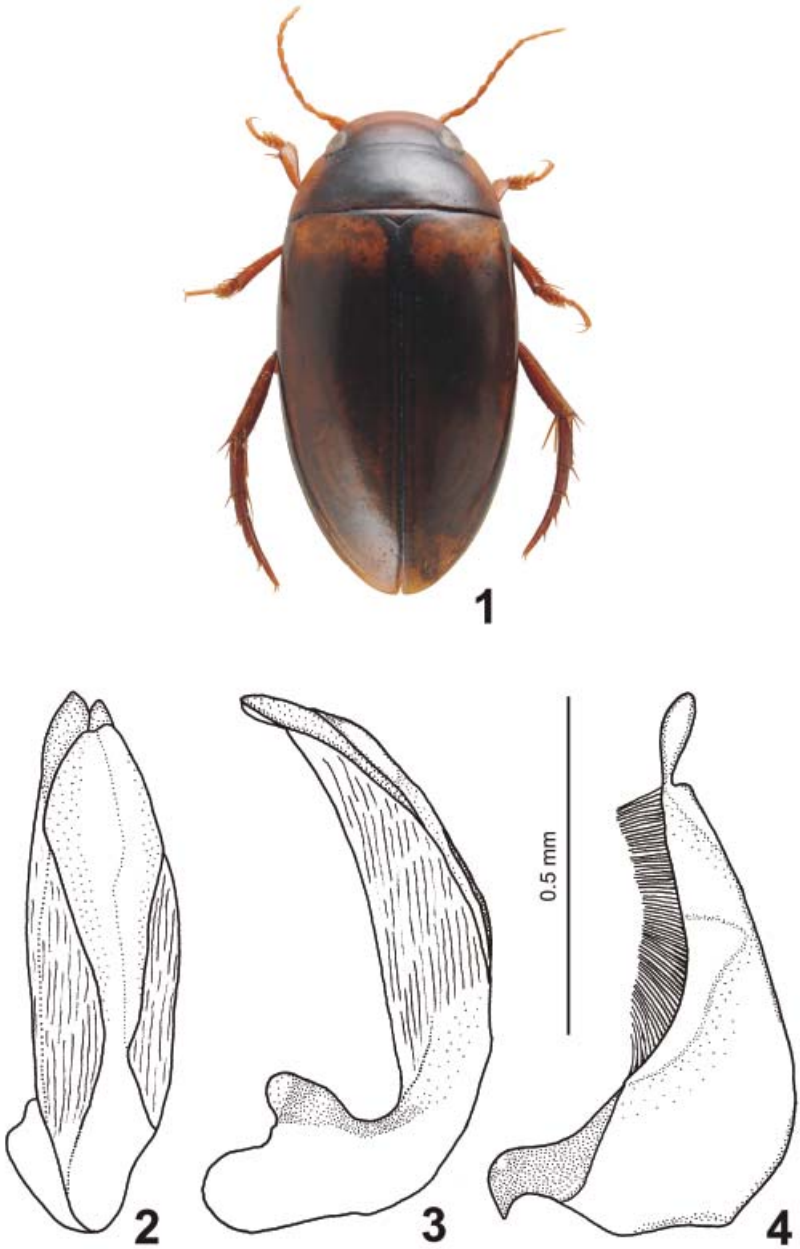
Surface sculpture. Head uniformly microreticulated, reticulation composed of moderately deeply impressed isodiametric meshes. Punctation composed of coarse setigerous punctures, and very small punctures spread sparsely on surface; rows of coarse punctures presented around inner margin of eyes and in small depression antero-laterally of eyes. Antenna with antennomeres long and slender.

Pronotum with lateral beading very thin and indistinct. Microreticulation similar to that of head. Punctation similar to that of head; row of coarse setigerous punctures presented along anterior margin, basal margin (except for baso-medially), and laterally close to sides. Indistinct longitudinal wrinkles presented baso-laterally.

Elytra without striae or strioles, microreticulation similar to that of head and pronotum. Punctation similar to that of head; coarse setigerous punctures form four indistinct longitudinal rows.

Ventral part. Finely microreticulated, with intermixed sparsely distributed very small punctures. Meshes isodiametric, except for metacoxae and abdominal ventrite I (longitudinal), abdominal ventrite II (diagonal), and abdominal ventrites III–IV (transverse). Prosternum obtusely keeled medially. Prosternal process lanceolate, bordered except for tip. Metepisterna ('metasternal wings') tongue-shaped, slender. Metacoxae and abdominal ventrites I–II with numerous striae, possibly used for stridulation. Indistinct transverse rows of large setigerous punctures are presented on abdominal sternites.

Male. Protarsomeres and mesotarsomeres 1–3 distinctly broadened, with adhesive discs on their ventral side. Median lobe of aedeagus slightly asymmetrical in ventral view, apically



Figs. 1–4. *Copelatus sibelaemontis* sp. nov. 1 – habitus; 2 – median lobe in ventral view; 3 – the same in lateral view; 4 – lateral lobe (paramere).

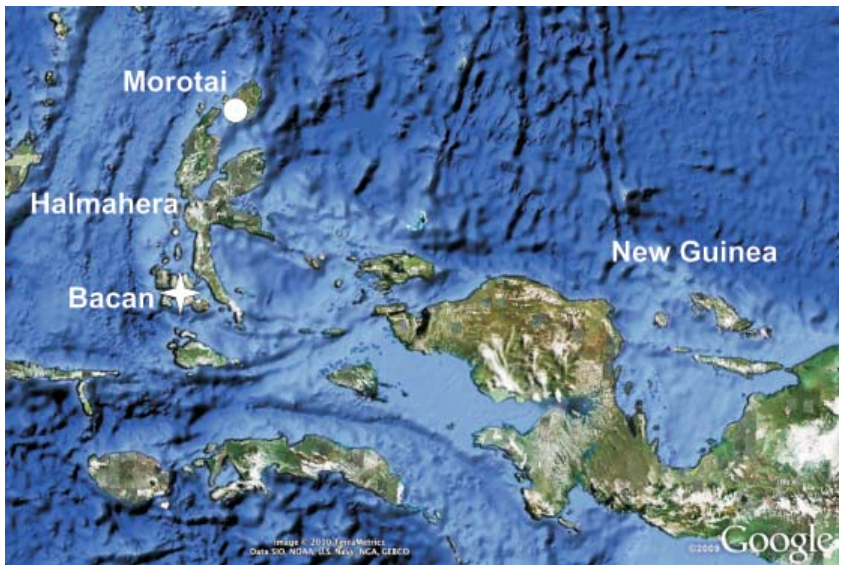


Fig. 5. Distribution of *Copelatus sibelaemontis* sp. nov.

bifid; inner lobe well developed (Fig. 2). In lateral view, median lobe broadest in middle; apex bent down (Fig. 3). Lateral lobe with broader base narrowing towards apex, setation on inner margin distinct (Fig. 4).

Female. Similar to male in habitus. Protarsomeres and mesotarsomeres not broadened.

Differential diagnosis. Readily characterized by the smooth elytra combined with shape of male genitalia (Figs. 2–4).

Etymology. The new species is named after the type locality – Sibela, and Latin word *mons* (genitive *montis*, masculinum) meaning mountain.

Collection circumstances. Specimens from Bacan were collected in a small stream (S. Jákl, pers. comm.). The species was associated in both localities with *Copelatus wallacei* J. Balfour-Browne, 1939, and on Bacan also with *C. ternatensis* Régimbart, 1899 and a species from the *Platynectes decastigma* Régimbart, 1899 complex.

Distribution. So far known from Bacan and Morotai Islands of Northern Maluku, Indonesia (Fig. 5). The occurrence in Halmahera Island is likely.

Notes. Specimens from Morotai and Bacan show > 4% *cox1* divergence, suggestive of interrupted gene flow. This, together with their insular distribution, could be taken as indicators of ongoing speciation. The occurrence of local endemism would be typical of running water species, usually with limited dispersal abilities (Ribera et al. 2001), although in this case the lack of apparent morphological differentiation between the specimens from two islands about 300 km distant from each other is noteworthy. We suggest to collect more material and study population-level processes in this very diverse archipelago.

Acknowledgements

We are obliged to Stanislav Jákl (Praha, Czech Republic) and Alexander Riedel (Karlsruhe, Germany) for placing specimens at our disposal. We are indebted to Pavel Sláma (Nové Hrady, Czech Republic) for line drawings. Thanks to Ignacio Ribera (Barcelona, Spain) and one anonymous reviewer for helpful comments on the manuscript. Richard Pyle (Honolulu, USA) kindly provided LSIDs for the new species and this paper. We thank CIPRES Portal v2.0 for computational resources. The study was partly supported by the Ministry of Culture of the Czech Republic (grant MK00002327201) and Deutsche Forschungsgemeinschaft (DFG BA2152/7-1).

References

BALKE M., PONS J., RIBERA I., SAGATA K. & VOGLER A. P. 2007: Infrequent and unidirectional colonization of hyperdiverse Papuadytes diving beetles in New Caledonia and New Guinea. *Molecular Phylogenetics and Evolution* **42**: 505–516.

BALKE M., RIBERA I. & VOGLER A. P. 2004: MtDNA phylogeny and biogeography of Copelatinae, a highly diverse group of tropical diving beetles (Dytiscidae). *Molecular Phylogenetics and Evolution* **32**: 866–880.

EDGAR R. C. 2004: MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

FELSENSTEIN J. 1995: Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

GUÉORGUIEV V. B. 1968: Essai de classification des coléoptères Dytiscidae. I. Tribus Copelatini (Colymbetinae). *Izvestiya na Zoologicheskiya Institut s Muzey* (Sofia) **28**: 5–45.

GUIGNOT F. 1961: Revision des hydrocanthares d'Afrique (Coleoptera Dytiscoidea). 3. *Annales du Musée Royal du Congo Belge, Série 8vo (Sciences Zoologiques)* **90**: 659–995.

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

NILSSON A. N. 2001: Dytiscidae (Coleoptera). *World Catalogue of Insects* **3**: 1–395.

NILSSON A. N. 2007: Exocelina Broun, 1886 is the valid name of Papuadytes Balke, 1998. *Latissimus* **23**: 33–34.

NILSSON A. N., BILARDO A. & ROCCHI S. 1997: A check list of Copelatus Erichson 1832 species (Coleoptera Dytiscidae) from Afrotropical and Madagascan regions with a review of published penis drawings. *Tropical Zoology* **10**: 11–48.

RIBERA I., BARRACLOUGH T. G. & VOGLER A. P. 2001: The effect of habitat type on speciation rates and range movements in aquatic beetles: inferences from species-level phylogenies. *Molecular Ecology* **10**: 721–735.

SHARP D. 1882: On aquatic carnivorous Coleoptera or Dytiscidae. *Scientific Transactions of the Royal Dublin Society, Series 2* **2**: 179–1003 + pls. 7–18.

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. *Annals of the Entomological Society of America* **87**: 651–701.

ZIMMERMANN A. 1919: Die Schwimmkäfer des Deutschen Entomologischen Museums in Berlin-Dahlem. *Archiv für Naturgeschichte* (A) **83 (12)** (1917): 68–249.

ZIMMERMANN A. 1934: Monographie der paläarktischen Dytisciden. V. Colymbetinae (1. Teil). *Koleopterologische Rundschau* **20**: 138–214.

ZWICKL D. J. 2006: *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.* PhD. thesis. The University of Texas, Austin, USA, x + 115 pp.