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A highly modified stygobiont diving beetle of the genus *Copelatus* (Coleoptera, Dytiscidae): taxonomy and cladistic analysis based on mitochondrial DNA sequences

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Abstract. We report the discovery of a new species of *Copelatus* that is morphologically highly modified for life in groundwater. *Copelatus abditus* sp.n. was collected from a 4 m deep bore in central Australia. It was placed in Copelatinae based on morphological evidence. This is the first known stygobiont diving beetle that does not belong to the subfamily Hydroporinae. Sequences from the mitochondrial DNA cytochrome oxidase 1, 16S rRNA, tRNAL and NADH dehydrogenase subunit 1 genes suggest that the species belongs to the subgenus *Papuadytes*, the morphological apomorphies of which are reduced in the new groundwater species. *Copelatus* (*Papuadytes*) *abditus* very much resembles other world stygobiont dytiscids, which all belong to the subfamily Hydroporinae. We suggest that this striking convergence is due to similar selective pressures imposed on all groundwater beetles. We suggest a scenario in which species from temporary habitats evade drought by entering the hyporheic zone, and may be driven to a subterranean existence by desertification of their ancestral habitats, as can be observed in Australia.

Introduction

First discovered in France (Abeille de Perrin, 1904), stygobiont or groundwater diving beetles are now known from many parts of the world. To date, fifty-eight species in sixteen accepted genera have been described, and ten are monotypic (Uéno, 1996; Spangler & Decu, 1998; Watts & Humphreys, 1999, 2000, 2001, 2003; Castro & Delgado, 2001; Nilsson, 2001; Appendix). Eyes are usually completely reduced in underground diving beetles, but recently two species with rudimentary eyes were discovered in Australia (Watts & Humphreys, 2003). Typically, the beetles are micropterous (=only veinless wing vestiges present; Thayer, 1992). Some species are rather large, the adults being up to 4.8 mm long (*Tjirtudessus magnificus* Watts & Humphreys, 2000), whereas others are hardly longer than 1 mm. Pupation is believed to take place in air-filled crevices, but the possibility of underwater pupation has not yet been ruled out (Spangler & Decu, 1998; Balke, 2003). All the species known thus far belong to the subfamily Hydroporinae (Alarie & Wewalka, 2001; Nilsson, 2001; Appendix), to which most of the smaller (< 5 mm) species of Dytiscidae belong.

In the arid zone of central Western Australia, a strikingly rich stygobiont diving beetle fauna was recently discovered in isolated calcrete (limestone) aquifers (Watts & Humphreys, 1999, 2000, 2001, 2003; Cooper *et al*., 2002). Forty-two species have been described to date, each of which appears to be unique to an individual calcrete aquifer, and dozens more species await description (Watts & Humphreys, 2003). This is the most diverse known stygobiont water and diving beetle fauna in the world and may well comprise more than a hundred species.

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Similar calcrete aquifers exist in other arid zone regions of northern Australia and following a preliminary survey of calcrete aquifers on Newhaven Station in the Northern Territory by one of the authors (WH), five remarkable specimens of diving beetle were discovered.

Here, we report the first nonhydroporine species of stygobiont Dytiscidae. Based on morphology, we could readily assign it to the subfamily Copelatinae (see description below), which in Australia is represented by *Copelatus* s.str. and *Copelatus* (*Papuadytes*). We analysed mitochondrial DNA sequences phylogenetically to assign our new species to either of these reliably.

Copelatinae

This subfamily of Dytiscidae currently contains 520 species (following Nilsson, 2001; several new species have since been described) in four genera: *Copelatus* Erichson, 1832 (468 spp.) (cosmopolitan, most species in tropics and subtropics); *Lacconectus* Motschulsky, 1855 (forty-three spp.) (oriental); *Aglymbus* Sharp, 1882 (twelve Neotropical, three Ethiopian, nine Madagascan); and *Agaporomorphus* Zimmermann, 1921 (five spp.) (Neotropical) (Miller, 2001; Nilsson, 2001).

Papuadytes, a subgenus of *Copelatus*, was described from New Guinea (Balke, 1998), and later reported from Australia and New Caledonia (Balke, 2001). Eight of the twenty-one Australian *Copelatus* have been assigned to *Papuadytes* to date (Balke, 2001). At present, *Copelatus* is the only Australian genus of the subfamily, but *Papuadytes* is perhaps a separate clade deserving generic status (see below).

The subgenus *Copelatus* (*Papuadytes*) is characterized by at least one morphological apomorphy: fourth male protarsomere ventrally on the anteriodistal angle with a seta that is stout and distinctly hook-shaped (Balke, 1998).

Materials and methods

The sampling site

The five known stygobiont specimens were collected from Camel Bore at Newhaven Station in central Australia using a small weighted plankton net lowered into the water table. Camel Bore lies east of the Great Sandy Desert in an area of aeolian sand overlying a calcrete plain adjacent to a salt lake (playa) in the intracratonic Ngalia Basin of the Northern Territory. Regionally, groundwater drains along palaeodrainage channels to discharge playas (Jacobson & Wischusen, 2001) that are often fringed by groundwater calcrete deposits (Humphreys, 1999, 2001). Bores in the area normally reach brackish groundwater several metres below the surface. The only information for Camel Bore is a water analysis from 1971: total dissolved solids 4480 $mg\,l^{-1}$ and pH 8.0. The nearby bore RN 15494, which replaced Camel Bore as a water source, intersected water at 4 m in cavernous calcrete (R. Read, pers. comm., 2002). Previously, Newhaven was a cattle station with low intensity use and is now owned by Birds Australia as a habitat reserve.

^{36}Cl isotopic data suggest that substantial recharge of the superficial aquifers in the Ngalia Basin occurs only during favourable interglacial climatic regimes; near the playa lakes in the Ngalia Basin the estimated groundwater residence time is often considerably longer than 80 ka (Jacobson & Wischusen, 2001). The general water quality of the area is low, with most samples in Tertiary sediments ($n = 124$) exceeding, often considerably, drinking water guidelines for F, SO_4, Cl, NO_3, Na and TDS.

Taxon sampling

Eight species of *Copelatus* were chosen for the phylogenetic analysis in order to cover broadly the taxonomic range of the genus in Australia (Table 1). The aim of our analysis

Table 1. Nine species of *Copelatus* and two outgroups used in the phylogenetic analysis. Further information about specimens can be obtained from the Australian Biological Tissue Collection (ABTC) using the given voucher numbers.

Genus species	ABTC	*n*	Location
**Copelatus abditus* sp.n.	–	2	Australia: Newhaven Station, NT
**Copelatus* sp.n.	–	2	Australia: Strachan, WA
**Copelatus australiae* Clark, 1863	9215F-CHS2	2	Australia: Chain of ponds, SA
**Copelatus glyptus* Guignot, 1954	9285F-CHS3	1	Australia: Wallaman Falls, QLD
**Copelatus melanarius* Sharp, 1882	9337F-CHS3	2	Australia: Bendolba, NSW
**Copelatus punctipennis* Lea, 1899	9219F-CHS2	2	Australia: Forreston, SA
†*Copelatus clarki* Sharp, 1882	9279F-CHS3	2	Australia: Blue Water, QLD
†*Copelatus irregularis* MacLeay, 1871	9284F-CHS3	2	Australia: Wallaman Falls, QLD
†*Copelatus nigrolineatus* Sharp, 1882	9272F-CHS3	2	Australia: Greenvale, QLD
Liodessus shuckardii (Clark, 1862)	75362	1	Australia: Maryborough, VIC
Dytiscus circumcinctus Ahrens, 1811	–	1	Germany: Berlin

*Subgenus *Papuadytes*.
†*Copelatus* s.str.
n, number of specimens sequenced for each species.

was to test the placement of the stygobiont species within either *Copelatus* s.str. or *Copelatus* (*Papuadytes*). A recent cladistic analysis of the Dytiscidae based on morphology placed Hydroporinae and Hydrodytinae as sister lineages to Copelatinae (Miller, 2001), whereas an analysis of 18S rRNA sequences of hydradephagan beetles by Ribera *et al.* (2002) placed a polyphyletic Copelatinae close to Dytiscinae and Laccophilinae. We chose a member of the Hydroporinae, *Liodessus shuckardii* (see Table 1), as well as *Dytiscus circumcinctus* (Dytiscinae) as outgroups for our phylogenetic analyses. We aimed to sequence two specimens per species to control against possible sequencing and contamination problems and errors in identification or labelling of samples during molecular procedures.

DNA extractions

The specimens were preserved in ordinary 96% ethanol and after 3–6 weeks transferred to 98–99% absolute ethanol or snap frozen in liquid nitrogen and then stored at −80 °C. DNA was extracted from the anterior half of a beetle cut in the middle or (the stygobiont species) from tissue around the genital capsule obtained during dissection for study of the copulatory apparatus. We used the DNAzol protocol (Chomczynski *et al.*, 1997) with slight modifications. Prior to centrifugation the homogenate was incubated overnight at room temperature with 2.5 µl of proteinase K (400 µg ml^{-1}). Vouchers are deposited in the South Australian Museum (SAMA) entomology collection.

Polymerase chain reaction (PCR) amplification, sequencing and alignment

Two regions of the mitochondrial genome were amplified and sequenced using PCR methods. A ~810 bp region of the 3′ end of the cytochrome oxidase 1 (CO1) gene was amplified using primers M202 (forward, 5′-CAA CAT TTA TTT TGA TTT TTT GG-3′, alias Jerry; Simon *et al.*, 1994) and M70 (reverse, 5′-TCC ATT GCA CTA ATC TGC CAT ATT A-3′, alias Pat; developed in the Harrison laboratory, see Simon *et al.*, 1994). An 820 bp segment containing 644 bp at the 3′ end of the large ribosomal subunit (16S rRNA) gene, the entire 76 bp of tRNAL and the first 100 bp of NADH dehydrogenase subunit 1 (ND1) were also amplified using the primers M14 (forward, 5′-CGC CTG TTT ATC AAA AAC AT-3′, 16Sar; Simon *et al.*, 1994) and M223 (reverse, 5′-GGT CCC TTA CGA ATT TGA ATA TAT CCT-3′, ND1A; Simon *et al.*, 1994).

Standard PCR amplifications included 1× reaction buffer (Perkin Elmer, Boston, MA, U.S.A.), 0.2 mM of each dNTP, 5 pM of each primer, 1 unit of Amplitaq Gold (Perkin Elmer) and 2 mM of $MgCl_2$ in a 50 µl reaction volume. PCR amplifications were carried out on an FTS-320 thermal cycler (Corbett Research, Sydney, Australia) for one cycle of 92 °C for 9 min and thirty-five cycles (94 °C, 45 s; 48 °C, 45 s; 72 °C, 60 s), followed by a final incubation step at 72 °C for 6 min. The PCR products were purified using the UltraClean® PCR clean-up DNA purification kit (MoBio Laboratories Inc., Carlsbad, CA, U.S.A.) according to the manufacturer's protocol.

Sequencing was performed using the ABI Prism® Big Dye Terminator Cycle sequencing kit (PE Applied Biosystems, Foster City, CA, U.S.A.) in 10 µl reaction volumes according to the manufacturer's instructions. PCR primers were used as sequencing primers and each fragment was sequenced on both strands. The reaction products were purified by ethanol precipitation (as specified by ABI) and sequenced on ABI 373 (version 3.0) automated DNA sequencers. Sequence files were edited using SEQED version 1.0.3 (Applied Biosystems) and a consensus of bidirectional sequencing was determined. The 16S rRNA and tRNA sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and the 16S rRNA sequences were also aligned with reference to a secondary structure model for beetle 16S rRNA (Buckley *et al.*, 2000). However, a few positions could not be aligned unambiguously, and these were excluded from the final analysis. Our matrix is available at http://www.waterbeetles.info/abditus.htm, excluded characters are: 16–21, 213–216, 240–242, 678–683. Sequences have been submitted to GenBank (accession numbers: AY353845, AY350898, AY368206–AY368221).

Phylogenetic analyses

Phylogenetic analyses of aligned sequence data were carried out using the parsimony program PAUP* version 4.0b10 (Swofford, 2002). The two datasets, CO1 and 16S/tRNAL/ND1, were combined for the final analysis. Parsimony analyses were performed using heuristic searches and the tree bisection reconnection method with 10 000 random addition sequences to search for the most parsimonious trees from different islands of trees. Bootstrapping with 10 000 pseudoreplicates and ten random additions per pseudoreplicate was used to examine the robustness of nodes in the analyses. Partitioned Bremer support values (Bremer, 1994) were established using TREEROT (Sorenson, 1996). We assessed the significance of the incongruence length difference (Farris *et al.*, 1994) with the partition homogeneity test as implemented in PAUP*, using 500 replicates of a heuristic search with 100 random addition replicates each. Base frequencies were provided by PAUP*.

Results

Phylogenetic analyses

The protein-coding CO1 sequences were not length variable, whereas the 16S sequences varied in length from 758 bp (*C. abditus*) to 767 bp (*C. punctipennis*). The aligned sequences consisted of 737 bp of CO1 and 782 bp of sequence hereafter referred to as 16S+, including 618 bp of 16S, 76 bp of tRNAL and 90 bp of ND1, the latter two

genes overlapping by three nucleotides. The sequences were combined for the phylogenetic analysis, giving a total dataset of 1499 bp of aligned sequence as twenty characters from variable regions were excluded (see Materials and methods). In total, 1022 characters were constant, 165 were uninformative and 312 (21%) were parsimony informative in our analysis. No significant heterogeneity of base frequencies across taxa was found ($\chi^2 = 13.86$, $P = 0.99$); mean frequencies were: A: 32%; T: 41%; G: 15%; C: 12%. The partition homogeneity test indicated no significant incongruence between the partitions 16S+ and CO1 ($P = 0.76$). We also partitioned our matrix as follows: 762 bp 16S+ (twenty excluded from the original alignment) and 737 bp CO1.

Parsimony analysis with all characters unweighted resulted in a single most parsimonious tree (tree length = 982 steps; consistency index (CI) = 0.63, retention index (RI) = 0.50, CI informative characters only = 0.55) (Fig. 1). Analysis of 16S+ alone yielded two trees of length 454 (CI = 0.69, RI = 0.60, CI informative characters only = 0.59), and analysis of CO1 only produced four trees of length 573 (CI = 0.58, RI = 0.52, CI informative characters only = 0.43).

Our analysis provides strong support for the placement of *C. abditus* within the subgenus *Papuadytes* of *Copelatus*, where it groups with the species *C. australiae* and *C. melanarius*, although weakly supported. However, subgenus *Papuadytes* including *C. abditus* is a well-supported clade, both measured by bootstrap (92) and partitioned Bremer support values (7.3/3.7 for the partitions 16S+ and CO1, respectively). We also find that the Australian *Copelatus* s.str. form a well-supported clade (bootstrap value 100, partitioned Bremer support value 18/7), whereas *Copelatus* s.str. + *Copelatus* (*Papuadytes*) are only weakly supported (bootstrap value 64, partitioned Bremer support value −1/3).

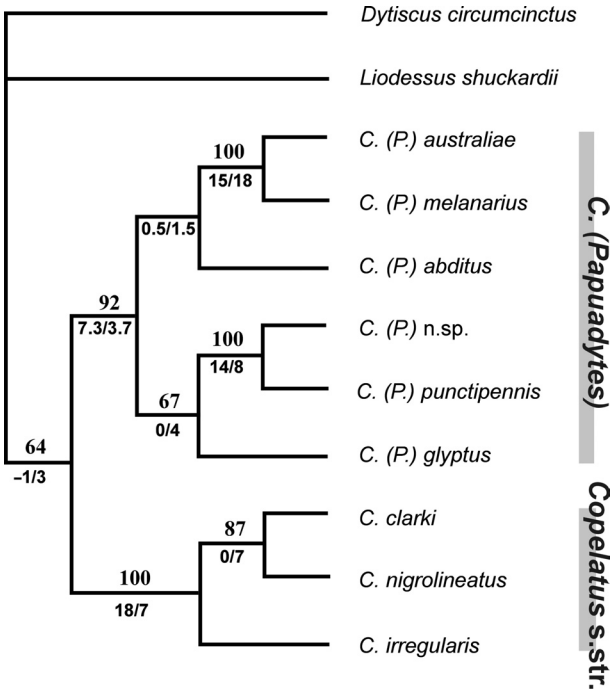

Fig. 1. Parsimony tree of length 982 resulting from a heuristic search with 10 000 random additions of 1499 bp of combined cytochrome oxidase 1 (CO1), and 16S rRNA/tRNAL/NADH dehydrogenase subunit 1 (ND1) (= 16S+) sequence data. Bootstrap percentages are shown above the branches; partitioned Bremer support values are shown below the branches (16S+/CO1). For tree statistics, see Results. Optimization used was 'only unambiguous'.

Taxonomy

Two morphological characters suggest that our stygobiont species belongs to Copelatinae rather than to Hydroporinae, in contrast to all other world stygobiont Dytiscidae (Appendix). The metacoxal lines closely approach each other caudally (Fig. 2B), which is a diagnostic character for Copelatinae, perhaps even an apomorphy (Miller, 2001). This state also occurs in the subfamily Hydrodytinae Miller, 2001, which is thus far known only from South America (metacoxal lines are reduced in some groups of Copelatinae: *Aglymbus*, *Lacconectus*). The paramere has a spiniferous distal stylus, which is an apomorphy for Copelatinae (Blake, unpublished). Two main groups of Copelatinae occur in the Australian region: *Copelatus* s.str. and *Copelatus* (*Papuadytes*) (Fig. 2F, G). We assumed that the stygobiont species belongs to one of these, but a placement based on morphological characters alone was not possible: the stygobiont species lacks a hooklike seta on the male tarsomere 4 (apomorphy for *Papuadytes*); it also lacks a strongly modified median lobe of the aedeagus (typical for many species groups of *Copelatus* s.str. for which as a whole, however, no apomorphies have yet been suggested). Therefore, we attempted a placement based on molecular phylogenetic information (see above).

Our analysis places the new species in subgenus *Papuadytes* (Fig. 1).

Genus *Copelatus* Erichson, 1832

Subgenus *Papuadytes* Balke, 1998

Copelatus (*Papuadytes*) *abditus*, sp.n. (Figs 2 and 3)

Type material. Holotype, ♂, AUSTRALIA: Northern Territory. Newhaven Station, Camel Bore, 4 m deep, BES 7296. NT Govt. RN 10151. 22°56′04′S; 131°14′23′E, 15 June 2001. Collected by W. F. Humphreys and A. Russ, in spirit. (Northern Territory Museum, Australia) (# NTM I 001173). Four paratypes, same locality, bore RN 15494, 19 August 2002, W. F. Humphreys and R. Read, dry mounted (Naturhistorisches Museum Wien, Austria; West

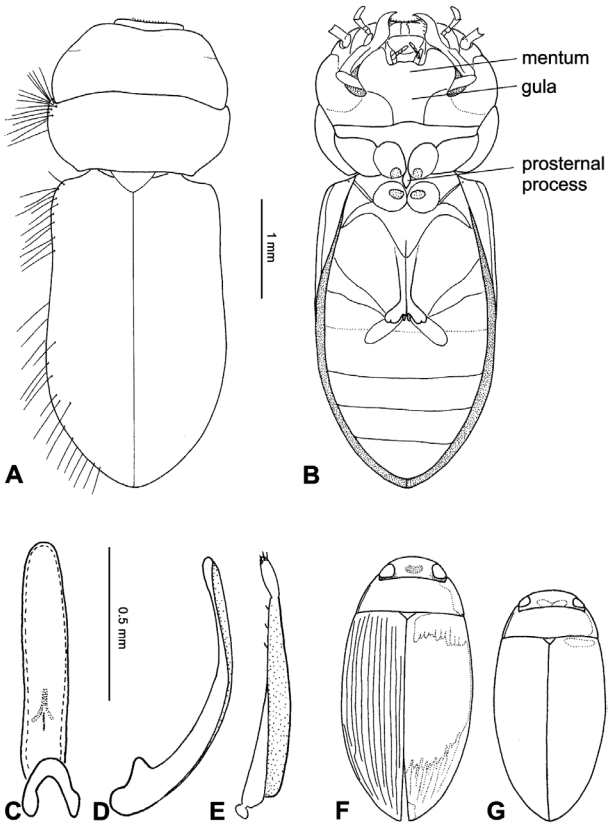

Fig. 2. *Copelatus abditus*. A, Habitus in dorsal view; B, in ventral view; C, median lobe of aedeagus in dorsal view; D, in lateral view; E, paramere in lateral, interior view. F, *Copelatus* (s.str.) *gestroi*, habitus in dorsal view. G, *Copelatus* (*Papuadytes*) *rasilis*, habitus in dorsal view.

Australian Museum, Perth; South Australian Museum, Adelaide).

Etymology. From the Latin, abditus – hidden, concealed.

Differential diagnosis. Characters found in this underground species not found in above-ground congeners are: head very large (Fig. 2A, F, G); eyes absent; mentum and gula fused; bases of pronotum and elytron strongly narrowed (Fig. 2A, F, G); prosternal process short, caudal end curved to dorsal; mesocoxae contiguous (Fig. 2B). *Copelatus* (*Papuadytes*) *abditus* is thus readily recognizable.

Description. Habitus. Head very large. Body outline interrupted by large angle between base of pronotum and base of elytron as both strongly narrowed (Fig. 2A). Elytral margins subparallel, apex gently rounded. Body rather flat. Fore- and midlegs long relative to surface species (length of middle femur plus tibia 1.86 mm; length of beetle/length of middle femur plus tibia 2.47; in *C. australiae* 3.36–3.46; $n = 5$).

Measurements. *Holotype*: Total length of beetle: 4.6 mm (head 0.6 mm; pronotum 0.8 mm; elytron 3.2 mm); greatest width 1.8 mm (pronotum 1.8 mm; base of pronotum 1.1 mm, between fore angles 1.7 mm; elytron in anterior third 1.7 mm, posterior third 1.8 mm). *Paratype* (*Vienna*): 4.4/0.6/0.7/3.1/1.7/1.7/1.1/1.6/1.5/1.6, respectively.

Colour. Entire beetle is orange and slightly translucent.

Surface sculpture. Dorsal surface without striae or strioles. Head and pronotum with microreticulation of regular polygonal meshes; head with few punctures, pronotum with several larger punctures of different sizes. Elytron with microreticulation of smaller, regular polygonal meshes, and comparatively densely punctate; diameter of punctures slightly larger than meshes, distance between punctures about 3–5 times their diameter. Metaventrite, metacoxa and abdominal segments with few punctures and microreticulation of more or less irregular polygonal meshes, these with distinct diagonal orientation on visible parts of abdominal segments I–III. Few diagonal cuts, or strioles, visible on segments I–III. Last visible sternite smooth, without lateral striae.

Structural features. Eyes absent. Pronotum lacking lateral bead. Pronotum and elytron with very long lateral setae (Fig. 2A). Hind wings represented by veinless vestiges. Mentum and gula fused, no suture visible (Fig. 2B). Prosternal process almond-shaped, strongly deflexed in dorsal direction; not reaching metaventrite. Mesocoxae contiguous. Metasternal process short, triangular, cranially pointed. Lateral processes of metaventrite ('metasternal wings') narrow, almost reaching lateral margin; metacoxa widely produced cranially (Fig. 2B). Metacoxal lines closely approximated caudally, as in other Copelatinae (where applicable); straight, and slightly diverging cranially, faint in front of metaventrite. Last visible ventrite gently rounded apically. Middle tibia and tarsus dorsally with a few long hairs; hind tibia and tarsus dorsally and ventrally with a fringe of long (swimming) hairs. Claws on all legs simple, slightly curved, in subequal pairs. Segments I–III of fore and middle tarsus stout but not expanded laterally. Segments I–III of fore and middle tarsus ventrally with 4 rows of stalked adhesive pads (tarsomere number to which stalks attached in parentheses): 3 (I)–4 (I)–4 (II)–4 (III). Segment IV of fore tarsus with a few setae on the anterior angles, lacking a hooklike seta. Protibia with margins straight, not sinuate.

Male genitalia. Median lobe of aedeagus simple (Fig. 2C, D). Parameres symmetrical; rather narrow, internal margin with only 3 or 4 short setae; stylus distally with a few short setae (Fig. 2E).

Habitat. The specimens were collected from a narrow (4 m) borehole into a relatively shallow calcareous aquifer. The bore and associated pastoral well yielded other stygal animals including Oniscidea: Scyphacidae (*Haloniscus* sp.n. 1),

Cyclopoida, Harpacticoida, Ostracoda, Gastropoda: Hydrobiidae aff. *Trochidrobia* sp.n. 1, Acarina: Unionicolidae (*Koenikea*? sp.) and *Tjirtudessus* sp.n. (Dytiscidae: Hydroporinae: Bidessini).

Distribution. Only known from the type locality.

Discussion

We used a combination of morphological examination and molecular phylogenetic analyses because the adaptation to a subterranean environment may lead to major modifications in morphology from surface ancestors (Smrž, 1983; Spangler & Decu, 1998). These modifications may result from convergent evolution and could, therefore, obscure phylogenetic relationships. In fact, the habitus of the newly discovered stygobiont *Copelatus* very much resembles stygobiont Hydroporinae from all around the world (see, e.g. Spangler & Decu, 1998; Watts & Humphreys, 2001) (Fig. 3). The general similarity of stygobiont Hydroporinae species led Smrž (1983) to introduce a new tribe for stygobiont species. However, his Siettitiini were discovered to be polyphyletic and formally synonymized with Hydroporini by Nilsson (2001).

The species described here is a highly modified species of *Copelatus*, subgenus *Papuadytes*. It is the first nonhydroporine stygobiont Dytiscidae known, perhaps illustrating the enormous diversity of Australia's underground ecosystems. The habitus similar to stygobiont Hydroporinae reflects the strong selective pressure imposed upon groundwater organisms in that environment. Typical features are: eyes absent; head often large compared with pronotum; base of pronotum and elytron strongly narrowed, beetle thus with interrupted lateral outline (Fig. 3); prosternal process short, not exceeding mesocoxae, mesocoxae thus contiguous or almost so; wings vestigial.

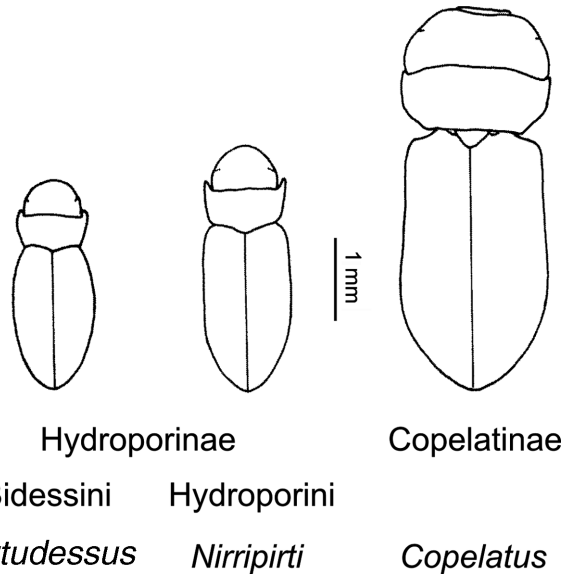

Fig. 3. Habitus and classification of stygobiont Dytiscidae. *Nirripirti hinzae* and *Tjirtudessus challaensis* after Watts & Humphreys (2001), illustrating convergent evolution of similar habitus.

Australia features a wide distribution of subsurface habitats and epigean groups possibly able to enter these groundwaters. This combined with Australia's long geological stability and its palaeoenvironmental history, specifically the recent desertification, were postulated factors predisposing Australia to be 'a significant "storehouse" of world subsurface biodiversity' (Boulton *et al*., 2003). This prediction is true for diving beetles. There are 209 described epigean species in Australia (Watts, 2002) and quite possibly more than a hundred stygobiont species (Watts & Humphreys, 2003; unpublished), by far the most diverse (known) fauna in the world (Appendix) (Boulton *et al*., 2003). The species of *Copelatus* (*Papuadytes*) are often associated with running water, where they occur frequently in the interstitial or hyporheic zone at stream margins (Balke, 1998). New Caledonian species (M. Balke and G. Wewalka, field observation) often occur in temporary streams, where they can be found in the moist gravel under larger stones when the rivers dry out completely. Lately, Leys and Watts (unpublished) found *C*. (*Papuadytes*) *australis* in groundwaters in South Australia. It is rarely collected and all other known specimens are epigean. It is possible that this is an example of a 'link' between epigean and subterranean fauna. A phylogenetic analysis is scheduled for the future. Also, it was recently found that some Australian epigean Dytiscidae of the tribe Bidessini occasionally enter the hyporheic zone to evade dry conditions (Watts, unpublished), and many closely related stygobiont species occur in regions that were once wet but are now desert (Leys *et al*., 2003; Watts & Humphreys, 2003).

Such strategies are a useful prerequisite to entering the subterranean habitat as a new, rather demanding adaptive zone which finally leads to a highly aberrant morphology. We postulate a scenario where *Copelatus* (*Papuadytes*) and other beetles inhabit temporary habitats such as small streams or wetlands and avoid floods or drought by seeking refuge in the hyporheic zone, the interface between groundwater and epigean habitats. Desertification of ancestral ranges may ultimately drive species into a stygobiont existence (Leys *et al*., 2003).

The phylogenetic analysis provides evidence for two major monophyletic groups of *Copelatus* species in Australia, i.e. *Copelatus* s.str. and *Copelatus* (*Papuadytes*). However, *Copelatus* as presently defined is only weakly supported and this issue will be addressed in more detail in a forthcoming analysis of world Copelatinae.

Our finding also extends the geographical range of stygobiont beetles discovered in Australia to a new region. To date, Australian stygobiont Dytiscidae were only known from central Western Australian calcrete aquifers. *Copelatus abditus* is the first record of dytiscid stygofauna from central Australia.

Finally, we have demonstrated the successful use of DNA sequence data as a powerful tool in aiding the classification

of organisms with deviating morphologies. Our checklist of stygobiont Dytiscidae reveals that eleven of the sixteen described genera were monobasic at the time of their description (Appendix). These genera were probably erected because specimens could not be assigned to existing genera based on their highly derived morphology. With the view that phylogenetics, and ultimately classification, consists more of searching for shared commonalities than searching for unique differences (Hennig, 1966), our results underline the importance of a phylogenetic approach to taxonomy. We suggest studying related groups carefully, in a combined morphological/molecular framework when necessary. This can help to determine whether new, higher level taxa should be introduced for morphologically derived species.

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Appendix

Checklist of stygobiont dytiscidae of the world

Listed are the species names published by April 2003 as well as twenty-five species in the press (Watts & Humphreys, 2003).

*Genera containing stygobiont species only;
**Genera containing interstitial species only

Copelatinae

Copelatus (*Papuadytes*) Balke, 1998
abditus sp.n. (Australia)

Hydroporinae

Bidessini
Bidessodes Régimbart, 1900
gutteridgei Watts & Humphreys, 2003 (Australia)
limestoneensis Watts & Humphreys, 2003 (Australia)
**Comaldessus* Spangler & Barr, 1995
stygius Spangler & Barr, 1995 (U.S.A.)
**Sinodytes* Spangler, 1996
hubbardi Spangler, 1996 (China)
**Tjirtudessus* Watts & Humphreys, 1999
= **Nirridessus* Watts & Humphreys, 1999 (n.syn. by Watts & Humphreys, 2003)
bialveus Watts & Humphreys, 2003 (Australia)
bigbellensis (Watts & Humphreys, 2000) (Australia)
challaensis (Watts & Humphreys, 2001) (Australia)
cueensis (Watts & Humphreys, 2000) (Australia)
cunyuensis Watts & Humphreys, 2003 (Australia)
eberhardi Watts & Humphreys, 1999 (Australia)
fridaywellensis (Watts & Humphreys, 2001) (Australia)
hahni Watts & Humphreys, 2000 (Australia)
hinkleri (Watts & Humphreys, 2000) (Australia)
jundeeensis Watts & Humphreys, 2003 (Australia)
karalundiensis Watts & Humphreys, 2003 (Australia)
lapostaae (Watts & Humphreys, 1999) (Australia)
macrotarsus Watts & Humphreys, 2003 (Australia)
magnificus Watts & Humphreys, 2000 (Australia)
masonensis (Watts & Humphreys, 2001) (Australia)
morgani (Watts & Humphreys, 2000) (Australia)
pinnaclesensis (Watts & Humphreys, 2001) (Australia)
pulpa (Watts & Humphreys, 1999) (Australia)
raesidensis Watts & Humphreys, 2001 (Australia)
silus Watts & Humphreys, 2003 (Australia)
sweetwatersensis Watts & Humphreys, 2003 (Australia)
wilunaensis Watts & Humphreys, 2003 (Australia)
windarraensis (Watts & Humphreys, 1999) (Australia)
yuinmeryensis Watts & Humphreys, 2003 (Australia)
**Trogloguignotus* Sanfilippo, 1958
concii Sanfilippo, 1958 (Venezuela)

Hydroporini
**Haideoporus* Young & Longley, 1976
texanus Young & Longley, 1976 (U.S.A.)
**Iberoporus* Castro & Delgado, 2001
cermenius Castro & Delgado, 2001 (Spain)
**Nirripirti* Watts & Humphreys, 2001 (Australia)
darlotensis Watts & Humphreys, 2003 (Australia)
fortisspina Watts & Humphreys, 2003 (Australia)
hamoni Watts & Humphreys, 2003 (Australia)
hinzeae Watts & Humphreys, 2001 (Australia)
killaraensis Watts & Humphreys, 2003 (Australia)
macrocephalus Watts & Humphreys, 2003 (Australia)
melroseensis Watts & Humphreys, 2003 (Australia)
milgunensis Watts & Humphreys, 2003 (Australia)
napperbyensis Watts & Humphreys, 2003 (Australia)
newhavenensis Watts & Humphreys, 2003 (Australia)
pentameres Watts & Humphreys, 2003 (Australia)
plutonicensis Watts & Humphreys, 2003 (Australia)
stegastos Watts & Humphreys, 2003 (Australia)
skaphites Watts & Humphreys, 2003 (Australia)
wedgeensis Watts & Humphreys, 2003 (Australia)
**Siamoporus* Spangler, 1996
deharvengi Spangler, 1996 (Thailand)
**Siettitia* Abeille de Perrin, 1904
avenionensis Guignot, 1925 (France)
balsetensis Abeille de Perrin, 1904 (France)
**Stygoporus* Larson & LaBonte, 1994
oregonensis Larson & LaBonte, 1994 (U.S.A.)
Hyphydrini
**Dimitshydrus* Uéno, 1996
typhlops Uéno, 1996 (Japan)
Hydroporinae incertae sedis
**Kintingka* Watts & Humphreys, 1999
kurutjutu Watts & Humphreys, 1999 (Australia)
**Kuschelydrus* Ordish, 1976
phraeticus Ordish, 1976 (New Zealand)
**Morimotoa* Uéno, 1957
gigantea Uéno, 1996 (Japan)
morimotoi Uéno, 1996 (Japan)
phraetica phraetica Uéno, 1957 (Japan)
phraetica miurai Uéno, 1957 (Japan)
**Phreatodessus* Ordish, 1976
hades Ordish, 1976 (New Zealand)
pluto Ordish, 1991 (New Zealand)

Species thought to be interstitial

Hydroporinae
Bidessini
***Crinodessus* Miller, 1997
amyae Miller, 1997 (U.S.A.)
***Glareadessus* Wewalka & Biström, 1998
franzi Wewalka & Biström, 1998 (Iran)
stocki Wewalka & Biström, 1998 (Oman)