

RESEARCH PAPER

A new, putatively semisubterranean, *Rhithrodytes* diving beetle from southwestern Sardinia (Coleoptera: Dytiscidae)

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Abstract. The diving beetle genus *Rhithrodytes* Bameul, 1989 is endemic to areas surrounding the Western Mediterranean Basin, and currently includes six lotic species; three each in Europe and North Africa. Here we describe a striking new species, *Rhithrodytes pantaleonii* sp. nov., discovered during recent fieldwork in southwestern Sardinia. The new species differs markedly from the only previously known *Rhithrodytes* from the Tyrrhenian Islands, *R. sexguttatus* (Aubé, 1838). The pale, flattened appearance of *R. pantaleonii* sp. nov., together with its small eyes and scarcity during collecting, all point to it being semisubterranean. A combination of mitochondrial and nuclear DNA sequences, obtained non-destructively from the holotype, allow us to reconstruct the evolutionary history and historical biogeography of this taxon and take the opportunity to publish the mitogenome of the new species. *Rhithrodytes pantaleonii* sp. nov. is shown to be sister to *R. sexguttatus*, these taxa apparently having diverged during the Miocene, when the Sulcis-Iglesiente region of southwestern Sardinia was separated from the rest of Corsico-Sardinia by the Sardinian Seaway. Our discovery adds to our understanding of the biogeography of the Tyrrhenian Islands, and further highlights the importance of the Sulcis-Iglesiente region as an area of endemism in its own right.

Key words. Coleoptera, Dytiscidae, Hydroporini, diving beetle, new species, semisubterranean, endemic, phylogeny, historical biogeography, mitogenome, Europe, Palearctic Region

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Introduction

On a recent field trip to Sardinia, together with other interesting finds, two specimens of an unexpected new dytiscid were discovered in residual pools in a small stream in the south-western part of the island. The beetle was immediately recognized as new in the field; a pale and oblong *Rhithrodytes* Bameul, 1989, very different from *R. sexguttatus* (Aubé, 1838), the only species of the genus then known from Sardinia (and also present in Corsica, Elba and Montecristo). The discovery of this new species further emphasises the unique biogeography of the Sulcis-Iglesiente region (south-west Sardinia, south-west of

a line from Cagliari to Guspini), discussed by AUDISIO et al. (2009) when describing *Hydraena rosannae* Audisio, Trizzino & De Biase, 2009, another water beetle apparently endemic to this area. It also highlights the fact that, despite centuries of study, our understanding of the freshwater fauna of the Western Mediterranean remains incomplete.

Rhithrodytes is a Western Mediterranean genus of hydroporine diving beetles, belonging to the subtribe Siettitiina, closely related to the epigeal/endogean *Iberoporus* Castro & Delgado, 2001, the strictly endogean *Siettitia* Abeille de Perrin, 1904 and very likely related to the strictly endogean *Etruscodytes* Mazza, Cianferoni & Rocchi, 2013 (RIBERA & REBOLEIRA 2019), all of which



are endemic to the Western Mediterranean area. At present *Rhithrodytes* includes six species, all essentially epigeal, with tendencies to occupying interstitial spaces in stream benthos (BAMEUL 1989, FERY 2016, RIBERA & REBOILERA 2019). The new species described here is the seventh and its pale, flat and elongated habitus, together with relatively small eyes, suggest that it is semisubterranean. Whilst some *Rhithrodytes* species, such as *R. bimaculatus* (Dufour, 1852) are most often found in springs and hyporheic habitats at the margins of streams, the new Sardinian species appears, from its morphology, to be further along the transition to interstitial life. Here we describe this new species on the basis of two specimens collected in June 2022, and establish its phylogenetic placement and evolutionary history using mitochondrial and nuclear DNA sequence data.

Material and methods

Taxon sampling, DNA extraction and sequencing. Beetles were collected with a D-framed pond net or kitchen sieve with 1 mm mesh. Both specimens were found after intense agitation of gravels close to steep banks.

Specimens were studied using Leica MZ8 and M205C stereomicroscopes, with LED gooseneck lights diffused using a tracing paper collar and tubes derived from opaque white plastic film canisters. Habitus photographs were taken with a Canon EOS 5D Mark IV camera fitted to a Leica M205C stereomicroscope, with a 1× objective lens. Specimens were illuminated with gooseneck lights, diffused with a film canister tube. Genitalia were mounted on glass slides in Kisser's glycerol gelatine (see RIEDEL 2005) and imaged using an Olympus CX31 microscope and Canon EOS 500D camera. All image stacks were produced by hand and combined using Zerene Stacker software (www.zerene.com).

DNA was extracted non-destructively using the Macherey-Nagel NucleoSpin Tissue Kit following manufacturer's instructions. Low-coverage whole-genome library preparation was conducted using the NEBNext Ultra II FS DNA kit, and sequencing performed using a NovaSeq 2000 platform to retrieve an estimated output of 30 million paired-end reads (StarSeq, Mainz, Germany). Data quality was checked in FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and adapters and low quality read ends trimmed using BBDuk (<https://jgi.doe.gov/data-and-tools/software-tools/bbtools/>). SPAdes v.3.15.3 (PRJIBELSKI et al. 2020) was used to assemble reads and contigs blasted (CAMACHO et al. 2009) against reference sequences of *cox1*, *rrnL*, 18S and histone 3 genes from other hydrophilic beetles at NCBI (CLARK et

al. 2016). We identified the mitochondrial genome, which was annotated using MitoS 2 WebServer (DONATH et al. 2019) and manually refined by comparison with other hydrophilic mitochondrial genomes (VILLASTRIGO et al. 2021). The mitochondrial genome and nuclear sequences were uploaded at NCBI, with the following accession numbers: mitochondrial genome: OP699163; 18S: OP700050; Histone 3: OP696834.

Phylogenetic analyses. We compiled DNA sequences of 4 molecular markers (*cox1*, *rrnL*, 18S, and histone 3) for the following genera related to the new species: *Iberoporus*, *Porhydrus* Guignot, 1945, *Rhithrodytes*, *Siettitia*, and *Stictonectes* Brinck, 1943. In addition, two *Graptodytes* Seidlitz, 1887 species were used as outgroups, resulting in a dataset containing 29 species. Model selection and partition schemes were conducted in PartitionFinder 2 (LANFEAR et al. 2017), followed by Bayesian Inference analysis in BEAST v1.10.4 (SUCHARD et al. 2018) using a Yule speciation prior and an uncorrelated lognormal clock per partition. This analysis was run during 100 million generations, and convergence was checked to estimate the burn-in fraction in Tracer v1.7.1 (RAMBAUT et al., 2018), which was set at 10%. A temporal framework was estimated using 5 secondary calibrations obtained from the phylogeny in VILLASTRIGO et al. (2021) – see Table 1.

Label data and abbreviations. Exact label data are cited for specimens. Slashes “/” indicate new line in label text. The following abbreviations are used in the text:

CTB	Collection Mario Toledo, Brescia, Italy;
ZSM	Zoologische Staatssammlung München, Munich, Germany;
BL	body length (front of labrum to elytral apices);
BL-H	body length minus head;
EL	elytral length (outer angle of shoulder to apex, parallel to suture);
EW	elytral width at widest point.

Results of molecular analysis

The mitochondrial genome of the new *Rhithrodytes* species was recovered almost completely, only missing sequence for tRNA-I. The mitochondrial genome follows the general order and orientation pattern of other known beetles (TIMMERMANS et al. 2016). Seven protein-coding genes (*cox1*, *cox2*, *cox3*, *nad2*, *nad3*, *nad4*, *nad5*, and *cob*) terminate with an incomplete stop codon (T- or TA-).

The best partition scheme as estimated by PartitionFinder 2 relied on one partition per gene marker as follows: 1) *cox1*: GTR+G+I, 2) *rrnL*: GTR+G+I, 3) 18S: JC+I and 4) histone 3: HKY+G. The phylogenetic reconstruction (Fig. 1) provides a highly supported phylogeny. The most recent ancestor of *Rhithrodytes* was dated to the

Table 1. Secondary calibrations used in Bayesian Inference analysis.

Genus	Age estimate <i>sensu</i> VILLASTRIGO et al. (2021)	Lognormal prior
<i>Graptodytes</i>	33.05 Ma [25.57–40.84]	Mean = 33.05; SD = 4.75
<i>Iberoporus</i>	18.2 Ma [13.73–22.52]	Mean = 18.2; SD = 2.8
<i>Porhydrus</i>	34.05 Ma [25.69–42.32]	Mean = 34.05; SD = 5.2
<i>Rhithrodytes</i>	19.78 Ma [15.13–24.74]	Mean = 19.78; SD = 3.1
<i>Stictonectes</i>	24.82 Ma [19.65–30]	Mean = 24.82; SD = 3.2

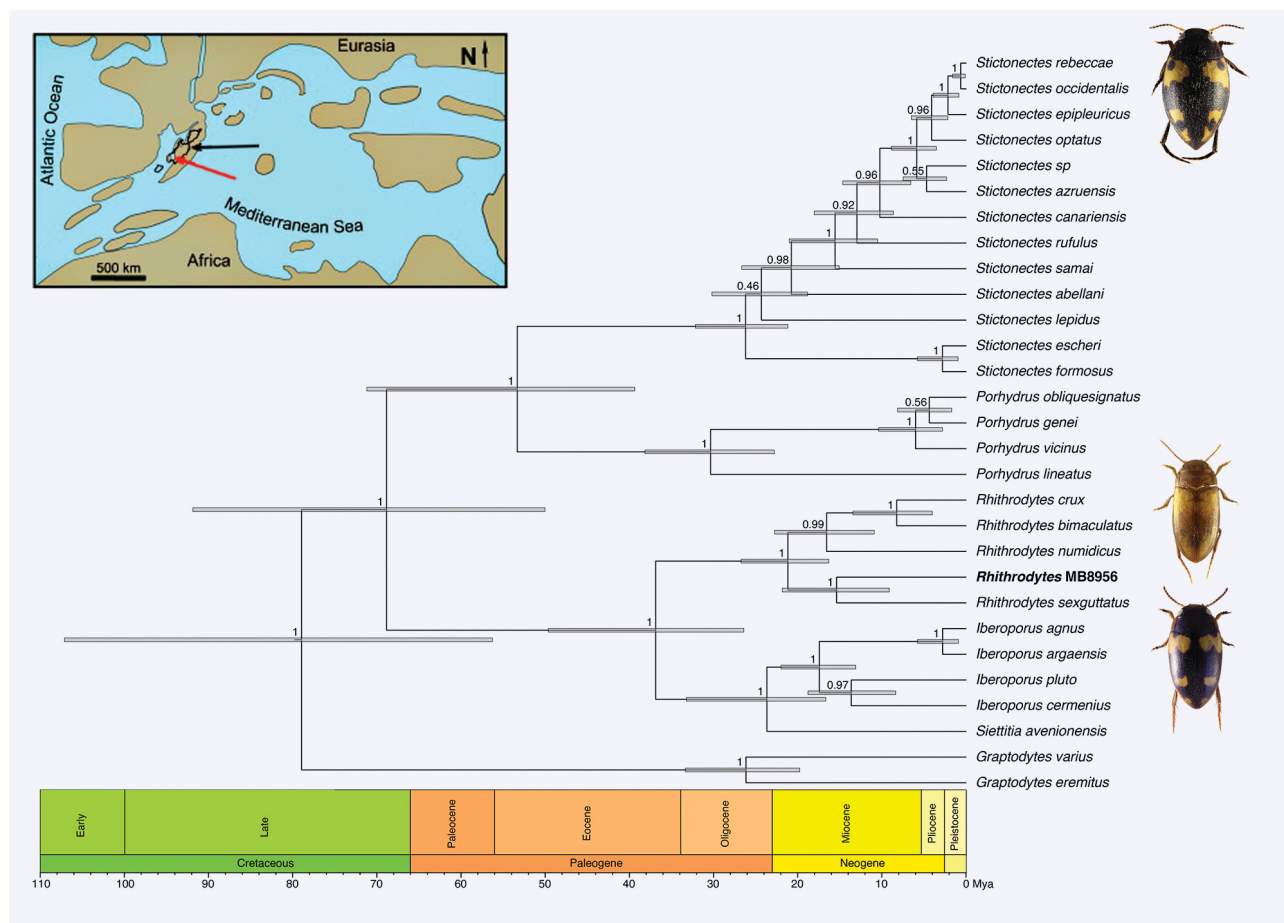


Fig. 1. Molecular phylogeny of *Rhithrodytes* and related genera of Hydroporini obtained with BEAST. Values beside nodes represent posterior probabilities, and 95% credibility intervals for estimated ages are shown with grey bars. Inset at top left shows the approximate distribution of land in the Mediterranean Basin in the Oligocene/Early Miocene (modified after ANDREUCCI et al. 2017); black arrow – Corsico-Sardinia, red arrow – Sardinian Seaway. Beetles at right hand side as follows, top to bottom: *Stictonectes rebecca* Bilton, 2013, *Rhithrodytes pantaleonii* sp. nov., *Rhithrodytes sexguttatus* (Aubé, 1838).

Miocene-Oligocene ca. 21.32 Ma (95% credibility interval: 16.32–26.72 Ma). The new *Rhithrodytes* species was sister to *R. sexguttatus*, the other Sardinian species of the genus, showing a COI sequence divergence of 15.19%. These two species diverged ca. 15.41 Ma (95% credibility interval: 9.13–21.82 Ma).

Taxonomy

Rhithrodytes pantaleonii sp. nov.

(Figs 1–5)

Type locality. Italy, Sardinia, Carbonia-Iglesias province, Riu Siuru 4 km NW Domusnovas, 39°21.55'N 08°37.14'E, 260 m a.s.l.

Type material. HOLOTYPE: ♂, labelled: “16.VI.2022 Italy, Sardinia, CI / Riu Siuru, 4 km NW Domusnovas / 39°21.55' N 08°37.14' E, 260 m / Balke, Bilton, Schizzerotto & Toledo leg.” (ZSM), with red holotype label and DNA voucher label: “DNA voucher MB8956” (ZSM). PARATYPE: ♂ same data as holotype and with red paratype label (CTB).

Description. *Habitus.* Body shape (Fig. 2) elongate-oblong, no discontinuity between posterior angles of pronotum and base of elytra, lateral sides of elytra almost parallel, maximum width just before middle of elytral length; dorsoventrally flat (Fig. 3A); colouration pale on both dorsal and ventral sides, ventral segments and parts of elytra almost transparent. Appendages uniformly reddish-yellow; eyes small (Fig. 4A).

Head relatively large in proportion, anterior margin forming a rather prominent, regular curve. Eyes apparently functional, but small in proportion to head (Fig. 4A); ommatidia irregular in size, maximum 9 in longest series. Surface weakly shining, with visibly impressed isodiametric microreticulation and sparse fine punctures (Fig. 4A). Colouration uniformly reddish-yellow. Antennomeres 3–10 as long as or longer than wide; terminal antennomere slightly darkened.

Pronotum subrectangular; lateral sides almost subparallel posteriorly, rounded anteriorly; weakly but visibly sinuate in lateral view. Anterior angles rather prominent; lateral rim well developed along each side. Anterior margin with impressed, transverse irregular puncture row; with sparse and fine punctures between lateral rims and disc; disc and scutellar process without punctures; surface with well impressed isodiametric microreticulation. Colouration reddish-yellow, with indistinct infuscations along anterior and posterior margins.

Elytra elongated, almost parallel-sided in anterior half, apical third regularly acuminate; lateral margins straight in lateral view, gently curving anteriorly over approximately basal sixth of length (Fig. 3A). Microreticulation isodiametric, more impressed than on head and pronotum, with fine and irregular discal punctures arranged longitudinally,

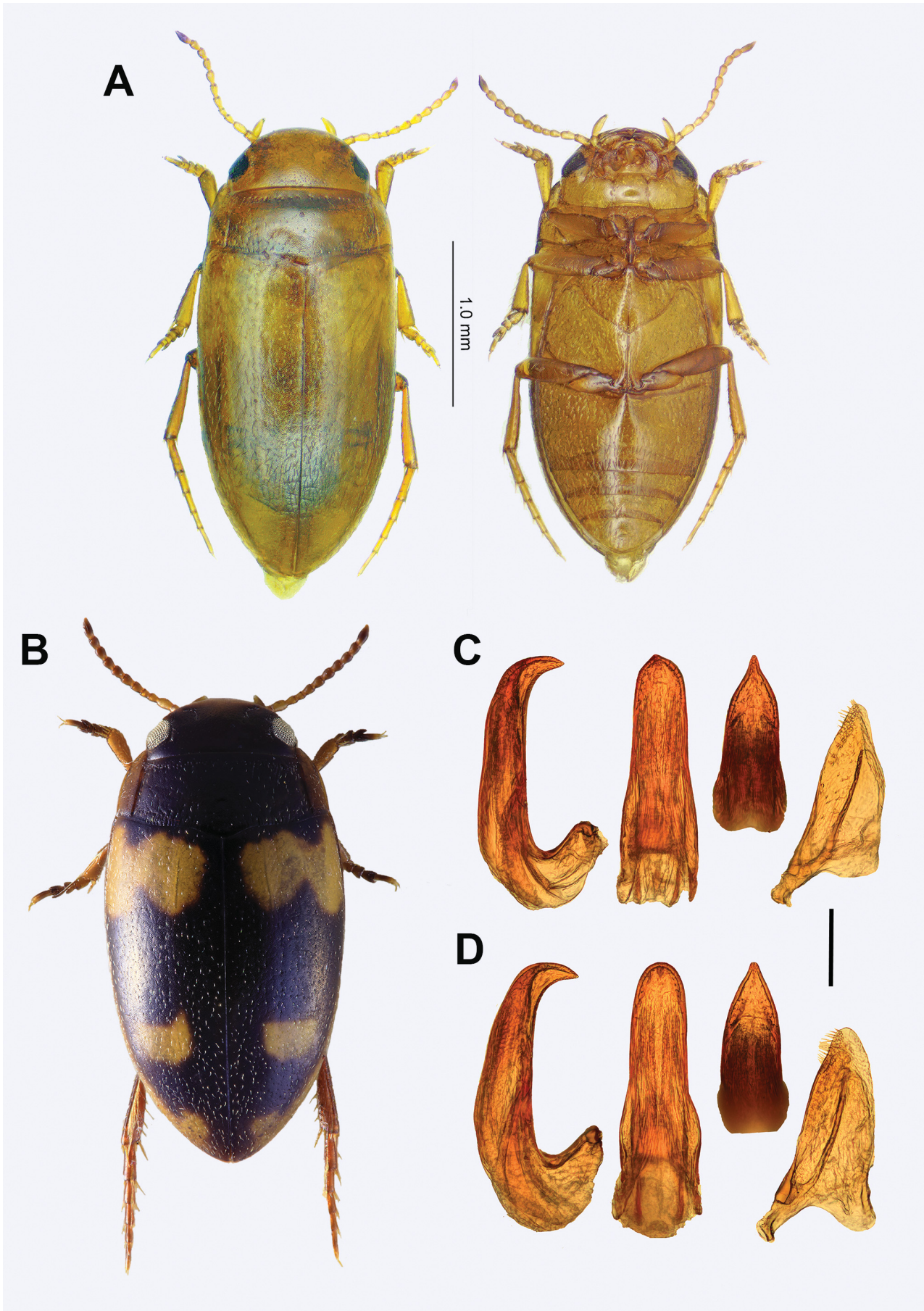


Fig. 2. Sardinian *Rhithrodytes* species. A – *R. pantaleonii* sp. nov., paratype, dorsal habitus; B – *R. sexguttatus* (Aubé, 1838), dorsal habitus; C – *R. pantaleonii* sp. nov., holotype, male genitalia; D – *R. sexguttatus* (Aubé, 1838), male genitalia. In C & D, images represent, from left to right: median lobe, lateral view; median lobe, ventral view; median lobe apex; paramere. Scale bars A & B = 1 mm; C & D = 100 µm.



Fig. 3. Sardinian *Rhithrodytes* species, lateral view. A – *R. pantaleonii* sp. nov., holotype; B – *R. sexguttatus* (Aubé, 1838). Scale bar = 1 mm.

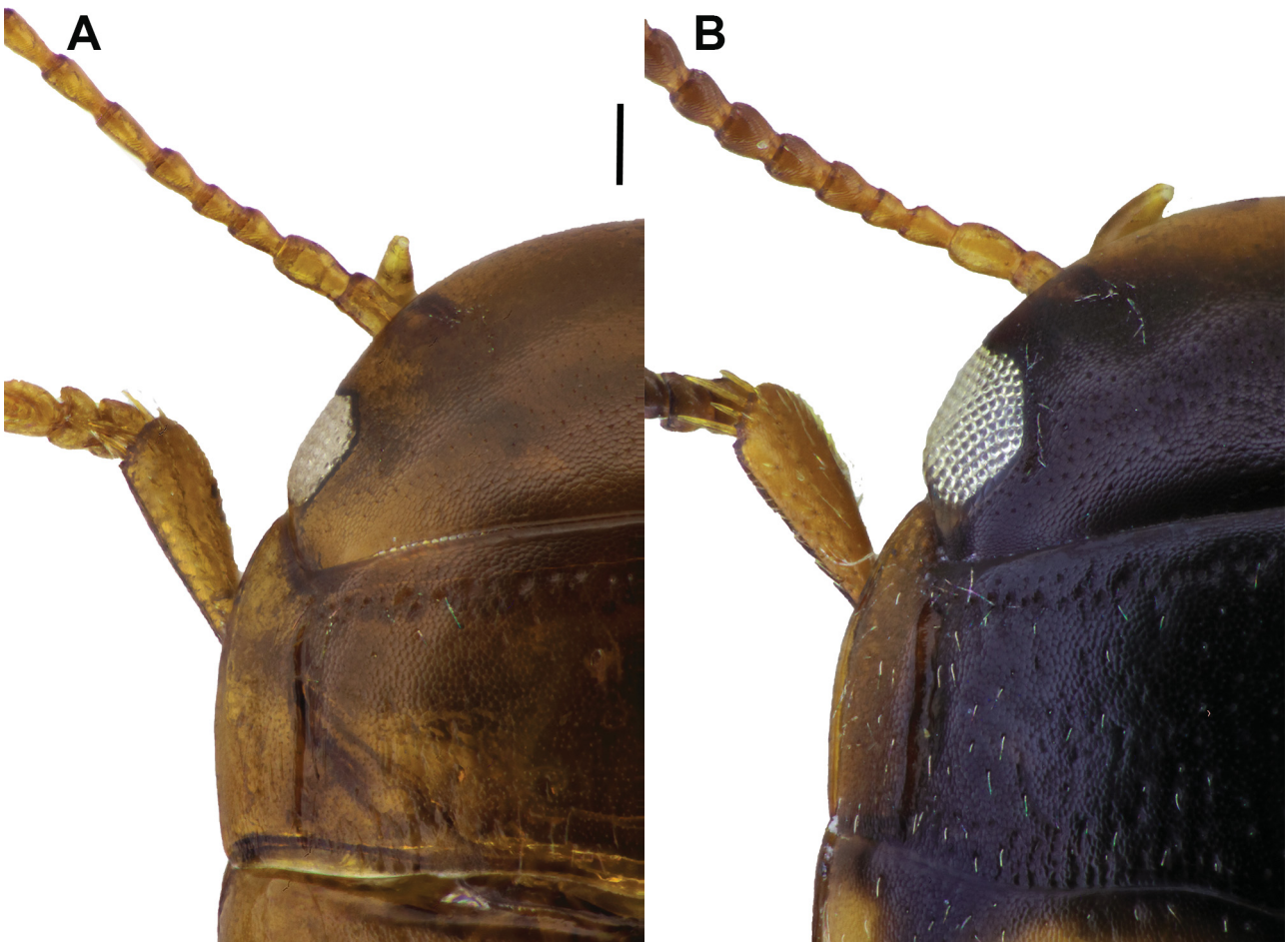


Fig. 4. Sardinian *Rhithrodytes* species, details of head and compound eyes. A – *R. pantaleonii* sp. nov., holotype; B – *R. sexguttatus* (Aubé, 1838). Scale bar = 100 μ m.

most of them bearing yellow decumbent setae. Colouration yellow with wide, poorly defined, weakly darker area posteriorly along suture and slightly expanded apically. Venation of metathoracic wings visible through elytra.

Legs. Metatibiae and metatarsi slender and quite long, with very sparse natatorial setae on inner face. Pro- and mesotarsi not strongly expanded; protarsal claws unmodified.

Venter (Fig. 2A) uniformly reddish-yellow; entirely isodiametrically microreticulated, with additional impressed pits. Prosternal process elongate, rounded apically, barely touching anteromedial metaventral process. Metacoxal lines clearly visible, reaching metaventricle. Epipleuron quite abruptly narrowed before mid-length; oblique carina visible near shoulder. Abdominal ventrites II and III fused with suture barely visible.

Male genitalia. Median lobe (Fig. 2C), straight in lateral view, with apical hook strongly developed; parallel-sided in ventral view, with acuminate tip visible at apex; acuminate tip strongly developed and clearly visible in ventro-apical view. Parameres (Fig. 2C) elongate, with narrow, rounded, setose apex.

Measurements. Holotype: BL = 2.7 mm; BL-H = 2.35; EW = 1.2 mm. Paratype: BL = 2.7 mm; BL-H = 2.35; EW = 1.2 mm.

Differential diagnosis. An oblong, flat, almost parallel-sided *Rhithrodytes* (Fig. 2), with small eyes in proportion to the head. Colouration uniformly pale yellowish-red-dish (note that the holotype in Figs 3 & 4 has darkened somewhat following DNA extraction; for more accurate representation of colour, see Fig. 2A). Natatory setae reduced. On the basis of these characters, *Rhithrodytes pantaleonii* sp. nov. has a very distinctive external morphology and cannot be confused with any other species of the genus. The male genitalia (Fig. 2C) are rather similar to those of *R. sexguttatus* (Fig. 2D), differing by the straighter profile of the median lobe in lateral view, with a narrower apical hook, by the narrower, more strongly acuminate apex of the median lobe in ventral view, and by the narrower apex of the parameres.

Etymology. The new species is dedicated to Prof. Roberto Pantaleoni (University of Sassari), who kindly supported our trip in Sardinia. It is a noun in the genitive case, standing in apposition.

Ecology. The Riu Siuru is a small, rocky lowland stream, flowing in a North-South direction over a calcareous substrate. Due the relative drought conditions in Spring-Summer 2022, we found the stream partially dried out and therefore focused on a ca. 15 m reach which had numerous residual pools and some flow (Fig. 5). Areas immediately above and below this stretch were found to be entirely dry. The new species was collected by digging between gravel and sand, very close to the water's edge and was very rare or at least difficult to find with hand nets. Other associated aquatic Coleoptera were Gyrinidae: *Gyrinus urinator* Illiger, 1808, *Gyrinus caspius* Ménétriés, 1832; Dytiscidae: *Agabus binotatus* Aubé, 1837, *A. rufulus* Fairmaire, 1859, *Colymbetes schildknechti* Dettner, 1983, *Meladema lepidoptera* Bilton & Ribera, 2017, *Bidessus minutissimus* (Germar, 1824), *B. saucius* (Desbrochers,

1871), *Hydroglyphus geminus* (Fabricius, 1792), *Hydroporus analis* Aubé, 1838, *Graptodytes exsanguis* (Bedel, 1925), *G. flavipes* (Olivier, 1795), *Stictonectes optatus* (Seidlitz, 1887), *Deronectes moestus moestus* (Fairmaire, 1858), *Scarodytes fuscitarsis* (Aubé, 1838); Hydrophilidae: *Laccobius neapolitanus* Rottenberg, 1874, *Anacaena globulus* (Paykull, 1798), *Limnohydrobius convexus* (Brullé, 1835); Hydraenidae: *Hydraena sardoa* Binaghi, 1961, *H. subsequens* Rey, 1886, *Limnebius perparvulus* Rey, 1884, *L. mucronatus* Baudi, 1872, *Ochthebius dilatatus* Stephens, 1829; Limnichidae: *Pelochares versicolor* (Waltl, 1838). Of these, *Graptodytes exsanguis* and *Hydraena sardoa* were the most abundant.

Discussion

The discovery of a new species of dytiscid in Sardinia in 2022 was most unexpected, as this family has previously been well-investigated on the island by a number of competent researchers. The only *Rhithrodytes* known from the island previously is *R. sexguttatus*, a common species in the island's streams and rivers, which is also abundant throughout Corsica (BAMEUL 1989) and on the islands of Elba and Montecristo (ROCCHI 2006). This species has also been recorded from a single locality in the Tuscan Apennines (ANGELINI 1984, ROCCHI 2006), where *R. crux* (Fabricius, 1792) is common, and recent investigations by FERY (2016) only found *R. crux* in this locality, meaning this record appears to be in error. The likely semisubterranean habits of the new species and the geological history of the Sulcis-Iglesiente region may together explain why this striking beetle has never been found previously. The type locality of the new species lies in the Monte Linas massif (the third highest mountains in Sardinia), in the Fluminese, an area well known for its karstic features, including caves, that likely supports considerable habitat for (semi)subterranean aquatic invertebrates. The habitus of *R. pantaleonii* sp. nov. is somewhat intermediate between that of other *Rhithrodytes* species and specialized subterranean relatives such as members of *Iberoporus*, *Settitia* and *Etruscodytes*. On this basis, coupled with the collecting circumstances, it seems reasonable to assume a semisubterranean lifestyle in this beetle. Semisubterranean diving beetles have been described from a number of parts of Europe and elsewhere and are, like *R. pantaleonii* sp. nov., typically partly depigmented compared to surface relatives, often with reduced eyes and a flattened, subparallel habitus (BILTON & FERY 1996, WEWALKA & BISTROM 1998, MANUEL 2013, BILTON & MLAMBO 2022). BILTON & FERY (1996) described a scenario for the possible evolution of phreatic life in Mediterranean Siettitiina, through an interstitial stage, already known in other species of *Rhithrodytes*, epigeal species of *Iberoporus* and some *Graptodytes* such as *G. exsanguis* and *G. fractus* (Sharp, 1882). *Rhithrodytes pantaleonii* sp. nov., with its pale, flattened habitus and small eyes may represent an intermediate step between interstitial and hypogean lifestyles. WATTS et al. (2016) made a similar case for an Australian species in a different subfamily, the Copelatinae, where morphology and collecting circumstances also suggested a transition stage from



Fig. 5. Type locality of *R. pantaleonii* sp. nov., Italy, Sardinia, Carbonia-Iglesias province, Riu Siuru 4 km NW Domusnovas, 39°21.55'N 08°37.14'E, 260 m a.s.l. A – Overview of stream with (left to right) Mario Toledo, Antonio Schizzerotto & David Bilton; B – Detail of microhabitat, *Rhithrodytes* collected from edges of right-hand bank. Photos Michael Balke.

epigean to stygobitic life. Sardinia has a rather complex geological history and the Sulcis-Iglesiente region has been entirely isolated from the rest of the island in the past. During the Late Oligocene and Early-Mid Miocene the Corsico-Sardinian Microplate was situated close to the southern margin of the Eurasian continent, and whilst Corsica and most of Sardinia formed a single landmass, the Sardinian Seaway isolated the Sulcis-Iglesiente region from central and eastern parts of the island (ANDREUCCI et

al. 2017, LONGHITANO et al. 2017, TELESCA et al. 2020). The estimated divergence dates for *R. pantaleonii* sp. nov. and *R. sexguttatus* derived from our BEAST analysis strongly suggest that these beetles were isolated on separate landmasses at this time: *R. pantaleonii* sp. nov. on the Sulcis-Iglesiente block; *R. sexguttatus* on the rest of Corsico-Sardinia. A relatively old origin for the divergence of these two *Rhithrodytes* species is in marked contrast to the much more recent estimates for the divergence of the

Hydraena evanescens (Rey, 1884) complex in Corsica and Sardinia, which appears to have occurred within the last four million years (AUDISIO et al. 2009, TRIZZINO et al. 2011). In these water beetles, the two Sardinian species occupy the Sulcis-Iglesiente region (*H. rosannae*) and the centre and north of the island (*H. tyrrhena* Binaghi, 1961), but appear to have speciated in the Pleistocene. Whilst the Sulcis-Iglesiente region would have united with the rest of Sardinia during the Messinian Salinity Crisis (5.96–5.33 mya), the Zanclean flood ca. 5.33 mya led to this area becoming a separate island again (CHERCHI & MONTADERT 1982, BOCCALETTI et al. 1990), this marine barrier only closing completely in the Pleistocene (ANDREUCCI et al. 2017, LONGHITANO et al. 2017, TELESCA et al. 2020). This second period of isolation may therefore have triggered the speciation of the two endemic *Hydraena*. The Sulcis-Iglesiente region supports endemics in a number of other animal groups (GRILL et al. 2007), including *Hydromantes* Gistel, 1848 salamanders (CIMMARUTA et al. 1998, LANZA et al. 2006, VAN DER MEIJDEN 2009, CHIARI et al. 2012) and *Percus* Bonelli, 1810 ground beetles (KETMAIER et al. 2003). In most cases there have been no explicit attempts to date the origin of the taxa concerned, although the relative phylogenetic isolation of the Sulcis-Iglesiente endemic *Hydromantes genei* (Temminck & Schlegel, 1838) suggests a relatively old divergence (VAN DER MEIJDEN 2009, CHIARI et al. 2012), as seen in the new *Rhithrodytes* described here. Clearly further molecular study of the endemic taxa of this region will be most illuminating.

Supplementary material

Supplementary data, consisting of the alignment used in our phylogenetic analysis is available in Zenodo under the DOI: 10.5281/zenodo.7657403.

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