

Origin of the interstitial isopod *Microcharon* (Crustacea, Microparasellidae) from the western Languedoc and the northern Pyrenees (France) with the description of two new species

Nicole COINEAU⁽¹⁾, Claude BOUTIN⁽²⁾, Malvina ARTHEAU⁽³⁾

⁽¹⁾ 67 Avenue du Puig del Mas, 66650 Banyuls-sur-Mer, France; e-mail: n.coineau@orange.fr

⁽²⁾ Université Paul Sabatier de Toulouse, Laboratoire Écologie fonctionnelle et Environnement (UMR n°5245, CNRS-UPS-INPT, ÉcoLab), Bât. 4R1, 118 route de Narbonne, 31062 Toulouse cedex 9, France. Present address: 48 rue Mouffetard, 75005 Paris, France; e-mail: claude.boutin@univ-tlse3.fr

⁽³⁾ Muséum d'Histoire Naturelle de Toulouse, 35 allées Jules Guesde, 31000 Toulouse, France

ABSTRACT

The interstitial groundwater genus *Microcharon* (Crustacea, Isopoda, Microparasellidae) is highly diversified in southern France. A new species, *Microcharon boulanouari* n. sp. is described from the Aude River, whereas specimens from the Lachein River in the central Pyrenees are reassigned to another species, *M. ariegensis* new to Science. *Microcharon boulanouari* n. sp. is closely related to the species of the group *rouchi* and may belong to the phylogenetic western Mediterranean lineage. The two-step model of colonization and evolution provides an understanding of the origin and age of this stygobite. *Microcharon boulanouari* n. sp. is derived from marine ancestors that lived in the interstitial littoral shallow bottoms of the Atlantic embayment which covered southwestern France at the very beginning of the early Eocene period. Both the regression of this gulf at the start of the Eocene and the Pyrenees uplift may have played a major role in the evolutionary history through vicariance of *Microcharon boulanouari* n. sp. and of the northern Pyrenean species of the group *rouchi*.

Key words: ground water, interstitial isopods, *Microcharon*, systematics, vicariance, western Languedoc, northern Pyrenees, palaeobiogeography

INTRODUCTION

Species of the minute crustacean isopod *Microcharon* (Microparasellidae) live in the fresh ground waters of river and aquifer sediments as well as in the interstitial water of marine sandy beaches and shallow bottom beaches (Lévi 1951, Spooner 1959, Bocquet 1970, Coineau 1986). Their ability to disperse is very feeble since they are holobenthic and exhibit no swimming larvae. Nevertheless, the genus *Microcharon* Karaman, 1933 has a worldwide distribution, from Polynesia and New Caledonia to Europe, the Mediterranean, the Caribbean and the Galapagos (Delamare Deboutteville 1960, Coineau 1986, Stock 1977, Galassi 1991, Galassi et al 1995a, b). More than 70 species are known from the Mediterranean basin alone both in the northern and the southern areas (Argano & Pesce 1979, Pesce & Galassi 1988a, b, 1990, Pesce & Tetè 1987, Galassi 1991, Coineau 1994, Galassi et al 1995a, b, Boulanouar et al 1995, Yacoubi Khebiza et al 1997, 1999, Stoch & Galassi 2002).

Inland species are derived from marine ancestors left in fresh ground waters by Tethys regressions (Delamare Deboutteville 1960, Coineau 1971, 1994, Stock 1977, Boutin 1993, Wägele 1990, Galassi 1991, Galassi et al 1995b, Coineau et al 2001, Stoch & Galassi 2002). Previous works

on phylogeny and biogeography of inland species have shown that species with more characters in an apomorphic state resulted from recent regressions whereas species exhibiting more plesiomorphic character states were left by ancient marine withdrawal during the late Cretaceous period (Coineau 1994, Coineau et al 1994, Galassi et al 1995b, Stoch & Galassi 2002). Vicariance evolution may have worked at different scales of space and time: at the scale of the Mediterranean, plate tectonics and large transgressions resulted in different lineages. At the regional and local scales, regressions and orogenesis were responsible for the differentiation of sister species (Coineau 1994).

The genus *Microcharon* is widespread in the southern ground waters of France (Ferreira et al 2003). Several species are located within the subterranean waters of the hydrographic system of the Rhône River (Coineau 1968, 1986, Dole & Coineau 1987). In southwestern France, only two species are known to occur. *Microcharon boui* Coineau, 1968 occurs in wells at Albi in the southern Massif central and *M. rouchi* Coineau, 1968 inhabits the ground waters of the Garonne and the Adour River hydrographic systems in the northern Pyrenees. Finally, *M. angelieri* Coineau, 1963 occurs in the phreatic waters of the Tech River in the Roussillon.

Therefore, the genus *Microcharon* is diversified in southern France. Different species are endemic to the various aquifer basins.

In the Roussillon and the western Languedoc regions (Fig. 1), four species new to Science have been discovered in the framework of the European PASCALIS Project in 2003 (Coineau et al 2005, Artheau 2006). One of these species from the ground waters of the Aude River is described hereafter. Moreover, the purpose of this paper is to provide an understanding of the origin of the representatives of the genus *Microcharon* from the western Languedoc and the northern Pyrenees within a distributional pattern and a palaeogeographic context.

MATERIAL AND METHODS

Sampling of specimens: All specimens were collected in wells using a Cvetkov net:

- More than 100 individuals with few males, many females and post-embryonic stages, from well ROU A37/D4 (X 456190- Y 47892810) at Trèbes, near the Aude River, August 5th 2003;
- 1 male, 11 females, 3 post-embryonic stages, from well ROU 139/D4 (X 436680 - Y 4767990) at Limoux, June 26th 2003;
- 1 male, from well ROU 138/D4-PN39 (X 436500 - Y 4754050) at Espéraza, June 26th 2003;
- 15 males, 11 females (with 1 ovigerous specimen), from well ROU 142/D4 (X 450190 -Y 4784380), at Périac-Carcassonne, August 4th 2003;
- 1 young male, 4 females, from well ROU 133/D4 (X 452870 - Y 4784460), Barette garden at Carcassonne, August 4th 2003;
- 4 females, 1 post-embryonic stage, from well ROU 143/D4 (X 483670 -Y4786830), at Ornai-sons, July 26th 2003.

Study of the new species

Several males and females have been completely dissected and mounted on slides in polyvinyl-lactophenol. Five specimens of the syntype series (individuals of Trèbes site) have been deposited at the Museum National d'Histoire Naturelle, Paris: MNHN-Is6302. Other specimens in authors' collections. Furthermore, the diversity of specimens of *Microcharon rouchi* from two different areas of the northern Pyrenees is re-examined.

Historical Biogeography

To assess the origin of the isopods, two types of data have been used. 1. The distributional pattern has been established considering the PASCALIS programme results and the distribution of the Pyrenean species (Coineau 1968). 2. Since freshwater species of *Microcharon* are derived from marine

ancestors, palaeogeographic data have been used and a relation between the extension of past embayments and the distribution area has been considered to date the ancestor entrance into the inland subterranean habitat. As for other marine interstitial relicts, the Two Step Model of Colonization and Evolution has been applied. This model is one of the scenarios providing an understanding of the colonization of interstitial inland subterranean waters by marine surface ancestors (Boutin & Coineau 1990, Notenboom 1991, Coineau & Boutin 1992, Holsinger 1994). During the first step, the surface epibenthic marine ancestor entered actively the interstitial sandy biotopes of the littoral shallow bottoms of the sea and evolved in minute interstitial isopods. This phase corresponded to an active dispersion and involved especially progenetic evolutionary processes. The second step involved a progressive and passive settlement of populations of the interstitial seashore crustacean into subterranean freshwater habitats during a regression of the sea. This last phase was described by Stock (1980) as the Regression Model Evolution. Vicariance evolutionary processes occurred as soon as the gene flow was interrupted between the new limnostygobiont and the marine populations remaining on the seashore. At the end of a regression period, a series of speciation events working successively, from the same marine ancestor up to the limnostygobionts, at each change of biotope and at each step of the colonization, resulted in several closely related species. When established in inland fresh waters, the new thalassoid populations continued to evolve mainly through vicariance, due to eventual geologic events acting as barriers responsible for divergences due to isolation of catchments and their stygobiotic populations within the distribution area.

Both palaeogeographical and paleontological results about the study region occupied now by the stygobionts have provided useful data.

RESULTS

Microcharon boulanouari n. sp.

Diagnosis of Microcharon boulanouari n. sp.: body segments stocky; distal plumose seta of antennule second article overreaching middle part of article 4; articles 5 and 6 of second antenna narrow and very long; pleotelson long with distal margin concave laterally, strongly convex in median part; female pleopod 2 long with lateral margins curved and distal margin straight; sympod of male pleopod 2 with inner distal corner slightly extended; exopod of third pleopod smooth; sympod of uropod slightly shorter than endopod.

Etymology.

The new species is dedicated to Dr M Boulanouar, Professor at the Ecole Normale Supérieure and at the University of Marrakesh for his important contribution to the knowledge of the different groups of groundwater isopods from Morocco.

Body length (excluding uropods): 1.60 - 1.87 mm in males, 1.68 - 1.90 mm in females. Body stocky. All segments of the body are wider than long, except the pleotelson.

Antennule (Fig. 2B). Six-segmented; first segment longer than wide, with 3 small setae and one distal plumose seta; second segment longer than wide, bearing 3 short plumose setae, 2 subdistal small setae and the long distal plumose seta overreaching the middle part of segment 4; following segments all together clearly shorter than the first two segments together; one short apical seta on segment 3, 1 distal plumose seta on segment 4; segments 5 and 6 longer than wide,

aesthetasc of segment 5 relatively short; segment 6 armed with 3 long setae, 1 long plumose seta and a long aesthetasc; no sexual dimorphism on the appendage.

Antenna (Fig. 2A). Exopodite with two setae; the last two peduncle segments very long; 8 to 10-articulated flagellum with all articles narrow and very long.

Right mandible (Fig. 2C). *Pars incisiva* armed with 4-5 apical teeth and without *lacinia mobilis*; 3 distally-denticulated spines and 4 short setae; *pars molaris* with 3 setae. Palp 3-segmented: segment 1 without armature, long segment 2 with 2 subdistal denticulated spines; segment 3 bearing 3 denticulated spines, decreasing in length from distal to proximal, and 2 rows of microsetulae.

Left mandible (Fig. 2D). Similar to the right counterpart except for *lacinia mobilis* with 4 small teeth and only 2 denticulated spines. Palp similar to the symmetric one.

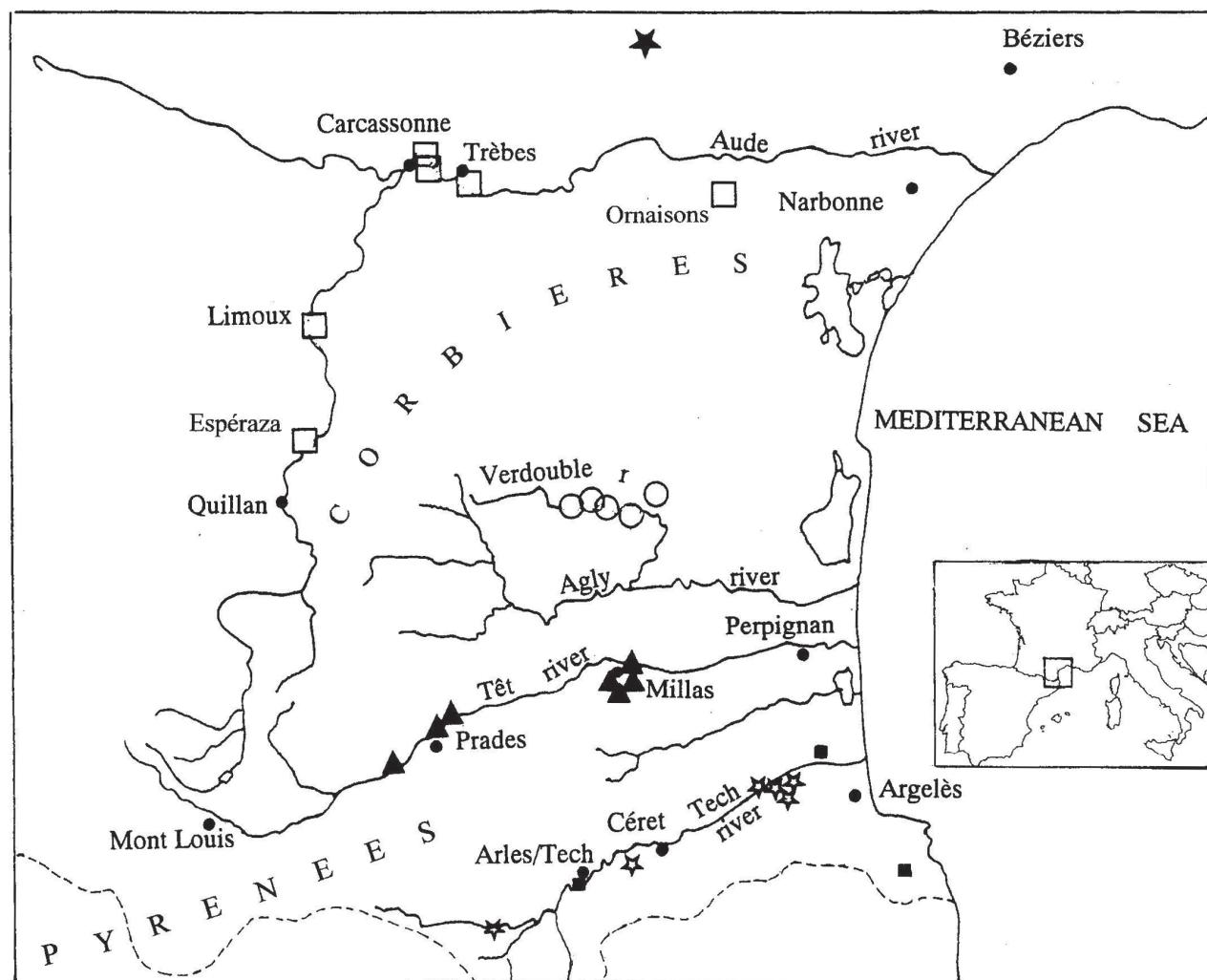


Fig. 1 - Distribution of the inland species of the genus *Microcharon* from the western Languedoc and the Roussillon. *M. boulanouari* n. sp. (open squares) from the Aude River; undescribed species new to science from subterranean waters of the Verdoulo River (open circles), the Têt River (filled triangles), and the Tech River (open stars). *M. angelieri* (filled squares).

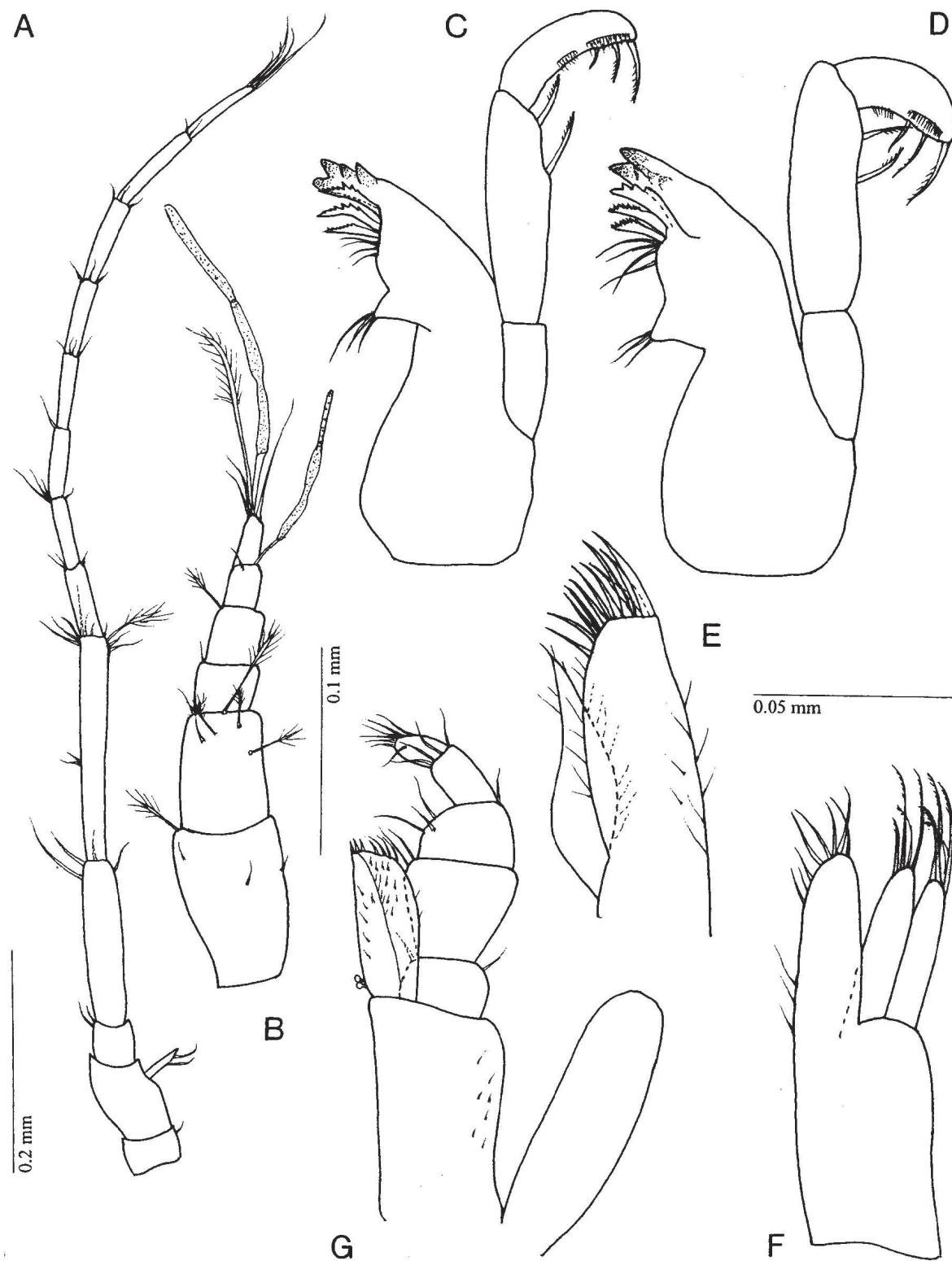


Fig. 2 - *Microcharon boulanouari* n. sp. A, antenna; B, antennule; C, right mandible; D, left mandible; E, maxillule; F, maxilla; G, maxilliped. Scale bars: 0.05 mm: C, D, E, F; 0.1mm: B, G; 0.2 mm: A.

Maxillule (Fig. 2E). Inner lobe narrow and pointed, with 1 distal and 2 subdistal thin setae. Outer lobe armed with 11 distal spines; seven and 8 small setae on inner and outer margins respectively.

Maxilla (Fig. 2F). Median and outer endites with 4 long pectinated setae; inner endite scarcely longer than the other, but wider and with 5 naked apical and 6 short marginal setae.

Maxilliped (Fig. 2G). Basipodal endite with 7 short apical spines, marginal setae and covered with numerous setulae; two coupling hooks on inner margin; articles 2 and 3 of palp wide; palp armature as in figure; epipodite apex reaching distal limit of palp article 1.

Pereiopods (Fig. 3). Basis moderately enlarged with small differences in armature (see figures); propod long and narrow in P6 and P7; dactylus with unequal claws, longest one not too much elongate.

Pleotelson (Fig. 4A) clearly longer than wide (width/length ratio $\geq 2/5$). Lateral margin almost straight with maximum width attained at proximal one-third of length; distal margin slightly concave laterally, strongly convex and rounded in median part. Chetotaxy as in Fig. 4A, with two subdistal plumose setae on dorsal surface

Male first pleopod (Fig. 4C) relatively long, with long exopods (according to Cvetkov 1968 terminology), separated along distal one-third of appendage length. Fold and hyaline fringe both reaching outer third of distal margin of exopod. Six setae located as in figure of which 2 subdistal setae very short, and 2 distal most longer and subequal.

Male second pleopod (Fig. 4B). Sympod moderately elongated, with outer margin regularly rounded and inner distal corner slightly marked and round; appendix masculina thin with chamfered edge tip and slightly overreaching sympod apex; exopod well developed and broad.

Female second pleopod (Fig. 4D, E) clearly longer than wide, lateral margins dorsally curved and forming a cavity containing pleopods 3 and 4; distal margin more or less straight, without seta.

Third pleopod (Fig. 4F). Endopod subovoid; exopod without setulae, first segment wide, second segment short and wide, reaching distal margin of endopod.

Uropods (Fig. 3E). Sympod scarcely shorter than endopod; the latter very long and narrow, with the normal subdistal and distal setae.

All specimens examined from the six localities exhibit a similar morphology.

Microcharon rouchi and *M. ariegensis* n. sp.

While comparing *Microcharon boulanouari* n. sp. with *M. rouchi* from several valleys of the Pyrenees, it appeared that the specimens from the Lachein River previously assigned to *M. rouchi* (see Coineau 1968) had to be separated from the individuals of the Basque Country and transferred into another new species, *Microcharon ariegensis* n. sp. on the basis of the differences already mentioned in 1968 (Compared to the individuals from the Basque Country, those from the Lachein River have the antennula aesthetascs longer, the

plumose seta of antennula segment 2 shorter, the pereiopodal claws shorter, the second article of pleopod III longer). The types are deposited at the Muséum National d'Histoire Naturelle, Paris: one specimen is designated as the lectotype of *Microcharon rouchi* from the Kakouetta River, number MNHN-Is6301. One individual is designated as the holotype of *M. ariegensis* n.sp. from the Lachein River, number MNN-Is6308.

Diagnosis of Microcharon ariegensis n. sp.

Distal plumose seta of antennula second segment very short; one of pereiopodal claws very long; pleotelson wider than body segments with rounded distal and lateral margins; distal part of sympod of male second pleopod wide with rounded outer corner; female pleopod 2 almost rounded and slightly longer than wide; setulae present along lateral margin of exopod of third pleopod.

Only specimens from the ground waters of the Pyrenean Kakouetta, Nive and Laran Rivers, from the French Basque Country, may be referred to *Microcharon rouchi*. *Microcharon ariegensis* n. sp. inhabits the ground waters of the Lachein River (Coineau 1968), and the Ariège and Hers Rivers in the central northern Pyrenees (Dumas 2000).

Remarks

Microcharon boulanouari n. sp. exhibits affinities with the Pyrenean species *M. rouchi* and *M. ariegensis* n. sp., especially the enlarged pleotelson and distal part of the male pleopod 1. However, compared with these two species, *M. boulanouari* n. sp. is longer and clearly more stocky. Lateral margins of the pleotelson are not curved as in *M. ariegensis* n. sp. (Fig. 5), so that this segment is not the widest of the body. The long plumose seta of antenna 1 second segment is very short in *M. rouchi* and *M. ariegensis* n. sp., while aesthetascs are shorter in *M. boulanouari* n. sp. The male first pleopod is clearly different from this appendage in *M. rouchi* and *M. ariegensis* n. sp. In the latter species, the fold and the hyaline fringe reach the median part of the distal margin of the exopod. In *M. boulanouari* n. sp., they reach this margin at a more lateral location; the distal part of the appendage is wider in *M. rouchi* and *M. ariegensis* n. sp. The female pleopod 2 is also very different in *M. boulanouari* n. sp.: it is more or less round in the Pyrenean species whereas it is clearly longer than wide in *M. boulanouari* n. sp. The length of exopod and endopod of the third pleopod is almost equal in *M. boulanouari* n. sp. vs endopod longer than exopod in the two other species. Furthermore, the outer margin of the exopod exhibits a row of microsetulae in *M. rouchi* and *M. ariegensis* n. sp.

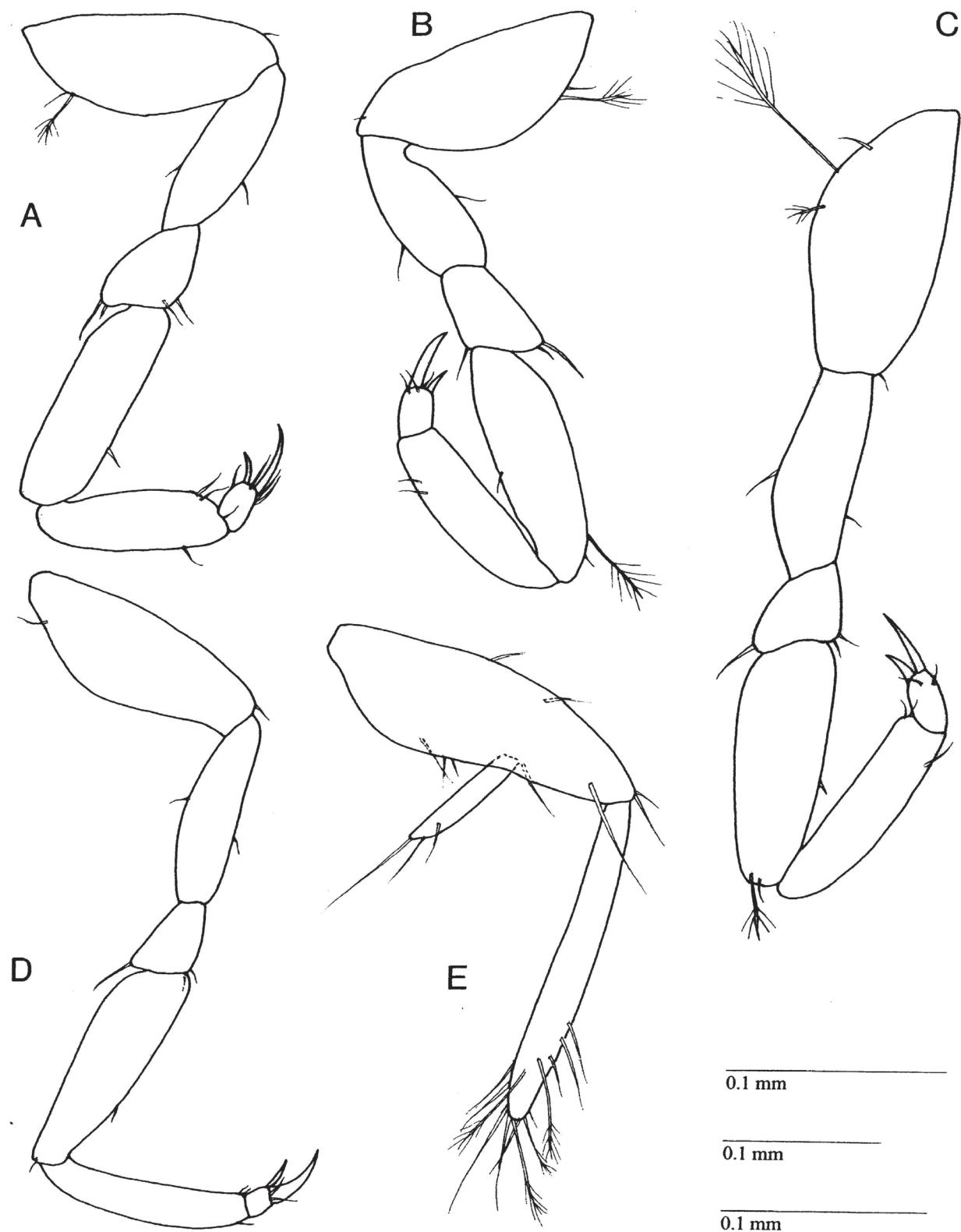


Fig. 3 - *Microcharon boulanouari* n. sp. A, pereiopod 1; B, pereiopod 3; C, pereiopod 4; D, pereiopod 7; E, uropod. Scale bars: top: P1 to P4; middle: P7; bottom: uropod.

(not present in *M. boulanouari* n. sp.). In addition, one of the pereiopod claws is clearly longer in the Pyrenean species.

Several characters, such as the thinner body shape, the longer pereiopodal claw, the detailed morphology of the male pleopod 1, the round and

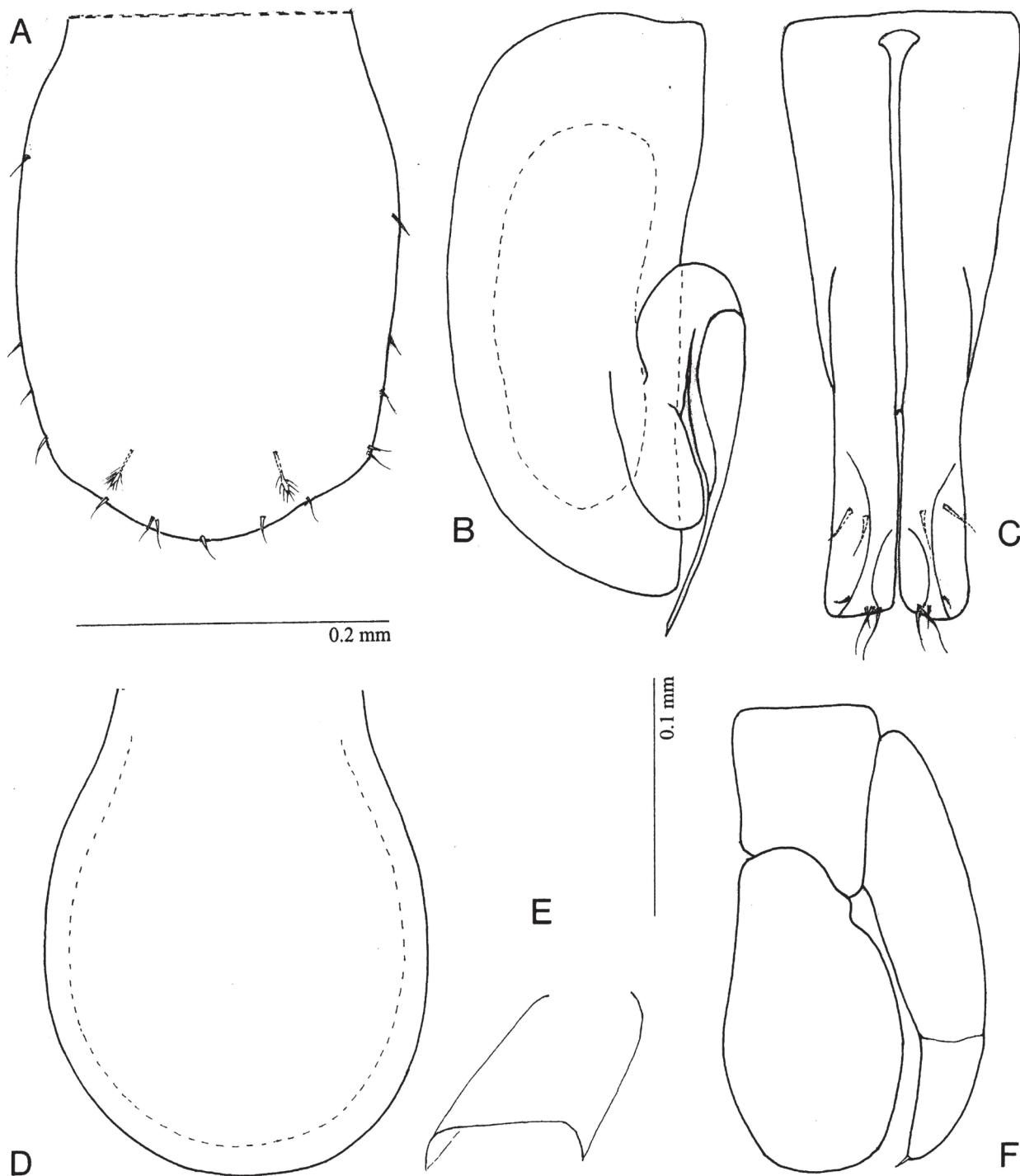


Fig. 4 - *Microcharon boulanouari* n. sp. A, pleotelson; B, male pleopod 2; C, male pleopod 1; D, female pleopod 2, ventral view; E, schematic 3D view of female pleopod 2; F, pleopod 3.

shorter female pleopod 2 are in an apomorphic state in *M. ariegensis* n. sp. and *M. rouchi*, in contrast to *M. boulanouari* n. sp.

Microcharon boulanouari n. sp. can be easily distinguished from the other French freshwater species, i. e. *M. angelieri*, *M. boui*, *M. juberthiei*, *M. doueti* and *M. reginae*, and from the Spanish Pyrenean species *M. longistylus* and *M. notenboomi*

(Coineau 1968, Dole & Coineau 1987, Pesce & Galassi 1988a) by the following characters:

- length of longest plumose seta of second segment of antenna 1 slightly overreaching median part of segment 4 (versus seta longer in Spanish species and shorter in other French species);
- body segments relatively wide and stocky;

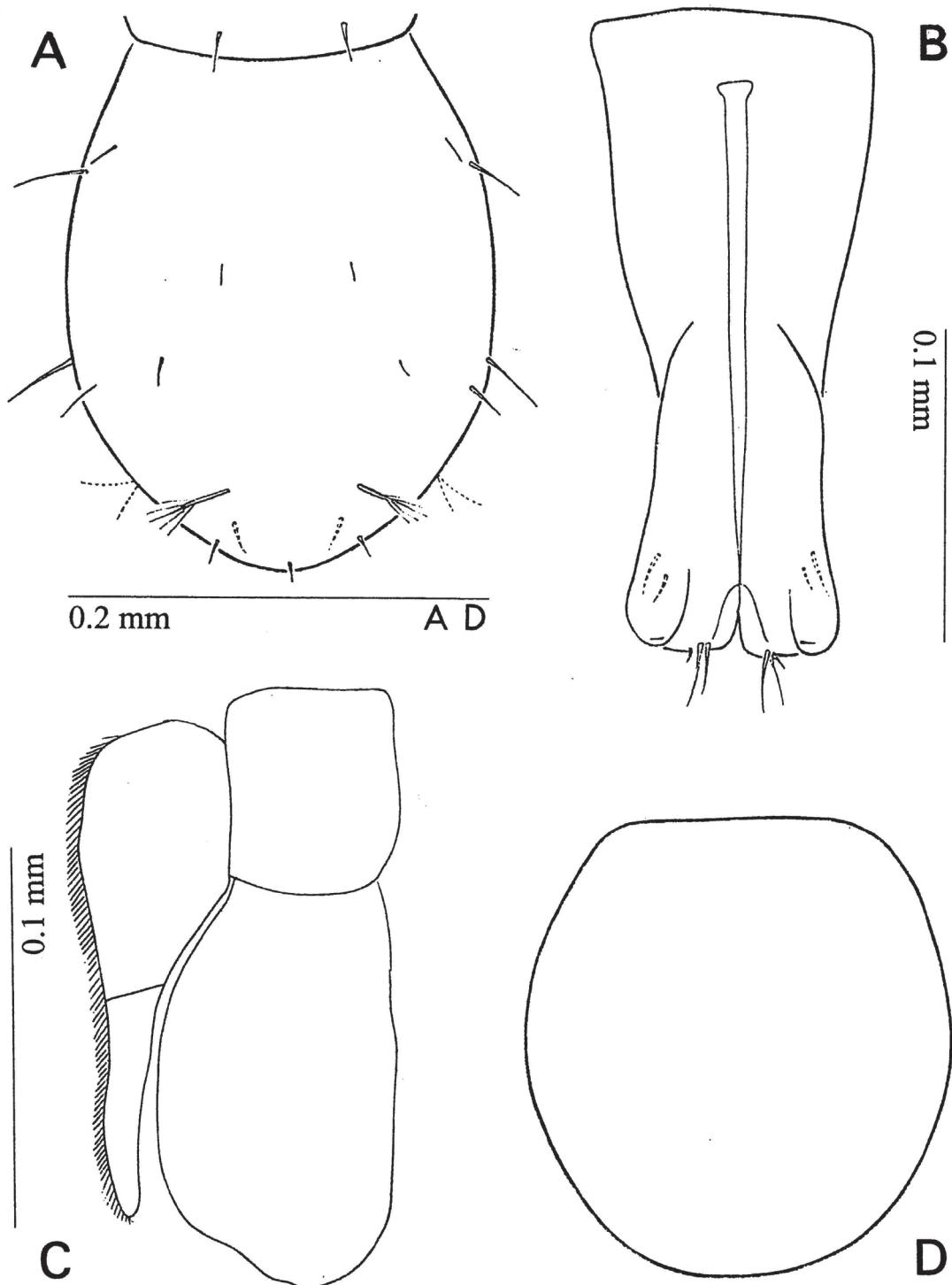


Fig. 5 - *Microcharon ariegensis* n. sp. A, pleotelson; B, male pleopod 2; C, pleopod 3; D, female pleopod 2.

- very long segment 5, and particularly 6 of antenna
- long pleotelson with characteristic distal margin;
- very long female pleopod 2 (vs more or less rounded appendage in other French species);
- sympod of male pleopod 2 not subovoid as in other French species, but with inner distal corner round and slightly extended.

Preliminary phylogenetic relationships

A phylogenetic study involving *Microcharon boulanouari* n. sp., *M. rouchi*, *M. ariegensis* n. sp., Moroccan, Algerian and Spanish species is in progress (Boulanour et al. in prep.). In the preliminary results of this study, and according to the phylogenetic relationships established within the freshwater species of *Microcharon* (Coineau 1994, Aït

Boughrous et al. 2007), *M. boulanouari* n. sp., *M. rouchi* and *M. ariegensis* n. sp. belong to the so-called “Western Mediterranean” group of species. More precisely, these three species share with members of this species group the most derived characters, i.e., female pleopod 2 without setae; uropods with the sympod shorter than the endopod; only three spines on the mandibular palp; and the inner distal corner of the sympod of male pleopod 2 not drawn out. The species *M. boulanouari* n. sp. is a sister species of the *rouchi-riegensis* group, whereas *M. rouchi* and *M. ariegensis* n. sp. are two sister species.

Historical biogeography

Distributional pattern: *M. boulanouari* n. sp. occurs in the ground waters of the Aude River at Espéraza, Limoux, Carcassonne, Trèbes, from upstream to downstream, and its Orbieu tributary at Ornaisons (Fig. 1). The Aude River flows within the western Languedoc in France. *M. ariegensis* n. sp. lives in the hyporheic waters of the Lachein, Hers and Ariège Rivers, in the northern Pyrenees, whereas *M. rouchi* inhabits the ground waters of the Nive, Larau and Kakouetta Rivers in the northern Pyrenean Basque Country.

All sites of the Aude basin inhabited by *Microcharon boulanouari* n. sp. are distributed in areas formerly covered by ancient marine gulfs. Upstream, the Aude River flows in the axial zone of the Pyrenees, while downstream, the floodplain is located within the former pre-Pyrenean trough excavated in the Senonian period (Bes 1988, Freytet 1973, Bousquet 1997) (Fig. 6). After the withdrawal of this Senonian Pyreneo-Provençal sea which was opened to the Atlantic Ocean (Debrand-Passard & Courbouleix 1984a, b, Plaziat 1981, 1983, 1984, Bilotte 1978, 1985), a new large Atlantic transgression developed towards the east up to Beziers within the north pre-Pyrenean trough, the Pyrenean domain, and its southern region in Spain during the Ilerdian (Late Paleocene-very early Eocene; Baceta et al 2005, Aguirre et al 2007, Gianetti 2010) (Fig. 7, 8). Almost the entire future Aude basin was covered by this Biscay embayment, as well as the Adour basin in the Aquitaine region (Tambareau 1972, Plaziat 1981, 1984, 1986, Alabouvette et al 1984, Bilotte et al 1989, Payros et al. 2006). The sea retreated definitively from the northern Pyrenean regions from the late Ilerdian to the Cuizian (= Early Eocene, 52-46 Myr (Odin & Odin 2002); Tambareau 1972, Tambareau et al 1997, Plaziat 1981, 1984, 1986, Freytet 1973). No further Atlantic transgression reached the Languedoc area of the Aude and the Ariège basins. The common ancestral populations of *Microcharon boulanouari* n. sp., *M. rouchi* and *M. ariegensis* n. sp. lived in the intersti-

tial littoral water or in the shallow bottoms of the Ilerdian marine gulf. They settled in fresh ground waters during the regression of this Ilerdian embayment (Fig. 9) from the late Ilerdian onwards, according to the second stage of the Two Step Model of Colonization and Evolution. In the region of the central Pyrenees, the Ilerdian gulf retreated from the Ariège area during the Cuisian (46 Myr), slightly after from the Aude basin. *M. ariegensis* n. sp. ancestral populations settled in fresh subterranean waters in the very early Eocene, whereas *M. rouchi* ancestors were left in fresh ground waters much later, when the sea retreated from the Basque-Country in the Eocene-Early Oligocene (34-30 Myr). The Pyrenean uplift at the end of the middle Eocene (i.e. end of the Lutetian, 42 Myr, during the Biarritzian and the Early Priabonian periods) (Durand-Delga 1980, Arthaud & Seguret 1981, Baceta et al 2005, Bilotte et al 2007, Scheibner et al 2007) induced subsequent erosion phenomena resulting in the development of the hydrographic systems of the Aude River, the Hers and the Ariège Rivers, and the Adour River, in separate valleys. At the same time, the primitively continuous ancestral population was divided in several isolated populations which further evolved independently by vicariance processes in each river catchment. Such vicariance evolution resulted in the closely related species *M. boulanouari* n. sp. in the Aude River ground waters on the one hand, and the two Pyrenean species *M. rouchi* from the Nive and the Larau Rivers (western northern Pyrenees, the Basque Country), and *M. ariegensis* n. sp. from the Lachein and the Ariège basins (northern central Pyrenees) on the other hand. Moreover, the sea retreated much later in the Basque Country. Different extant species live today in the three different hydrographic basins and are endemic to each basin. They are rhoendemics sensu Myers & De Grave (2000).

DISCUSSION

One of our goals was to describe the new species *Microcharon boulanouari* and to compare it with other Spanish and French species of the genus in order to get reliable characters for phylogenetic analyses of several groups of species from the Mediterranean. *M. boulanouari* n. sp., from the Aude River ground waters, displays several characters in a plesiomorphic state whereas these characters are in an apomorphic condition in the two Pyrenean species *M. ariegensis* n. sp. and *M. rouchi* from the Ariège and the French Basque Country regions. These morphological differences may reflect the significant role played by the marine regression of the Ilerdian gulf on the speciation. It occurred by

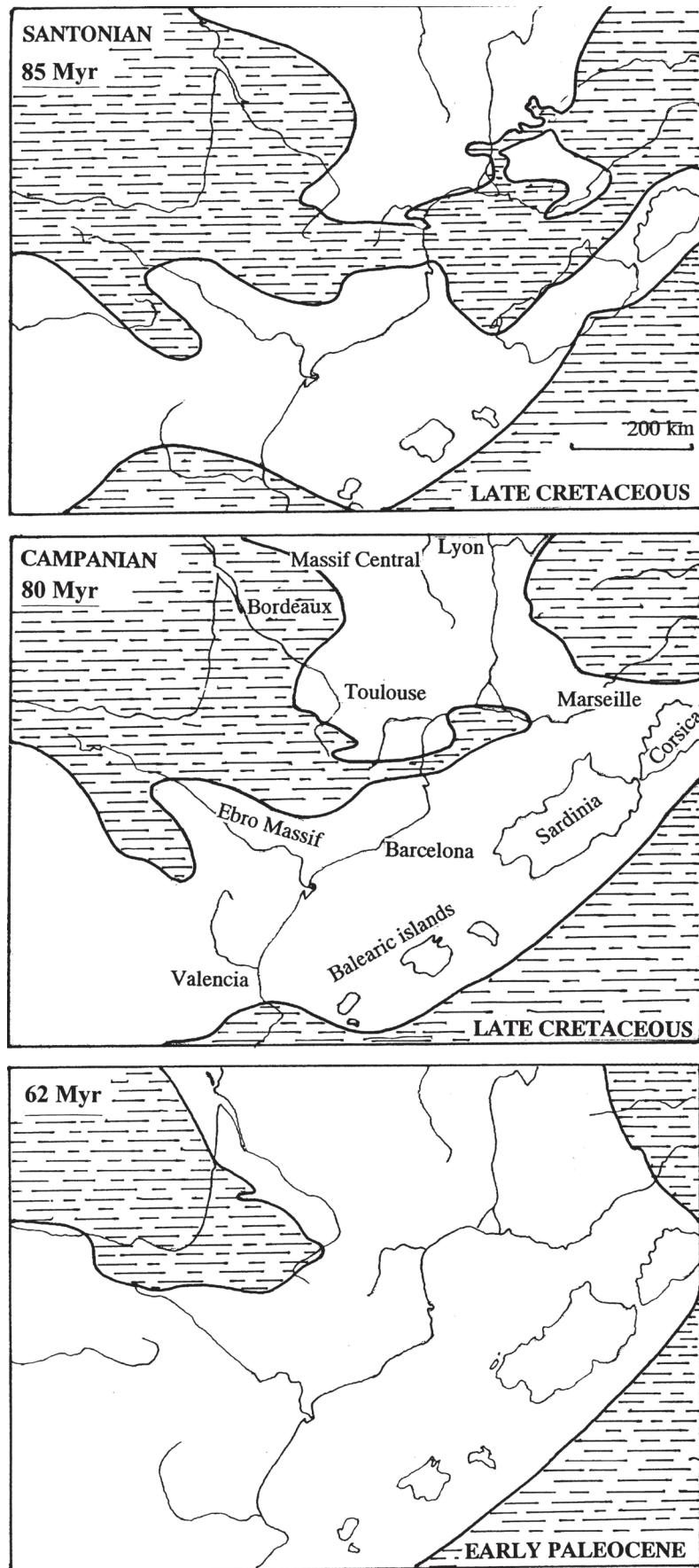


Fig. 6 - Southwestern France and northeastern Spain during the Santonian (top), the Campanian (middle) and the Early Paleocene (bottom): extension of the marine embayments (hatched areas). After Plaziat 1981 and Debrand-Passard & Couberleix 1984a, b, modified.

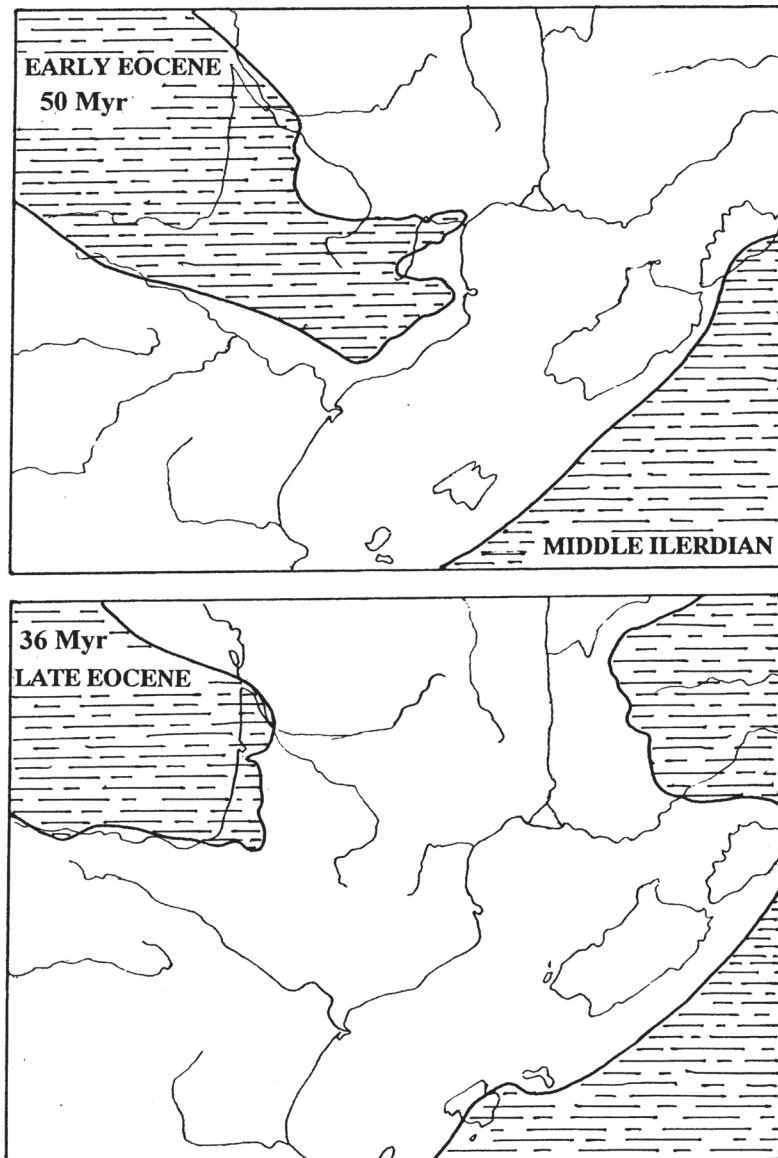


Fig. 7 - Soutwestern France and northeastern Spain during the Early Eocene (Ilerdian period, top) and the late Eocene (bottom): extension of the marine gulfs (hatched areas). After Plaziat, 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

successive stages from the East to the West (Plaziat 1981, 1986). The distributional pattern of these species, that are marine relicts, corresponds to the successive locations of the shoreline. The ancestors of *M. boulanouari* n. sp. stranded first into fresh ground waters while the ancestral populations of the Pyrenean species remained for a longer time in the unstable environment of the marine shallow bottoms or of the littoral interstitial water before their entrance into subsurface water-bodies later. Such a changing environment induced new selection pressures that may have triggered subsequent rapid evolutionary change. In contrast, at the same time the ancestors of *M. boulanouari* evolved in the more stable environment of the inland ground waters (Gibert et al. 1994). So that the extant species

retains plesiomorphic character states. This species may have evolved at a lower rate. Similarly, various authors have reported that an increase of evolutionary rates is related to environmental instability and stress, from the molecular scale to that of biogeography (Parsons 1991, Taddei et al 1997, Stearns 2002, Boutin & Coineau 1991, 2000, Ruokolainen et al 2009, Venditti & Pagel 2010). Stearns & Hoekstra (2002) added that stasis is the “result of lack of change in the environment, and therefore in selection pressure”. At the molecular scale, Wägele et al (2003) concluded also that the evolution of Asellota isopods is faster in marine than in freshwater habitats and that the low number of substitutions seen in sequences of freshwater species is caused by slow rates of adaptive morphological evolution.

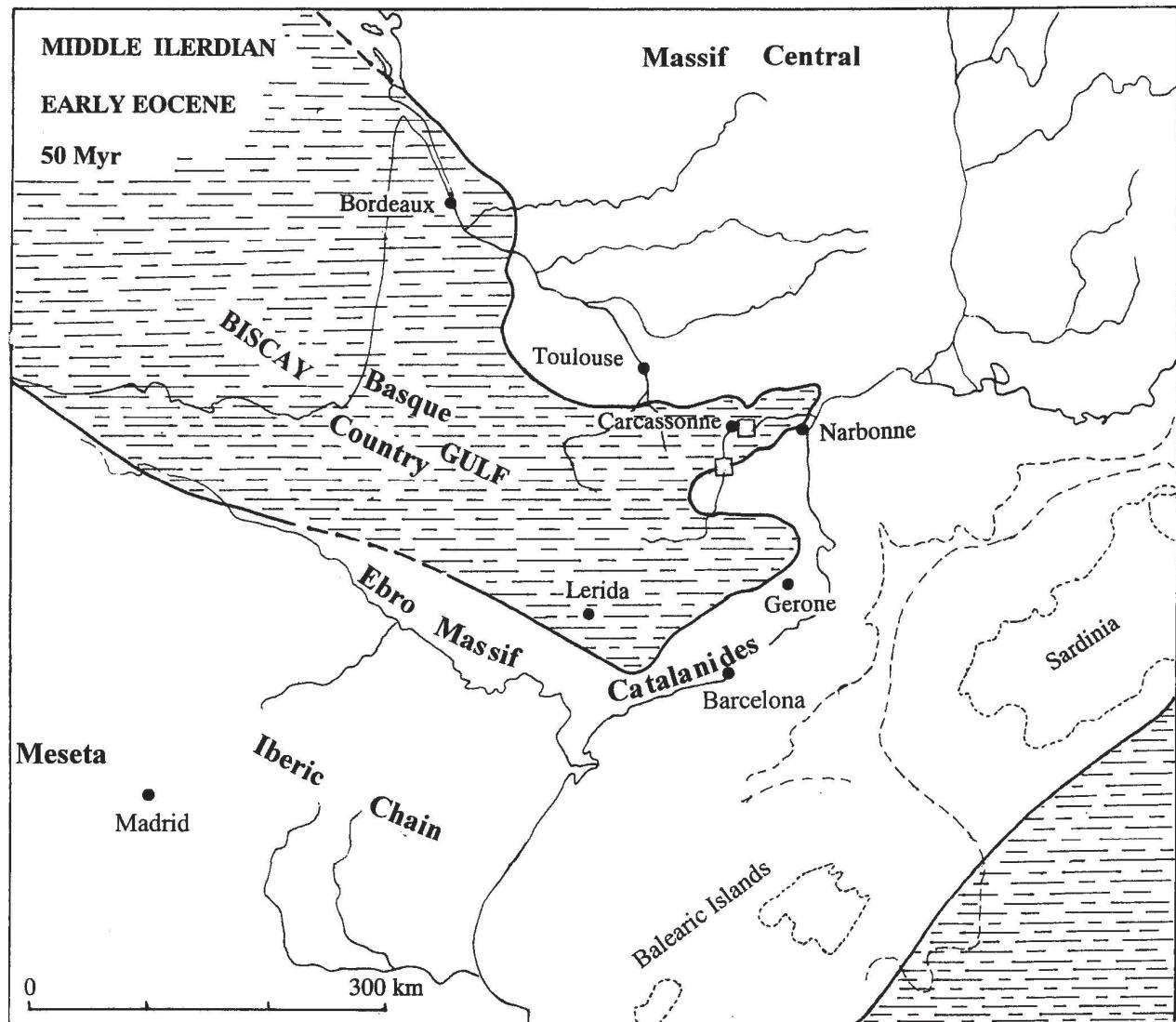


Fig. 8 - Southwestern France and northeastern Spain during the early Eocene period: extension of the marine Biscay gulf (hatched) and distribution of *Microcharon boulanouari* n. sp. (open squares). After Plaziat 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

Besides successive environmental changes, *Microcharon* diversification in southwestern France is also linked to the isolation of ancestral populations due to the Pyrenean orogenesis. Subsequent vicariant processes in the different new river catchments resulted in endemic species occurring in only one hydrographic system. A high level of endemism is one of the general patterns emerging in the outcome of the large-scale European survey of groundwater biodiversity in the framework of the European PAS-CALIS project (Gibert & Culver 2009, Gibert et al 2009, Deharveng et al 2009). Trontelj et al (2009) states that this pronounced endemism “is caused by strong hydrographical isolation and low dispersal abilities of their inhabitants”. Small ranges of stygobionts are the rule and “ranges over 200 km are extremely rare”. *Microcharon* species that are

restricted to one hydrographic system, even to one tributary, may belong to the so-called “narrow or short-range endemics” (Eberhard et al 2009).

According to Trontelj et al (2009), stygobiont ranges seem to “reflect historical rather than current hydrogeological conditions”. Therefore, geological history is an important determinant of species richness. The central Pyrenees are one of the four hotspots of groundwater biodiversity identified in Europe (Deharveng et al. 2009). Therefore, filling the gap of sampling coverage in the aquifers between the eastern and the western Pyrenees may give the opportunity to discover new species also derived from the Ilerdian marine gulf. Such species will allow to test sensu K Popper our palaeobiogeographic hypotheses.

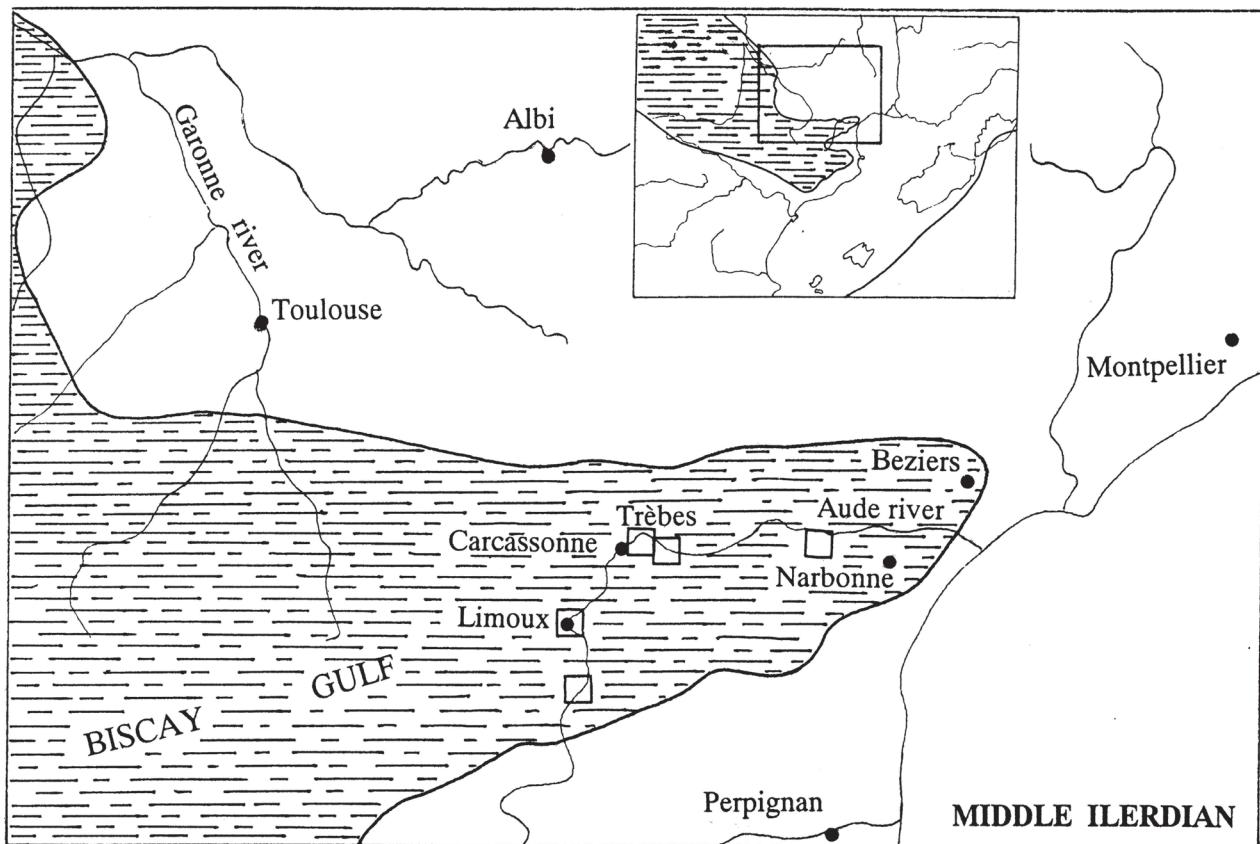


Fig. 9 - Extension of the Ilerian embayment in the western Languedoc (hatched area) and location of *Microcharon boulanouari* n. sp. sites (open squares). After Plaziat 1981 and Debrand-Passard & Courbouleix 1984a, b, modified.

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Shape variation of *Aegla schmitti* (Crustacea, Decapoda, Aeglidae) associated to superficial and subterranean stream reaches

Camile Sorbo FERNANDES ^(1,*), Maria Elina BICHUETTE ⁽¹⁾

⁽¹⁾ Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva (DEBE), Universidade Federal de São Carlos - UFSCar. Via Washington Luís, km 235, São Carlos, SP, Brazil, 13565-905

*Corresponding author: camilesorbofernandes@yahoo.com.br

ABSTRACT

The species *Aegla schmitti* is relatively abundant in epigean streams in the Upper Ribeira Basin, southeastern Brazil and has a few records in caves. In this study, we questioned if those individuals would be an accidental presence or could be populations already established in cave streams? In this case, would morphological variations be associated with superficial and subterranean stream reaches? In this exploratory survey we tried to answer these questions by assessing the shape variation of cephalothorax using landmark-based geometric morphometrics. Different shapes were found for both environments, evidencing a successful hypogean colonization. The area effect was discarded as being the unique factor responsible for the divergence, therefore, for the first time in the literature, here we report morphological modifications associated with subterranean colonization in troglobites. We also conceived and discussed some hypotheses about the colonization.

Key words: caves; morphometrics; subterranean colonization; troglobites

INTRODUCTION

The species *Aegla schmitti* Hobbs III, 1979 is widely distributed in the Upper Ribeira Valley karstic area, southeastern Brazil (Rocha and Bueno 2004) and has been recorded in epigean and rarely recorded in hypogean stream reaches. In this last case, it was reported in a non linear pattern of distribution in Temimina I and II, Santana, Calcário Branco and Água Suja caves, which are separated by insoluble rocks in the studied area (see Moracchioli 1994; Bond-Buckup and Buckup 1994 and Rocha and Bueno 2004 for occurrence and geological map available at Sallun and Sallun Filho 2009).

Although not yet classified as a facultative cavernicole (= troglobite *sensu* Barr 1968) depigmented specimens of *Aegla schmitti* were mentioned by Moracchioli (1994) in a subterranean environment. Laboratory experiments resulted in gradual depigmentation in individuals maintained in a regime of darkness and without the addition of carotenoids in their diet, in a period of about a year. The absence of light and low content of carotenoids (available only in vegetal debris) is similar to the conditions found in cave streams. Even though long term studies are lacking, it is believed that this species could be capable of establishing viable populations in subterranean as well as superficial stream reaches (Moracchioli *Op. cit.*).

When a species occurs in both environments (as *A. schmitti*) it is not always clear to what is its degree of dependence on the subterranean environment, as well as it is not clear the degree of connectivity that is achieved among those subpopulations. As a matter of fact, dif-

ferent degrees of specialization to subterranean realm can even be observed in subpopulations, from the same biological species, as a consequence of the connectivity between them and the adaptability to each specific habitat condition (Wilkens 1988). In this regard, authors as Trajano et al (2009), Trajano (2012) and Poulson (2010) have already applied the concept of source-sink metapopulation (*sensu* Harrisson 1991) to subterranean populations.

In his classic book "On Growth and Form", Thompson (1917) discussed how morphology is related to mechanical and physiological efficiency in live organisms thus resulting ultimately from natural selection. In subterranean environments, not only the selective regimen (or even the relaxation of the selection), but the stochastic factors, besides geomorphology and historic factors, also play a fundamental role. Indeed, the colonization process itself subjects the subterranean fauna to genetic drift and founder effect (see founder principle, Mayr 1942) by initially reducing the genetic variability, while geomorphology influences the population connectivity (see Barr 1967; Wilkens 1988; 2010).

In fact, character states associated with isolation in subterranean environment often show up convergently in the most diverse taxonomic groups (Wilkens 2010). Morphologically, several troglobites have a more slender and elongated body and appendages than the closely related epigean species (Barr 1968; Culver 1982; Christiansen 2005). Such elongation of appendages has been explained as being sensory compensation (*e.g.* Mejía-Ortiz et al 2006), while the adaptive value of body elongation and slenderness seems to be associated with specific

habits and/or habitats (Barr 1968; Christiansen 2005; Trontelj et al. 2012).

Herein, we discuss the morphology of *Aegla schmitti* with an ecological evolutionary approach, searching for morphological modifications that could be attributed to life in subterranean environments, associated or not to subterranean selective regimes.

The chosen method was Landmarks-based geometric morphometrics, which consists of shape analysis by means of anatomical reference points, after eliminating effects of non-shape variation (position, orientation and scale of specimens) (Adams 1999; Giri and Loy 2008).

This exploratory technique has been successfully applied in studies with subterranean organisms like Reis et al (2006), in epigean and subterranean populations of armoured catfishes *Ancistrus cryptopthalmus* (Reis, 1987) and subterranean *Rhamdiopsis krugi* Bockmann and Castro 2010 (B. Rantin, in prep.). Both studies associated population divergences to isolation patterns between fish populations occurring in different cave systems (*A. cryptopthalmus*) or different limestone lenses (*R. krugi*).

Considering this, our purpose is to discuss two issues related to cave colonization of *A. schmitti*, by means of an exploratory analysis of shape: would those individuals be accidental or are populations already established in cave streams? In this case, would shape variations be associated to superficial and subterranean stream reaches? We also propose and discuss some colonization hypotheses.

METHODS

A. schmitti specimens were collected between March 2009 and August 2010, in subterranean and epigean stream reaches located at Parque Estadual Turístico do Alto Ribeira (PETAR) karstic area, São Paulo State, southeastern Brazil (Fig. 1).

Despite several field surveys in the area, including all caves with records of occurrence, individuals were captured only in Santana Cave ($24^{\circ}31'51"S$ and $48^{\circ}42'06"W$), and Betari River ($24^{\circ}31'14"S$ and $48^{\circ}41'43"W$, in a stream reach immediately downstream from outflow of the resurgence of Santana Cave river).

The collected specimens were deposited in the scientific collection of Laboratório de Estudos Subterrâneos, Universidade Federal de São Carlos – UFSCar (Numbers 1038 and 1039). Loans of individuals were also obtained from Museu de Zoologia from Universidade de São Paulo (MZUSP) and Laboratório de Estudos de Eglídeos from Universidade de São Paulo (LEEUSP) scientific collections.

Only adult specimens were used for geometric morphometrics, in a total of 24 males and two females from Santana Cave; 84 males and 50 females from Betari River; two males and two females from Temimina II Cave and three females from Água Suja Cave. The last two

localities were not analyzed because of the small size of the sample. Although it seems to be a small sample to undertake the geometric morphometrics, it is more than the minimum adequate to the number of coordinates (see Monteiro and Reis 1999). Also, after several collections we could not catch more individuals which can indicate that these populations are threatened. Therefore, more samplings are not justified.

The specimens were oriented by the dorsal region and, then, photographed using a digital camera (SONY-H7). The images were converted to TPS format with TPSUtil version 1.44 (Rohlf 2009) and the homologous coordinates were obtained using TPSDig 2 version 2.12 (Rohlf 2008 a). A total of 11 landmarks were digitized on each specimen (Fig. 2) and pixels were transformed in millimeters by means of a scale inserted in the picture. Also, the repeatability of the landmarks was checked by means of Analysis of Variance (ANOVA) in Generalized Procrustes Analysis (GPA, Bookstein 1986) aligned coordinates of 30 images, marked three times.

Subsequently, each configuration was submitted to symmetrization, as described in Mardia et al (2000), to reduce the influence of variations related to asymmetry and avoid inflation of degrees of freedom. It was done creating a reflected configuration, by multiplying the x coordinate of the landmarks by -1 and then calculating a consensus configuration between original and reflected landmarks. This procedure was done in R version 2.11 (R Development Core Team 2009). All posterior analyses were undertaken with transformed coordinates.

We chose GPA (Bookstein 1986; Adams et al 2004) as the method of superimposition to remove non-shape variations, computed by TPSRelw version 1.46 (Rohlf 2008 b). In the same software we performed a Relative Warps Analysis. To have all landmarks in the same scale, the alpha selected was equal to zero, most suitable for exploratory investigations (Rohlf 1993).

For testing whether the chosen landmarks would be influenced by sexual dimorphism, a Non-parametric Multivariate Analysis of Variance (NPMANOVA), using Euclidean distance (Anderson 2001) was applied in the relative warps scores matrix (S' matrix) between males and females, only in the first nine principal components different to zero, to remove redundant information.

Since there was influence from sexual dimorphism, only males were tested for shape differences between Santana Cave and Betari River. Thus, a Non-parametric Multivariate Analysis of Variance (NPMANOVA), using Euclidean distance (Anderson 2001) was applied in the relative warps scores matrix (S' matrix) between Santana Cave and Betari River, in the first nine principal components different from zero.

All statistical analyses were computed using Past version 2.01 (Hammer et al 2001). Also, thin-plate splines analysis was done using Morpheus beta version (Slice 2002), using consensus configurations of males from Betari River and Santana Cave.

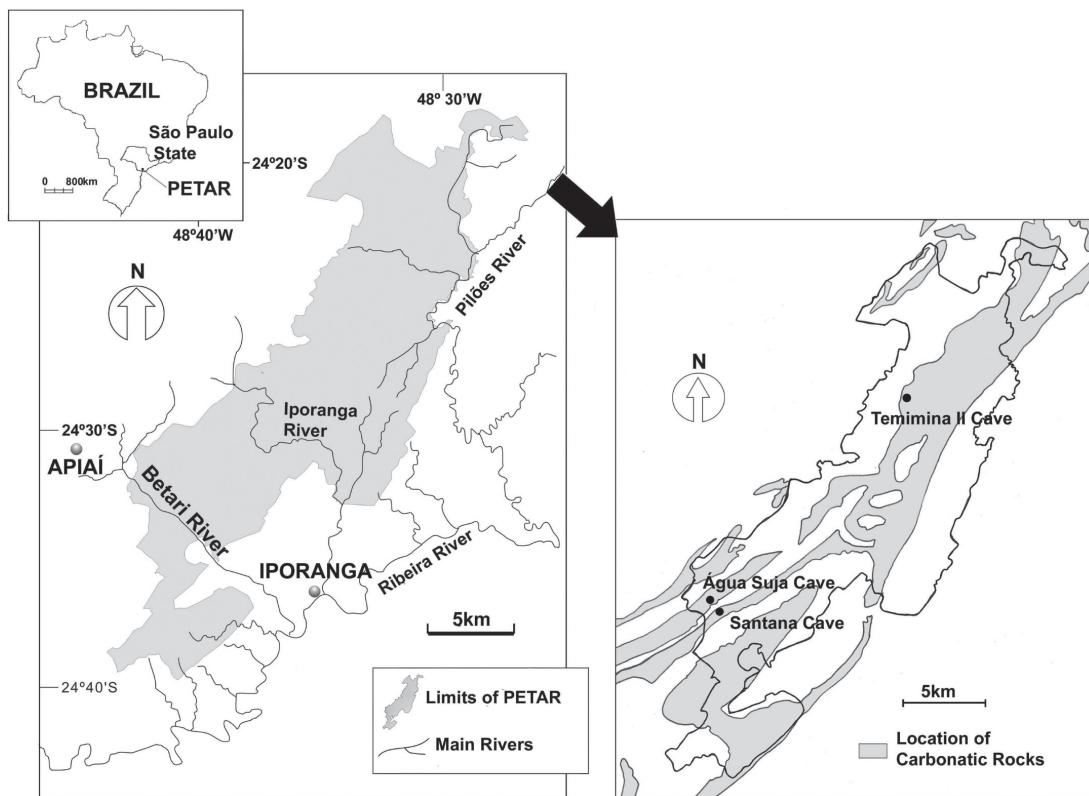


Fig. 1 - Limits of Parque Estadual Turístico do Alto Ribeira (PETAR) and location of limestone and caves in the study area. Modified from Karmann and Ferrari (2002).

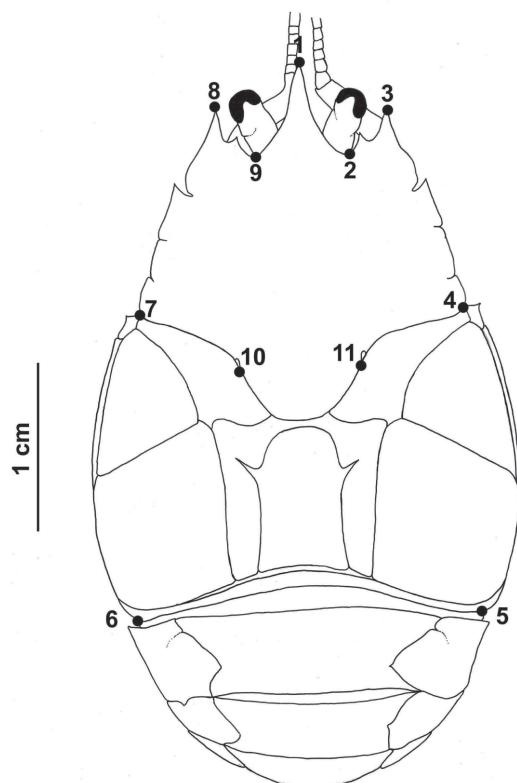


Fig. 2 - Dorsal view of *Aegla schmitti* showing the landmarks used in geometric morphometric analyses. 1 tip of the rostrum; 2 and 9 outer basis of eyestalk; 3 and 8 tip of anterolateral spine; 4 and 7 precervical width; 5 and 6 cephalothorax posterior region (marked where abdomen begins); 10 and 11 cervical groove apodeme.

RESULTS

The selected landmarks were repeatable and suitable for subsequent analyses ($F=2.738E-10$; $p=1$). Also, as they were influenced by sexual dimorphism, males and females were not grouped in later statistical analyses ($F=66.67$ e $p<0.0001$).

There was a continuous and statistically significant morphological divergence between individuals from Santana Cave and Betari river ($F=4.847$; $p=0.003$). Moreover, though individuals from Betari river have been obtained next to the Santana Cave resurgence, they were more similar to the river specimens deposited in scientific collections (MZUSP and LEEUSP) (Fig. 3). The first two relative warps calculated between males from cave and river, with $\alpha=0$ and uniform component included added up a total of 67.49% of the variance (first relative warp: 48.50%; second relative warp: 18.98%).

The landmarks responsible for the greatest variation on the first principal component were those related to eyestalk position and anterolateral spines (corresponding to numbers 2, 3, 8 and 9). Cave individuals had, as a rule, narrower cephalothorax and rostrum and a pre-cervical area more elongated than the epigean individuals, as illustrated by thin-plate splines diagrams (Fig. 4).

DISCUSSION

The epigean individuals sampled for this study, even being from a site next to the cave, showed greater similarities with the ones from the river, obtained from scientific collections (thus coming from different stream reaches and different time of collection). In fact, our results showed that not only the individuals from the river and from the caves were different, but also showed that a reduced gene flow between the extremes of distribution, as it was proposed by Vanzolini (1992) as being an Area Effect phenomenon, is not the unique factor influencing shape variation.

As demonstrated by Moracchioli (1994) in laboratory experiments, depigmentation in *A. schmitti* occurred in a period of about one year under dark conditions and low supplement of carotenoids in diet. As a consequence, the individuals of *A. schmitti* showing various degrees of depigmentation she found in subterranean stream reaches would be living for a relative period of time in this environment, and the depigmentation possibly occurred since in decapods it is associated with carotenoid availability on feeding (Maguire Jr. 1961; Wolfe and Cornwell 1964), which is often scarce in caves. Such facts, besides other records of this species in caves (Bond-Buckup and Buckup 1994; Rocha and Bueno 2004 and ours) showed that

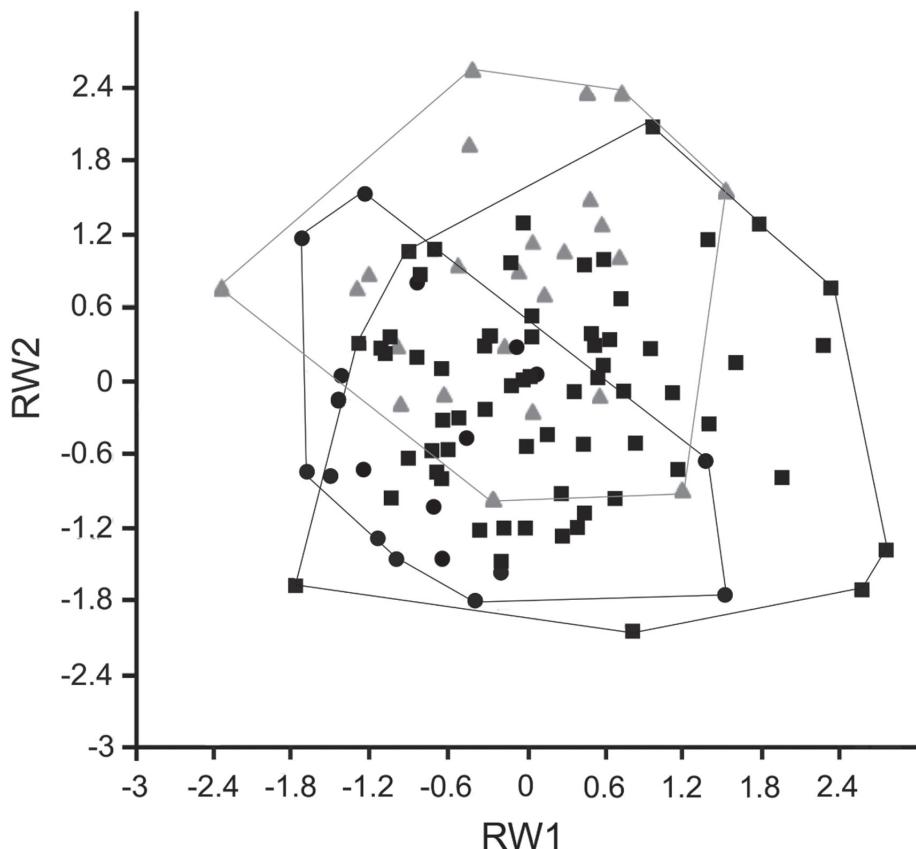


Fig. 3 - Relative Warps for values of $\alpha=0$ of *A. schmitti* males from Betari River sampled specimens (circles), Betari River from scientific collections (squares) and from Santana Cave (triangles). RW1 = Relative warp 1 e RW2 = Relative warp 2.

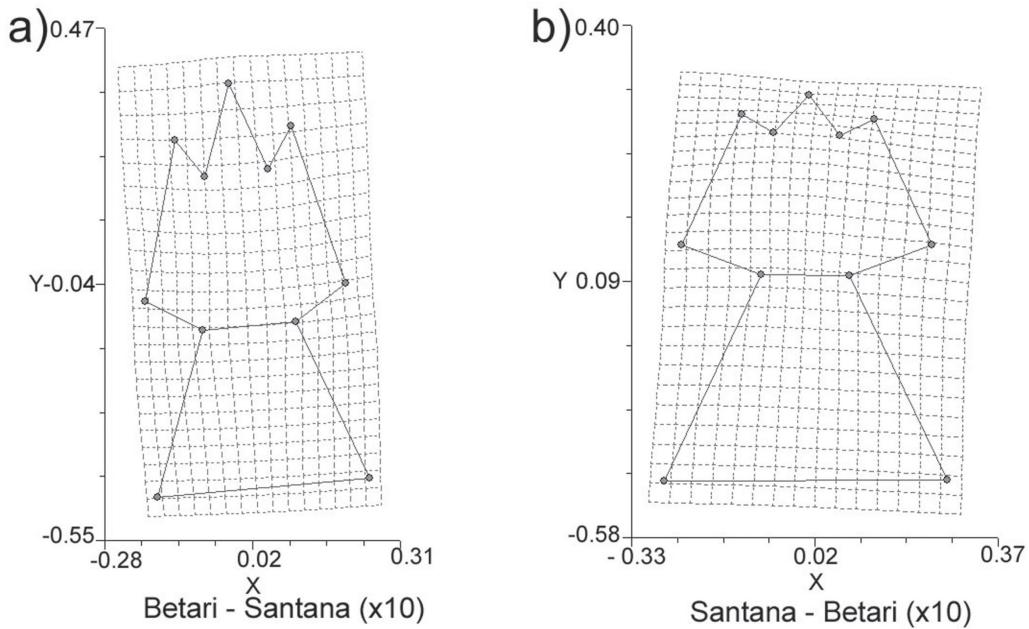


Fig. 4 -Thin plate splines diagrams showing deformations of a reference in a target configuration. (a) Betari River deformed in Santana Cave; (b) Santana Cave in Betari River. Results magnified 10 times for visualization of shape differences.

A. schmitti at least can establish and survive for a long period in this environment.

Nevertheless, as we said in the introduction, the records of *A. schmitti* in caves (Moracchioli 1994; Bond-Buckup and Buckup 1994; Rocha and Bueno 2004) show up in a non linear pattern, with the only exception of Temimina I and II caves, located very closely to each other in the same limestone outcrop. This pattern evidences the absence of a subterranean dispersion for this species (Barr 1967). So, although being capable of survival for a long period in subterranean stream reaches, the pattern demonstrates that the source population lies in epigean river.

Our results showed a continuous pattern of morphological divergence (Relative Warps Analysis, Fig. 3) which is consistent with a gene flow limited to occasional exchanges. Considering the distribution, the morphological divergence, and the fact that well established populations in past were not found in the present time (e.g. Temimina II and Água Suja Caves), we hypothesize that *A. schmitti* follows a source-sink metapopulation dynamics in the study area (*sensu* Harrisson 1991). Therefore, as the subterranean stream reaches are more restrictive to the establishment and reproduction of the species, the river population would be a source of new genes by means of migratory individuals, maintaining the peripheral population of Santana Cave as well as the other subterranean sink populations which were not found in the moment we sampled.

Sink populations correspond to groups of individuals stranded in habitats which are less than suitable. When the concept of source-sink populations is applied to the usual ecological-evolutionary classification of subterranean or-

ganisms, only the source populations are considered (Trajano 2012). According to this new definition, *A. schmitti* is classified as a troglobene (source populations in epigean river). Otherwise, using the traditional classification (populations established and reproducing in both environments), the status of troglophilic should be attributed.

Historical factors could also have influenced the observed morphological pattern. A set of geological and paleontological evidences (Ab'Saber 1977; Ledru 1993; Karmann 1994; Cruz et al 2005) shows that the study area was under Pleistocene climatic fluctuations as well as successive drainage alterations. Drier periods may have reduced streams levels, promoting successive scenarios of coalescence and isolation of Ribeira Valley waters (evidences from the troglobitic fish *Pimelodella kronei* (Ribeiro 1907), *sensu* Trajano and Britski 1992). Therefore, the aquatic environment fragmentation, associated with the isolation of troglophilic populations in subterranean environment, would enable the evolutionary processes to act, accumulating genetic differences during isolation and later introgressing with epigean population (Trajano 1995).

As *A. schmitti* is capable of establishing itself in subterranean environment, it must be investigated if and how climatic fluctuations (causing drainage alterations) would influence the genetic frequencies in these populations. Xu et al (2009), in a molecular study with *Aegla alacalufi* Jara and Lopez 1981 from Chile, reported divergences between populations supposedly under Pleistocene climatic fluctuations and those in refuge areas. In *Sclerobunus*, Banks 1893, a genus of Opiliones, it seems that the evolution of weakly troglomorphic forms (*i.e.*, with slightly reduced pigmentation, slightly elongated

appendages and no reduction in eye mound) is consistent with Pleistocene effects model (Derkarabetian et al 2010). According to Barnosky (2005) and Xu et al (2009), Quaternary climatic changes may have promoted a genetic change at population level. It is expected that anagenetic evolution in caves is accelerated in relation to that observed in epigean refuges, once subterranean ecosystems, depending on allochthonous food intake, may lose a great deal of diversity due to drastic food shortage (Trajano 1995).

Associated with partial reproductive isolation, a heritable higher capability to express specific responses to the environmental conditions can be expected to be favoured by natural selection (Stearns 1983; Romero and Green 2005). Experimental studies with tetracharacin fishes *Astyanax fasciatus* (Cuvier 1819) (Romero 2002) and epigean crayfishes *Cambarus tenebrosus* Hay 1902 (Cooper et al 2001) demonstrated that some troglomorphic forms differ from epigean ancestors not only genetically but, at least partially, also in environmentally induced features (Romero 2002; Romero and Green 2005).

The Aeglidae exhibits high intraspecific variation, which suggests they are capable of responding to different environmental conditions (see Giri and Collins 2004; Giri and Loy 2008). It is expected that in many caves, subjected to pronounced seasonality related to rainy cycles (Bichuette and Trajano 2003), plastic genotypes are selected, as environmentally induced phenotypes have higher probability to adapt to changes in environmental conditions than genetically fixed ones (Whiteman 1994; Stearns 1983; Romero and Green 2005).

Furthermore, genetic adaptation to specific habitats would favor those individuals with different morphological and physiological features between two environments, the ecotypes (Romero and Green 2005). Giri and Loy (2008) compared *Aegla neuquensis* Schmitt 1942 from lakes and rivers and reported the existence of ecotypes associated to both. Also, the cephalothorax shapes of river individuals were more variable than in the lake ones, which are features possibly associated to environmental conditions, predators and population connectivity.

The slenderness of cephalothorax and rostrum and, also, elongation of the pre-cervical area observed in the cave individuals from our study is similar to the morphological modifications already found in troglobitic (= obligate) cave fauna when compared to closely related epigean species. This trend towards body elongation and slenderness was observed in different areas and lineages of troglobites, evidencing that these traits are homoplasic cave-dependent features under adaptive control. Some authors associated these traits with habitat conditions and life habits, as an adaptation to squeezing through narrow crevices (Barr 1968; Hobbs 2000; Christiansen 2005; Trontelj et al 2012). This hypothesis is not applicable to our case, as *A. schmitti* occurs in a base-level subterranean stream reach thus not corroborating adaptation to narrow spaces. Nevertheless, the above mentioned trend

is somehow convergently similar to traits found in cave obligate fauna and, therefore, it seems to be associated to subterranean colonization whether resulting from subterranean selective regime, ecotypes selection or phenotypic plasticity.

Small populations, mainly inside caves, can lose alleles by genetic drift, if gene flow with surrounding populations does not occur compensating this loss (Barr 1967). There is an increased preoccupation about *Aegla* in the study area as their natural habitats are being impacted (Moraes and Molander 2004; Cotta et al 2006) and a drastic population reduction in all troglobites was already observed (K. Maia, in press.). Our unsuccessful collections, not observed in previous studies (Moracchioli 1994; Bond-Buckup and Buckup 1994; Rocha and Bueno 2004) showed that *A. schmitti* could also be threatened.

In summary, our results indicated when mainly associated with epigean stream reaches, *A. schmitti* is not only capable of colonizing and establishing itself in the subterranean environment, but also that the morphological differences are associated with subterranean colonization. Further studies are needed to elucidate the ecological-evolutionary processes lying behind the apparent morphological convergence with troglobites. Also, due the partial isolation, effective populational size studies would be useful to evaluate the viability of the Santana Cave population and propose future conservation policies.

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Molecular divergence and evolutionary relationships among Aemodogryllinae from Southern China, Laos and Thailand (Orthoptera, Rhaphidophoridae)

Valerio KETMAIER^(1,2,*), Claudio DI RUSSO⁽¹⁾, Mauro RAMPINI⁽¹⁾,
Nadine BERNHARDT⁽²⁾, Ralph TIEDEMANN⁽²⁾, Marina COBOLLI⁽¹⁾

⁽¹⁾ Dipartimento di Biologia e Biotecnologie “Charles Darwin”, Università di Roma “La Sapienza”, V.le dell’Università 32, I-00185 Roma, Italy

⁽²⁾ Unit of Evolutionary Biology/Systematic Zoology, Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24-25, Haus 25, D-14476, Potsdam, Germany; email: ketmaier@uni-potsdam.de

*Corresponding author: ketmaier@uni-potsdam.de

ABSTRACT

In this study we screened for sequence polymorphisms at one mitochondrial (Cytochrome Oxidase subunit I) and one nuclear (Internal Transcribed Spacer 1) gene 33 populations of the cave cricket genera *Diestrammena*, *Paradiestrammena*, *Eutachycines* and *Paratachycines* from Southern China (three Provinces: Jiangxi, Guangdong and Guizhou), Laos and Thailand. Twenty-five of these populations were assigned to the genus *Diestrammena*, subgenus *Gymnaeta*, while the remaining eight belonged to the genera *Paradiestrammena* (3), *Eutachycines* (3) and *Paratachycines* (2). The degree of troglomorphosis varies among them; some populations are blind and depigmented, some have fully developed eyes, while some others show intermediate characteristics. Phylogenetic searches carried out on the two gene partitions separately revealed multiple cases of incongruence but only three of them were statistically significant and were hence removed from the subsequent analyses based on the combined data set. Our data do not support *Diestrammena* as monophyletic while representatives of *Paradiestrammena*, *Eutachycines* and *Paratachycines* were clustered together; the validity of some nominal species was confirmed molecularly but we also revealed a large number of deeply divergent lineages. Populations with the same degree of troglomorphosis do not cluster together. We identified five major clades; divergence among them (and in a few circumstances also within them) is always higher than the DNA barcode threshold for intraspecific comparisons in insects. In two circumstances, the same clades (III and V) are co-distributed in geographically distinct areas (Provinces). This geographical distribution might be explained by envisioning an evolutionary scenario based on zones of secondary admixture following epigean dispersal among lineages that diverged in allopatry.

Key words: Aemodogryllinae, cave cricket, mitochondrial DNA, nuclear DNA, secondary contact.

INTRODUCTION

Southern China, with about 2.6 million km², hosts one of the largest karstic areas of the world and a vast – yet largely unexplored – biodiversity. Starting from the early ‘90s, a number of biospeleological expeditions have been organized in the area (Latella and Zorzin 2008). These were joint efforts carried out by Chinese, French, Italian and Slovenian teams. Even though these expeditions could visit only a small fraction of the caves in the area, the amount of speleological and biological information they gathered is massive and still in the course of being processed. Among others, cave crickets belonging to the subfamily Aemodogryllinae (Rhaphidophoridae) were retrieved in quite a large number (Rampini et al 2008).

The family is distributed worldwide and includes epigean representatives (mostly living in the litter of tropical forests) and many subterranean forms confined mostly to temperate areas where conditions for epigean life are favorable only seasonally. In the Mediterranean area two genera are present (*Dolichopoda* and *Troglophilus*); both have been object of extensive evolutionary research (Al-

legrucci et al 2011; Ketmaier et al 2010 and references therein). Allegrucci et al (2010) produced a biogeographic reconstruction of the evolutionary relationships among Rhaphidophorids from the Southern End of the World.

Here, we took advantage of the cave crickets collected in Southern China in three Provinces (Jiangxi, Guangdong and Guizhou) to assess their degree of genetic divergence and to produce a preliminary phylogeographic hypothesis on the diversification of the group in the area. We additionally included in our study cave crickets collected in Laos and Thailand. A detailed morphological assessment of these organisms (Rampini et al 2008) revealed that most of the samples at our disposal belong to the genus *Diestrammena*. In particular, Southern Chinese populations could be readily identified as belonging to the subgenus *Gymnaeta* because of the genital morphology and because they lack spines on the hind femurs; samples from Laos and Thailand were attributed to the genera *Eutachycines*, *Paradiestrammena* and *Paratachycines* (Storozenko 1991; Gorochov 1994; Gorochov 2002). Some populations could be identified at the species level (see Table 1); other populations were taxa new to science while

some others are still awaiting a formal specific attribution (Rampini et al 2008). All these taxa show a varying degree of adaptation to life in caves (troglomorphosis); some are blind and with reduced body pigments, some have reduced eyes while others have fully developed eyes and a pigmented body. We analyzed sequence polymorphisms at partial sequences of two genes, the mitochondrial (mtDNA) Cytochrome Oxidase subunit I (COI) gene and the nuclear (nDNA) Internal Transcribed Spacer 1 (ITS1). These markers have already proved useful in unveiling evolutionary relationships at the taxonomic level tackled in the present study in the cave cricket genus *Troglophilus* (Ketmaier et al 2010). We specifically ask whether the different genera could be validated molecularly, whether populations with the same degree of troglomorphosis cluster together and whether populations from the same geographic area are also genetically close.

MATERIALS AND METHODS

Sampling

Study samples were collected from twenty-nine caves from three South Chinese Provinces (Guizhou, Guangdong

and Jiangxi) and from Thailand and Laos. When feasible, sampled individuals were assigned to nominal species following Gorochov et al (2006) and Rampini et al (2008). In two cases we found the occurrence of different species or genera in the same cave (i. e. *Diestrammena chenui* and *Diestrammena ferecaeca* in An Ja Da Dong from Guizhou, and *Eutachycines cassani* and *Paradiestrammena vernalis* in the Maria Cassan cave from Laos). Furthermore, in two caves from Guangdong (Pi Pa Dong and Xian Yen Dong) we found individuals with different degrees of troglomorphosis; we kept these individuals distinct (i.e. not lumped in a single population) in the statistical treatment of data. At total of 93 individuals were collected and analyzed genetically. Details on the sampled localities and populations can be found in Table 1 and Fig. 1. Samples were collected by and preserved in 70% ethanol; part of the caudal femur muscle was used for the genetic work. Analyzed individuals are deposited in the M. Rampini collection (Department of Biology and Biotechnology "C. Darwin", University of Rome "Sapienza", Rome, Italy).

Genetics

Total genomic DNA was extracted following Ketmaier et al (2008). We amplified by Polymerase Chain

Table 1 - Populations and species (whenever a specific attribution was feasible) of Aemodogryllinae analyzed molecularly. For each taxon we indicate the Southern Chinese province and cave of origin and the population code used in the study. The last column shows the sample size (N). Underlined caves are those where two genetically divergent lineages co-exist.

Taxon	Province*	Cave	Code**	N
<i>D. (Gymnaeta) sp.</i>	Jiangxi	Hengli Dong	C23c	2
<i>D. (Gymnaeta) sp.</i>	Guangdong	Tan Shui Long Yan Dong	C10b	6
<i>D. (Gymnaeta) sp.</i>	Guangdong	Wei Yan Dong	C12b	3
<i>D. (Gymnaeta) sp.</i>	Guangdong	Jing Long Dong	C21b	3
<i>D. (Gymnaeta) sp.</i>	Guangdong	Shi Shi Qiao Dong	C16b	15
<i>D. (Gymnaeta) sp.</i>	Guangdong	Pi Pa Dong	C17b	5
<i>D. (Gymnaeta) sp.</i>	Guangdong	Ting Xia Wo Dong	C14b	9
<i>D. (Gymnaeta) sp.</i>	Guangdong	Xian Yen Dong	C19b	4
<i>D. (Gymnaeta) sp.</i>	Guangdong	Chao Yan Dong	C9b	1
<i>D. (Gymnaeta) sp.</i>	Guangdong	Da Qiao Dong	C22a	3
<i>D. (Gymnaeta) sp.</i>	Guangdong	Pi Pa Dong	C18a	2
<i>D. (Gymnaeta) sp.</i>	Guangdong	Xian Yen Dong	C20a	3
<i>D. (Gymnaeta) sp.</i>	Guizhou	Yun Dong	C1b	1
<i>D. (Gymnaeta) aspes</i>	Guizhou	Di E Dong	B1.0b	2
<i>D. (Gymnaeta) aspes</i>	Guizhou	Wangma Dong	B1.1b	3
<i>D. (Gymnaeta) chenui</i>	Guizhou	An Ja Da Dong	B6.1b	1
<i>D. (Gymnaeta) latellai</i>	Guizhou	Shachang Dong	C8b	1
<i>D. (Gymnaeta) ferecaeca</i>	Guizhou	Xiao Dong	B4.8.2a	1
<i>D. (Gymnaeta) ferecaeca</i>	Guizhou	Tiao Shuz Dong	B4.17a	9
<i>D. (Gymnaeta) sp.</i>	Guizhou	Tu Shi Dong	C4a	1
<i>D. (Gymnaeta) sp.</i>	Guizhou	Xiao Dong	C11a	1
<i>D. (Gymnaeta) sp.</i>	Guizhou	Yin Jia Dong	C5a	1
<i>D. (Gymnaeta) ferecaeca</i>	Guizhou	Tang Bian Da Dong	B1.2a	2
<i>D. (Gymnaeta) ferecaeca</i>	Guizhou	An Ja Da Dong	B5.0.1c	5
<i>D. (Gymnaeta) sp.</i>	Laos	Tham Kang	S4a	1
<i>Eutachycines sp.</i>	Laos	Mahaxay Cave	S12a	1
<i>Eutachycines cassani</i>	Laos	Maria Cassan Cave	S15c	1
<i>Eutachycines cassani</i>	Laos	Tham Nja Kay Khia	S6c	1
<i>Paradiestrammena vernalis</i>	Laos	Ban Tham	S9b	1

Taxon	Province*	Cave	Code**	N
<i>Paradiestrammena vernalis</i>	Laos	Marian Cassan Cave	S11b	1
<i>Paradiestrammena vernalis</i>	Laos	Tham Deua	S13b	1
<i>Paratachycines thailandensis</i>	Thailand	Tam Lod	S1b	1
<i>Paratachycines thailandensis</i>	Thailand	Christmas Cave	S2a	1

* Southern Chinese provinces only; it does not apply to populations from Laos and Thailand.

** a = small eyes; b = normal eyes; c = eyes absent.

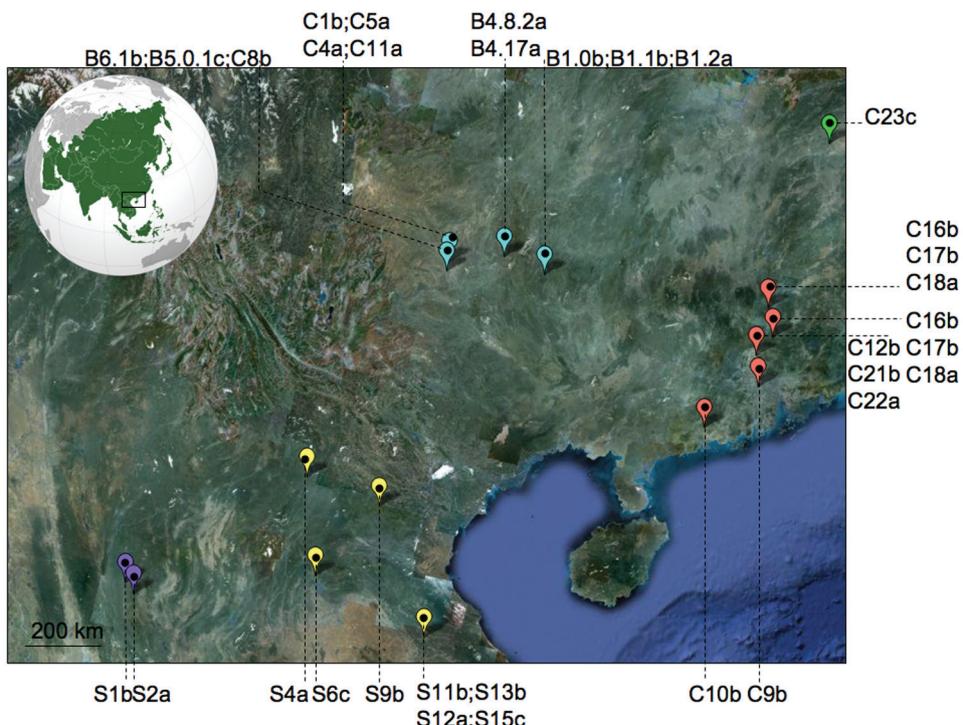


Fig. 1 - Geographic distribution of the localities sampled for the study. Population codes are as in Table 1. Green is Jiangxi, red is Guangdong, light blue is Guizhou (Southern Chinese Provinces) while Laos and Thailand are indicated in yellow and violet, respectively.

Reaction (PCR) a fragment of the mitochondrial DNA (mtDNA) region encoding for the Cytochrome Oxidase subunit I gene (COI) and a fragment of the nuclear DNA (nDNA) Internal Transcribed Spacer 1 (ITS1). For COI we used the same set of primers as in Ketmaier et al (2008) while for the ITS1 gene we adopted the primer pair Cas5p8sB1d/ Cas18sF1 developed by Ji et al (2003). Double-stranded PCR amplifications were performed in a 50 μ L reaction volume containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl₂, each dNTP at 2.5 mM, each primer at 1 mM, genomic DNA (10-100 ng) and 5 units of AmpliTaq (Applied Biosystems) following the conditions detailed in Ketmaier et al (2010). For some samples with a low quality of the total DNA, the COI and ITS1 fragments were obtained using a nested PCR

approach. For the COI gene we used the primers UEA1 and UEA10 (Lunt et al 1996) for the first PCR round and the same primer pairs as above as internal primers for the nested PCR. The primer pair Cas5p8sB1d/ Cas18sF1 were used for the initial PCR for the ITS1 gene while we designed ex-novo an internal primer pair (ITS-f: 5'- GCGGTCGCTGA TGTCA-3'; ITS-r: 5'- CT-GCAGTTCACATGTCGA-3') for the nested PCR. In either case, PCR reactions were prepared as detailed above but with a different thermal profile (initial denaturation at 98° for 30 sec followed by 30 cycles each consisting of a denaturation step at 98° for 10 sec, annealing at 50° for 20 sec, extension at 72° for 30 sec followed by a final elongation at 72° for 5 min). PCR products were purified using the NucleoSpin® Extract II (Macherey-Nagel).

Purified PCR products were directly sequenced on an automated ABI 3100 sequencer (Applied Biosystems) following the manufacturers' protocols. To promote accuracy, each PCR product was sequenced in both directions.

Sequences were edited with Sequencher 4.6 (Gene Code Corporation, Ann Arbor, MI). COI sequences were aligned by eye following the guide provided by the reading frame; for the ITS1 gene we used ClustalX (Thompson et al 1997) with default parameters. Minor adjustments to the alignment were done by eye.

Haplotypes (both genes combined) were identified using the program TCS (Clement et al 2000). We used PAUP* 4.0b10 to calculate base frequencies and to test for base frequency homogeneity across taxa (χ^2 test); PAUP was also used to calculate COI-based Kimura 2-parameter (K2P) distance values among and within the main clades identified phylogenetically. The occurrence of saturation of sequences in the data set was visually evaluated by plotting the absolute number of transitions (Ti) and transversions (Tv) against the uncorrected-*p* distance values. This analysis was performed on four different data partitions: the two genes separately, the two genes combined and, for the COI gene, on 3rd codon positions only. The two data sets were analyzed phylogenetically with the Southern Italian Rhaphidophorid species *Troglophilus andreinii* as outgroup (Ketmaier et al 2002) by maximum parsimony (MP; heuristic searches, ACCTRAN character-state optimization, 100 random step-wise additions, TBR branch-swapping algorithm) (Farris 1970), maximum likelihood (ML; heuristic searches, 100 random stepwise additions, TBR branch swapping algorithm) (Felsenstein 1981), Neighbor-Joining (NJ) (Saitou and Nei 1987) and Bayesian methods (Rannala and Yang 1996; Mau and Newton 1997; Larget and Simon 1999; Mau et al 1999; Huelsenbeck 2000). MP, ML and NJ analyses were performed using PAUP* 4.0b10 (Swofford 2002); Bayesian analysis was carried out using MRBAYES 3.1 (Ronquist and Huelsenbeck 2003). MP searches were run giving equal weight to all substitutions. We ran the ML analyses on PAUP* 4.0B10 after having determined the best model of DNA substitutions that fit our data using MODELTEST (Posada and Crandall 1998). MODELTEST selected the GTR + I + G model as the best fitting for both the COI and ITS1 data partitions (shape parameters $\alpha = 0.454$ and 1.993 for COI and ITS1, respectively). We used this model with the partition-specific settings to run all our ML analyses. NJ analyses were carried out on ML distances calculated with the same settings used for the ML analyses. For the Bayesian approach, we employed the same model of sequence evolution as in the ML searches; for COI we allowed site-specific rate variation partitioned by codon positions. MRBAYES was run for 2 million generations with a sampling frequency of 100 generations. We ran one cold and three heated Markov chains. From the 20000 trees found, we

discarded the first 10% ("burn-in") in order to include only trees for which convergence of the Markov chain had been reached.

We followed the approach detailed in van der Niet et al (2008) to identify incongruence between the two data sets. We initially produced strict consensus topologies from separate parsimony analyses based on either data set and inspected them visually. A haplotype (or clade) was identified as a source of incongruence if its removal solved the incongruence. At each step, we removed from the alignment a single identified source of incongruence and we produced a new, pruned, alignment. This procedure was reiterated until no incongruence was left. We then started adding these incongruent haplotypes or clades individually to the pruned combined alignment. Congruence between COI and ITS1 partitions in the alignment was tested with the Length Difference (ILD) test (Farris et al. 1994) in PAUP* 4.0B10. We ran the ILD test after having removed the outgroups and non-informative characters, with 1000 randomizations and 50 stepwise random addition sequence replicates (RASR). If the ILD test showed that adding a given haplotype (or clade) was not the source of a significant incongruence ($P_{ILD} < 0.05$) we kept it in the alignment, otherwise we removed it again. We then assessed whether conflicting positions of incongruent sequences were robust in both data sets separately by running Bayesian searches with the parameters and settings described above. The placements of incongruence were assumed to be robust if supported by posterior probability (PP) values of 75% or greater in both Bayesian analyses. We then plotted the PP values supporting conflicting placements (both genes separately) against the *P*-value of the ILD test. In doing so we were hence able to identify, for each gene, the critical bootstrap value at which the ILD test returns a significant result and, ultimately, to distinguish non-complex from complex cases of incongruence. The complex cases of incongruence were removed from the final alignment and phylogenetic searches run with the same methods as detailed for the two genes separately. The ML and Bayesian searches were based on the re-estimated GTR + I + G model of sequence (in MODELTEST) with a shape parameter of $\alpha = 1.545$. The competing placements of complex cases of incongruence were added to the obtained phylogenetic hypothesis by hand.

RESULTS

Sequence variation

For each of the 93 individuals included in the study we sequenced 1246 base pair (bp) of DNA; of these 543 bp belonged to the mtDNA COI gene and 703 bp to the nDNA ITS1 gene. Electropherograms resulting from direct sequencing of the ITS1 gene showed no evidence of

ambiguous base callings rendering cloning unnecessary. Table 2 shows the base frequencies of the different data partitions and the result of χ^2 tests for base frequency homogeneity across taxa. The four bases have very similar frequencies for the ITS1 gene, while COI sequences are anti-G biased and A+T rich especially in third codon positions, as expected for a protein coding mitochondrial gene. No data partition violated the homogeneity of base frequencies across taxa ($0.512 < P_{\chi^2} < 1.000$).

Fig. 2 shows the saturation plots for the two genes separately and combined and for COI 3rd codon positions. Transversions tend to increase linearly with the increase of genetic divergence whereas a slight saturation of trans-

sitions is evident at the higher levels of genetic divergence especially for COI 3rd codon positions.

Sequences of the two genes combined defined a total of 37 unique haplotypes for the 93 individuals included in the study; the absolute frequency of these haplotypes in the different populations is shown in Table 3. Very few haplotypes are shared among different populations while six populations present more than one haplotype. Haplotype 5 is noteworthy in that three individuals from two provinces (B4.8.2a from Guizhou; C12b and C21B from Guangdong) with different degrees of troglomorphosis (B4.8.2a has small eyes while C12b and C21B have both fully developed eyes) share it.

Table 2 - Base frequencies and results of the χ^2 tests for base frequency homogeneity across taxa in the two genes analyzed in the study. Values are shown for COI and ITS1 separately and combined; for COI analyses were conducted on each codon position (1st, 2nd and 3rd) and on all codon positions (all).

Partition	Base frequencies (%)				χ^2 values	P_{χ^2}
	A	C	G	T		
ITS1	25.1	24.1	25.5	25.3	185.8	0.999
COI- 1 st	28.7	17.2	26.4	27.7	50.1	1.000
COI-2 nd	20.3	22.9	17.0	39.9	19.3	1.000
COI- 3 rd	46.9	12.4	5.4	35.4	283.6	0.512
COI- all	32.0	17.5	16.2	34.3	144.4	1.000
ITS1+COI	28.6	20.8	20.8	29.8	203.3	0.999

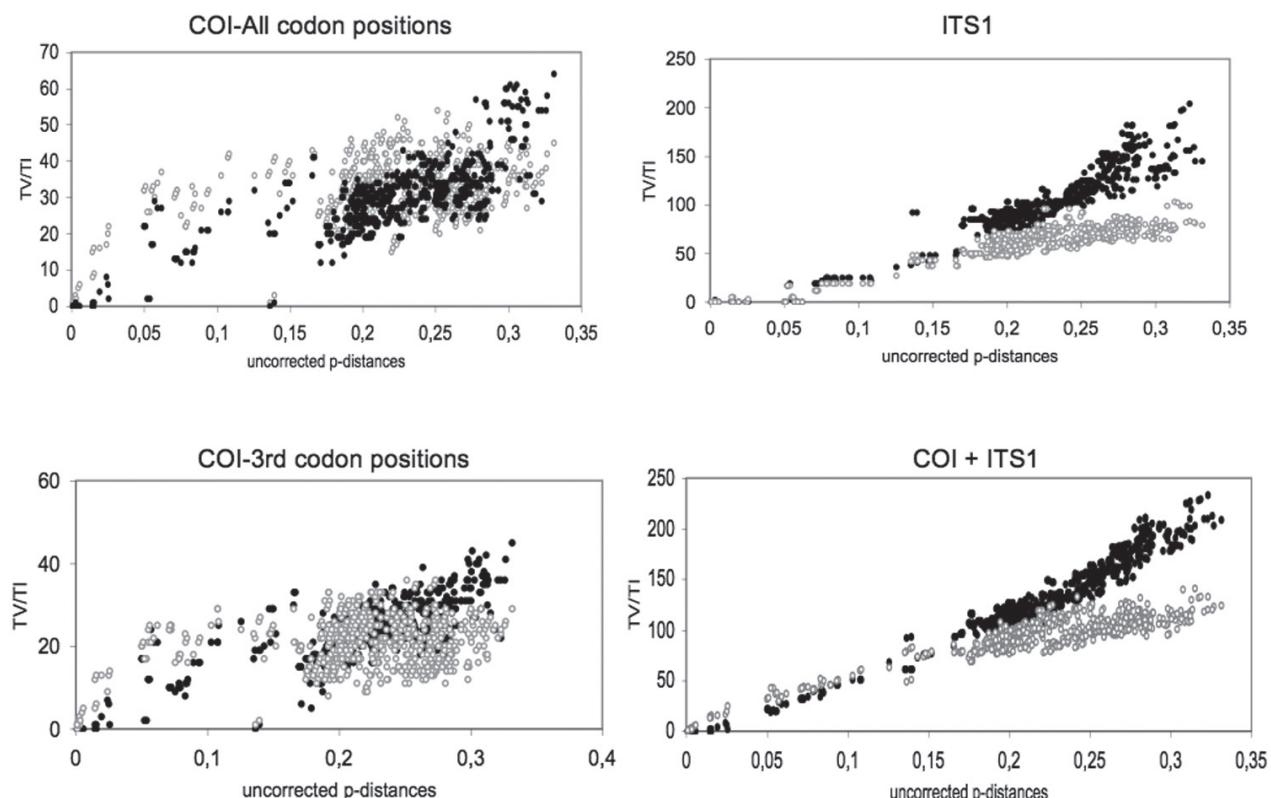


Fig. 2 - Plots of the uncorrected p values against the observed number of transitions (white dots) and transversions (black dots) on all pairs of sequences on each gene separately and both genes combined. For the COI gene we analyzed all codon positions together and 3rd codon positions only.

Table 3 - Absolute haplotype frequencies in the populations and species included in the study. Population codes are as in Table 1.

Sources of incongruence and phylogenetic relationships

Plotting of the P -value returned by the ILD test against the critical PP value (75%) revealed three complex sources of incongruence (Fig. 3), which corresponded to the populations B1.2a, B4.17a and C1b. All the other non-congruent placements ($P_{\text{ILD}} < 0.05$) were not supported by the corresponding PP value (PP < 75%; i.e. non-complex incongruent taxa). It should be noted here that since a PP value of 75% is considered a weak support to relationships in a Bayesian framework (Alfaro et al. 2003), our approach is quite conservative in identifying the main sources of incongruence between the two markers.

Fig. 4 shows the Bayesian tree based on the combined data set pruned of the three complex incongruent populations and summarizes the result of the MP and NJ searches. The Bayesian, MP and NJ topologies were largely similar to one another; incongruent taxa were placed in the position that they held in the topologies resulting from separate analyses of COI and ITS dataset (not shown). Representatives of the genus *Diestrammena* (subgenus *Gymnaeta*) do not cluster in a monophyletic clade since *Eutachycines*, *Paratachycines* and *Paradiestrammena* are deeply embedded within it. Similarly, haplotypes are not grouped in the phylogenetic tree according to the degree of eye development. Two (B5.0.1.c and C23c) of four blind taxa are rather basal in the tree while S6c and S15c are embedded in clade clade IV (see below); many supported clades repeatedly cluster together populations with fully developed and reduced eyes. Five major clades (labeled I-V in Fig. 4) could be identified phylogenetically. Clades I and II are restricted to the Guangdong Province only. Conversely, clades III and V include the Guangdong and Guizhou Provinces,

clade IV is spread across locations from Thailand and Laos and includes *Eutachycines*, *Paratachycines* and *Paradiestrammena*. Finally, clade V covers two provinces but at the within-clade level populations do not necessarily group according to their geographic proximity. Average COI-based K2P divergence among clades ranges between 7.9% (SD = 2%; clade I vs. clade II) and 13.7% (SD = 1.9%; clade IV vs. clade V); at the within-clade level K2P is the lowest within clade II (3%; SD = 1%) and the highest within clade IV (12.6%; SD = 4.3%).

DISCUSSION

We detected three complex sources of incongruence between the COI and the ITS data sets; while this figure is relatively little yet discrepancies between different gene trees should be evaluated carefully when attempting to produce a species tree by combining different genes. Gene and species trees tend to be in good agreement with each other when population sizes are relatively small relative to the branch lengths of a given phylogeny. Our tree of Fig. 4 contains many clades with relatively long branches; no information exists on the demography of these cave crickets but populations of subterranean organisms are seldom exceedingly large and this could explain why only three lineages show irreconcilable placements in the COI and ITS trees. These could be due to the fact that the common ancestry of gene copies extends deeper in time than the splitting event(s). Another explanation could invoke saturation of substitutions especially in the COI partition

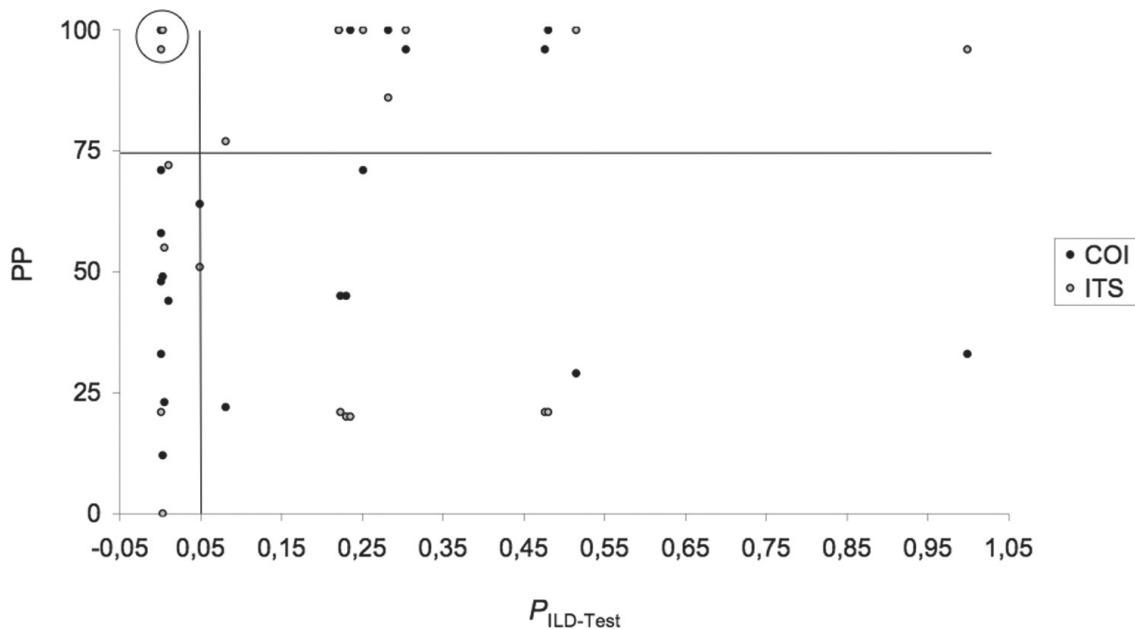


Fig. 3 - Relationship between the critical Posterior Probability (PP) value (75%) and the P -values returned from the ILD-test in the two gene partitions. Complex cases of incongruence are those above the PP threshold and with a $P_{\text{ILD_test}} < 0.05$ (circled dots). The horizontal line is at 75% PP, the vertical line at $P_{\text{ILD_test}} = 0.05$.

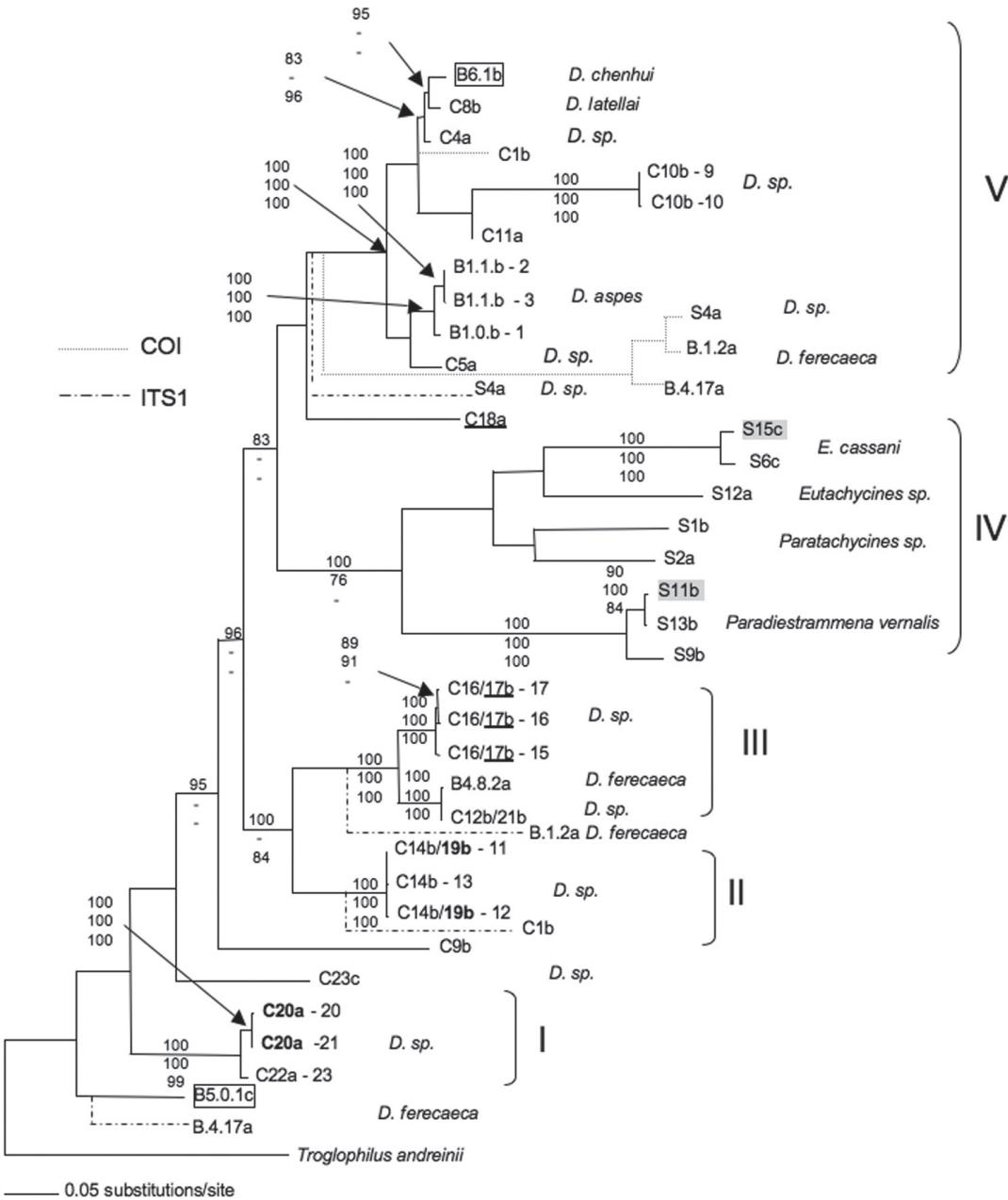


Fig. 4 - Evolutionary relationships among populations and species of Aemodogryllinae included in the study. The tree is a Bayesian phylogram; values at nodes are PP support for the Bayesian search (first value) and bootstrap supports for MP and NJ (second and third value; see text for details on the phylogenetic analyses). Only support values > 75% are shown. Underlined haplotypes were found in the Pi Pa Dong cave; bold haplotypes co-occur in the Xian Yen Dong cave; boxed haplotypes are from the An Ja Da Dong cave while haplotypes shaded in gray are from the Maria Cassan cave. Dotted (COI) and dashed (ITS1) branches represent cases of incongruence that could not be reconciled and are positioned in the tree according to the placements yielded by the phylogenetic searches conducted on the two genes separately. Five major clades are identified (see text for details).

that caused an increase in the amount of homoplasy. Finally, and perhaps more realistically, an incomplete taxon sampling should be considered as a likely reason. The cave cricket diversity in the area considered in this study is poorly known and new taxa are constantly being described (Rampini et al 2008; Zhang and Liu 2009). It is hence very likely that our taxon sampling

is far from being complete and this could have affected the accuracy of our phylogenetic reconstruction.

Molecular systematics and phylogeography

Keeping in mind the limitations intrinsic to our study in terms of representativeness of taxa, a few considerations can nonetheless be done. The genus *Diestrammena*

(subgenus *Gymnaeta*) does not form a monophyletic cluster. The other genera included in the study (*Eutachycines*, *Paratachycines* and *Paradiestrammena*) are firmly clustered in clade IV, which is deeply embedded within the subgenus *Gymnaeta*. It shouldn't be overlooked that this is the first molecular study considering cave crickets from Laos and Thailand. COI-based K2P divergence within clade IV is 12.6% (SD = 4.1%) thus higher than the 7.64% K2P value below which Virgilio et al. (2010) could assign intraspecific comparisons in 95% of the ca. 16000 DNA barcodes they analyzed in insects. Within the subgenus *Gymnaeta* at least four clades (I-III and V in Fig. 4) could be identified molecularly; the only comparison close to the 7.64% K2P threshold is clade I vs. II (7.9% SD = 0.2%); the remaining comparisons always gave values higher than 8.3%. At the within clade level, clade V exceeds the 7.64% threshold (9.7% SD = 4.3%). Based on our data and on the above pieces of evidence, we conclude that the morphological characters identifying the subgenus *Gymnaeta* as such (Storozhenko 1991; Gorochov 1994) are questionable. More noticeably, the subgenus *Gymnaeta* as currently defined hosts a number of deeply divergent lineages whose systematics awaits proper consideration. At the species level, genetic data cluster all *D. (G.) aspes* haplotypes in a single clade; this is not the case with *D. (G.) ferecaeca*. This nominal taxon is distributed scatteringly in the tree of Fig. 4 and is one source of incongruence. According to Gorochov et al. (2006) *D. ferecaeca* exhibits a great deal of morphological variability such that different subspecies have been identified. We are inclined to suspect that such a

morphological variability might conceal lineages more divergent than expected at the subspecific level. *D. (G.) chenhui* and *D. (G.) latellai* are genetically close; both species are from medium to large bodied and have well-developed eyes (Rampini et al 2008). In spite of these morphological similarities, it seems unlikely for the two species to be sister taxa. As a matter of fact *D. (G.) solida* and *D. (G.) zorzini* are morphologically and geographically closer respectively to *D. (G.) chenhui* and *D. (G.) latellai* than the latter are to one another. To fully resolve relationships among species we would have needed to screen molecularly *D. (G.) solida* and *D. (G.) zorzini* but we had no access to those species for our study.

The phylogenetic position of two of the four blind taxa (B5.0.1c and C23c), rather basal in the tree of Fig. 4, was not anticipated. Theoretically speaking, blind taxa are expected to end up in a given phylogenetic tree as terminal rather than basal branches with the latter being occupied by less cave-adapted lineages (Sbordoni et al 2000). This is because troglobionts are thought to derive from troglobile, trogloxene or even surface ancestors. An evolutionary scenario with a troglobitic taxon giving origin to a surface lineage is hard to envision, let alone this happening at least twice within the same group as suggested by the relationships in Fig. 4. We are more inclined to suspect that, once again, an incomplete taxon sampling is responsible for the observed pattern.

Fig. 5 is a schematic representation of genetic and geographic relationships among the five major clades of Fig. 4. It is evident that some clades identified phylogenetically are not restricted to a single area but they are spread across

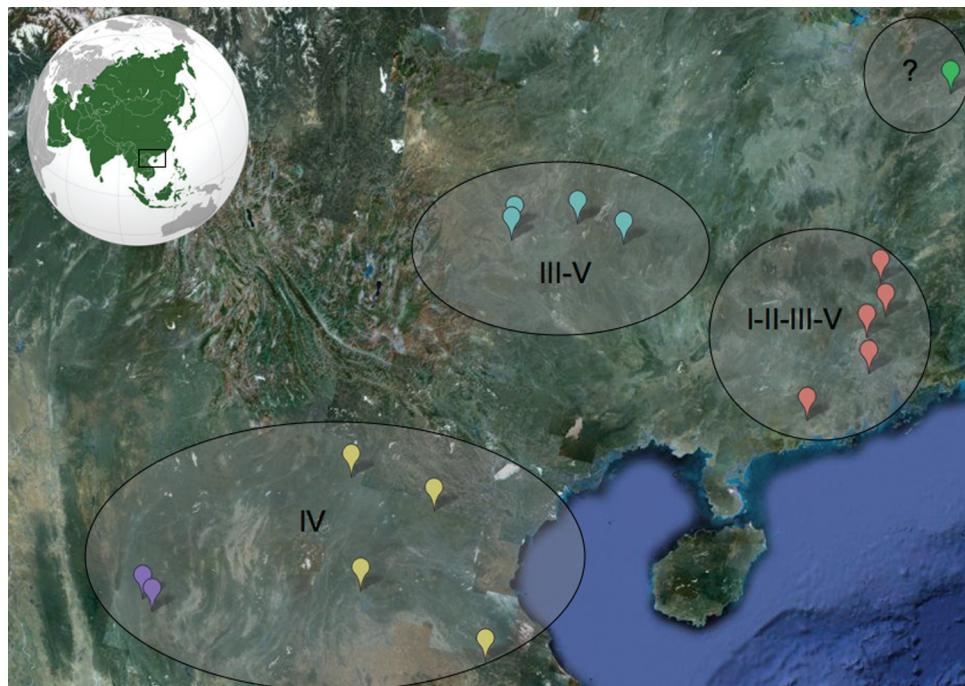


Fig. 5 - Geographic distribution of the main five clades identified phylogenetically in Aemodogryllinae representatives. Colors follow the same logic as in Fig. 1.

two or more areas. Clades III and V co-occur in Guizhou (with a number of phylogenetically independent lineages) and Guangdong. This pattern is brought to an extreme in four caves (An Ja Da Dong, Pi Pa Dong, Maria Cassan and Xian Yen Dong), which host two divergent lineages each (see Tables 1 and 2 and Fig. 4 for details). It is noteworthy that lineages occurring within the same cave have different degrees of eye development (well developed vs. blind) suggesting a) a different timing in the colonization of the caves (older for the blind form, more recent for the form with developed eyes) and, b) divergence in the ecological niche, possibly related to the exploitation of different sections of the cave; this pattern has already been observed in *Diestrammena* (Di Russo and Rampini 2005).

All in all, the sympatric occurrence of genetically divergent lineages should be taken as an indication of secondary contact among lineages that have acquired their own characteristics allopatrically (Avise 2000). The alternating climatic conditions of the area during the Quaternary are likely to have strongly influenced the evolutionary history of these crickets. Although the area was never glaciated (Li et al 2007), throughout the Quaternary, phases of warm and humid climate alternated with colder and drier periods. Rhaphidophorids have quite strict requirements as far as environmental humidity is concerned because they cannot withstand dry conditions. It is thus reasonable to envision a scenario where the Quaternary dry and cold periods forced these organisms to seek refuge in the subterranean environment hindering surface gene flow. This led to local divergence and, if the time span was long enough, to the acquisition of troglobitic traits (Sbordoni et al 2000; Ketmaier et al 2002, 2010; Rampini et al 2008). The milder phases of the Quaternary (which partially still persists) allowed epigean dispersal and presumably brought into secondary contacts those lineages that had been evolving in allopatry. Such surface dispersal was limited to those groups that hadn't evolved a tight dependency on caves and would explain the occurrence within the same cave system of lineages divergent both genetically and in terms of the degree of troglomorphosis.

As a conclusive remark, we want to reiterate our awareness on the fact that this study has its own limitations geographically and in terms of coverage of taxa. Nonetheless, our genetic data have unveiled a complex and multifaceted evolutionary scenario that could be used as a backbone to thoroughly revise the taxonomic arrangements of the group in the area and as a starting point for more detailed research based on a larger number of populations and molecular markers.

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Three new hyporheic water mite species from Australia (Acari: Hydrachnidia)

Harry SMIT ^(1,*)

⁽¹⁾ Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands; E-mail harry.smit@naturalis.nl

* corresponding author

ABSTRACT

Three new hyporheic water mite species are described from Australia, viz. *Wandesia minuta*, *Partidomomonia elongata* and *Mellamunda tasmanica*. With the description of these three species, some 50 hyporheic water mites species are known from Australia. In this paper the first *Partidomomonia* species for Tasmania is described, the third *Wandesia* species for Australia as well as the second species of the genus *Mellamunda*.

Key words: Taxonomy, *Wandesia*, *Partidomomonia*, *Mellamunda*

INTRODUCTION

The Australian water mite fauna is rich in hyporheic species. Thus far, 47 species of certain hyporheic origin are known, with four additional species of possible hyporheic origin (Smit 2007). However, this number might increase due to the fact that Cook (1986) described many new species which are a mixture of epigean and hyporheic species. Therefore the habitat of quite a number of species is not known with certainty. Moreover, many parts of Australia have not been examined for hyporheic water mites.

In this paper three new species will be described from Tasmania and Victoria.

METHODS

All material was collected by the author and this is not repeated in the text. The hyporheic water mites were collected with a pump according to Boulton et al (1992). This is an adapted Bou-Rouch pump, but much lighter, and therefore easier to use in the field. One hyporheic species was collected by hand-netting. Abbreviations used: PI-PV = palp segment 1-5; I-leg- 4-6 = first leg segments 4-6; TMAG = Tasmanian Museum and Art Gallery, Hobart; NMV = Museum Victoria, Melbourne; RMNH = Netherlands Centre for Biodiversity Naturalis, Leiden; NP = National Park; asl = above sea level.

RESULTS

Family Hydryphantidae Piersig

Subfamily Wandesiinae Schwoerbel

Genus *Wandesia* Schechtel, 1912

Wandesia (Pseudowandesia) minuta sp. nov.

(Figs. 1-4)

Material examined

Holotype male, Moleside Creek, Lower Glenelg NP, Victoria, Australia, 38°04.336'S 141°17.507'E, alt. 25 m asl, 3-iv-2008 (NMV).

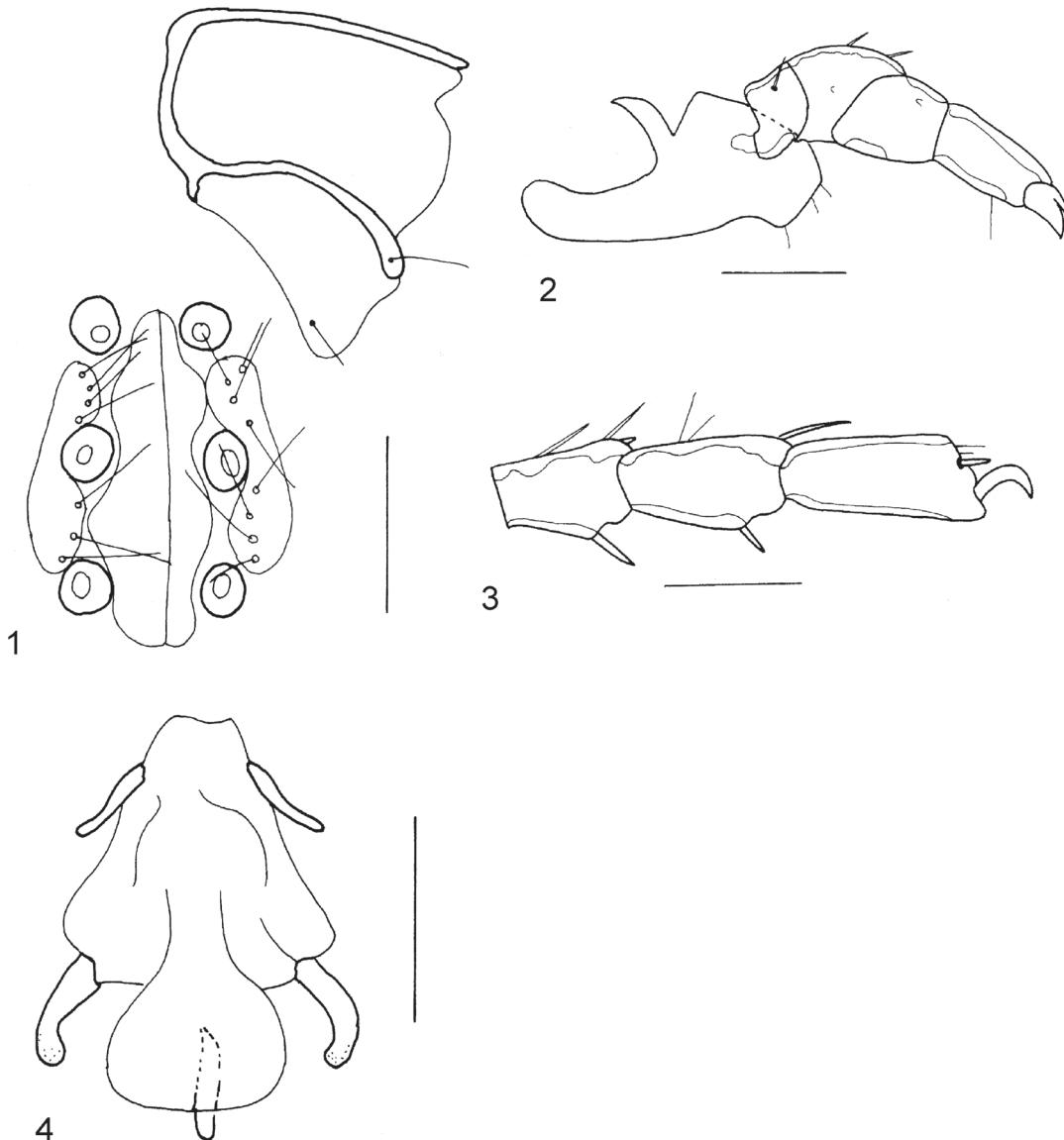
Diagnosis

Idiosoma small (871 long), fourth coxal plates with a concave medial margin.

Description

Male: Idiosoma 871 long, dorsum without platelets, eyes absent. Third coxal plates rectangular, fourth coxal plates with a concave medial margin. Gonopore 96 long. Genital field with three pairs of acetabula, these not stalked. Genital field with a pair of sclerites with 7-8 stiff setae. Genital skeleton 987 long, proximal arms with minute papillae. Lengths of PI-PV: 22, 49, 28, 52, 26; palp stocky, especially PIV. Lengths of I-leg-4-6: 50, 62, 62. Lengths of IV-leg-5-6: 82, 68. Legs without swimming setae.

Female: Unknown.



Figs 1-4 - *Wandesia minuta* sp. nov., holotype male: 1 = genital field + third and fourth coxae; 2 = palp; 3 = I-leg-4-6; 4 = genital skeleton. Scale bars 50 µm.

Etymology

Named for its relatively small size.

Remarks

Thus far, 13 species of the subgenus *Pseudowandesia* (K.O. Viets 1987; www.watermite.org viewed on February 1, 2012) are known worldwide. The subgenus has been found in Europe, Argentina, India, Australia and Siberia, including two from Australia: *W. glareosa* Weigmann & Schminke, 1970 and *W. troma* Cook, 1986. All known species are much larger (>1400) than the new species described here. The two known Australian species have the fourth coxal plates with a convex medial margin and the medial margin of the third coxal plates is relatively much larger. Moreover, *W. troma* has a slender palp, while the male of *W. glareosa* has a genital field with two pairs of setal sclerites.

Although the holotype has not been collected with a groundwater pump, it is certainly of hyporheic origin. Most wandesiiine species live in interstitial habitats (Cook 1974). Under large stones one can find small-scale exchange of the stream and the hyporheic zone (Boulton et al 2010).

Family Momoniidae K. Viets
Genus *Partidomomonia* Cook, 1983

Thus far, four *Partidomomonia* species are known, one from New Zealand (Cook 1983) and three from Australia (Harvey 1990; Smit 2007). The Australian species were reported from Victoria (two species) and Queensland (one species). The first species from Tasmania is described below.

Partidomomonia elongata sp. nov.
(Figs. 5-10)

Material examined

Holotype male, Franklin River, hyporheic, at crossing with road A10, Franklin-Gordon Wild Rivers NP, Tasmania, Australia, 42°12.916'S 146°01.170'E, 26-iii-2008 (TMAG). Paratypes: one male (RMNH), one female (TMAG), same data as holotype; one male, Apsley River, hyporheic, at waterhole, Douglas Apsley NP, Tasmania, Australia, 41°51.758'S 148°11.186'E, 28-iii-2008 (RMNH).

Diagnosis

Idiosoma elongated, distance of anterior pair of acetabula and central pair of acetabula larger than distance between two posterior pairs, PIV stocky, I-leg-5 and -6 slender.

Description

Male: Idiosoma pale yellow, ventrally 713 (713-778) long and 462 (458-559) wide, dorsally 652 (656-721) long. Dorsum with two unpaired plates, three larger pairs plates and five smaller paired plates. Large posterior unpaired plate 373 (365-397) long and 300 (292-356) wide; eyes present, lying below integument. Anterior coxal plates extending beyond anterior idiosoma margin. Suture lines of coxae indistinct. Genital field with three pairs of acetabula lying in the gonopore, distance of anterior pair of acetabula and central pair of acetabula larger than distance between two posterior pairs. Gonopore 80 long. Lengths of PI-PV: 21, 62, 36, 72, 44. PIV stocky and ventrally expanded, with a short, stout seta and a thin seta. Lengths of I-leg-4-6: 54, 254, 207. I-leg-5 and -6 relatively slender, claw 118 long, heavy posteroventral setae distanced. Lengths of IV-leg-4-6: 162, 227, 174. Legs without swimming setae.

Female: Idiosoma pale yellow, ventrally 770 long and 535 wide, dorsally 705 long. Dorsum with two unpaired plates, three larger pairs plates and five smaller paired plates. Large posterior unpaired plate 389 long and 348 wide; eyes present, lying below integument. Anterior coxal plates extending beyond anterior idiosoma margin. Suture lines of coxae indistinct. Genital field with three pairs of acetabula lying on a separate sclerite, distance of anterior pair of acetabula and central pair of acetabula larger than distance between two posterior pairs. Gonopore 150 long. Lengths of PI-PV: 24, 66, 40, 76, 46; palp as in male. Lengths of I-leg-4-6: 90, 271, 235; first leg as in male. Lengths of IV-leg-4-6: 162, 219, 170.

Etymology

Named for the elongated idiosoma.

Remarks

The new species is close to *Partidomomonia blythi* Harvey, 1990 (in parentheses data of *P. blythi*), but dif-

fers in its larger size (less than 450), the configuration on the acetabula (acetabula equidistant from each other) and I-leg-5 longer than I-leg-6 (same length). Both *P. cabanandra* Harvey, 1990 and *P. tertia* Smit, 2007 have a slender PIV and a less elongated idiosoma shape, while in *P. tertia* the lateroglandularia 5 are fused with the large posterior dorsal plate (free in the new species).

Family Athienemanniidae K. Viets
Subfamily Notomundamellinae Cook
Mellamunda Harvey, 1988

Revised diagnosis

Male genital field with 5-6 acetabula lying in the gonopore and 3-9 acetabula fused with the ventral shield. Eyes not lying in capsules but below the integument. See further Harvey (1988). IV-leg not or slightly modified.

Mellamunda tasmanica sp. nov.
(Figs. 11-16)

Material examined

Holotype male, Franklin River, hyporheic, at crossing with road A10, Franklin-Gordon Wild Rivers NP, Tasmania, Australia, 42°12.916'S 146°01.170'E, 26-iii-2008 (TMAG).

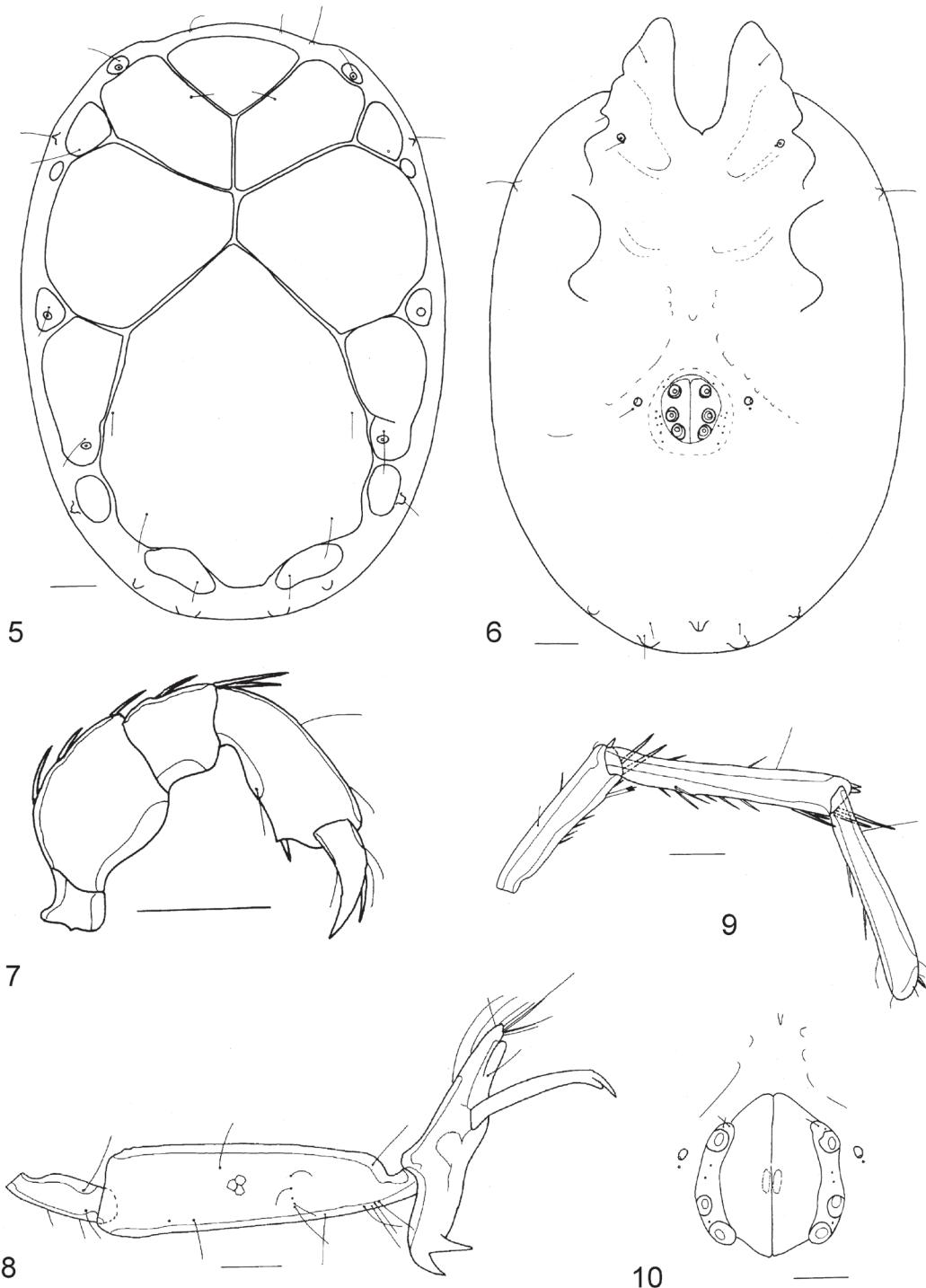
Diagnosis

Male genital field with six pairs of acetabula lying in the gonopore and three pairs fused with the ventral shield.

Description

Male: Idiosoma brownish, dorsal and ventral shields present. Ventrum 640 long, dorsum 640 long, width of idiosoma 397. Dorsal shield complete, 518 long and 348 wide, with three pairs of glandularia. Eyes not in capsules but lying below the integument. First coxal plates extending beyond anterior idiosoma margin. Suture lines of coxae indistinct, lateral suture lines obsolete. First and second coxae fused, third and fourth coxae medially close to each other. Genital field with six pairs of acetabula lying in the gonopore and three pairs of acetabula fused with the ventral shield. Ventroglandularia 2 absent, only accompanying seta present. Lengths of PI-PV: 30, 74, 48, 78, 32. PII with three setae in anterodorsal corner, PIII with a long seta on medial side, PIV rotated (and therefore not completely visible in illustrations) with one very long seta and one shorter seta, PV blunt. Lengths of I-leg-4-6: 86, 120, 108; I-leg-5 ventrally with two long, thin setae. Lengths of III-leg-3-6: 62, 80, 116, 96; III-leg-3 and -4 expanded ventrally, latter segment with two stout setae and one hyaline spatulate seta. Lengths of IV-leg-5-6: 176, 154. Legs without swimming setae, although IV-leg-5 and -6 ventrally with 4-6 long, thin setae.

Female: Unknown.



Figs 5-10 - *Partidomomonia elongata* sp. nov., holotype male 5-9, paratype female 10; 5 = dorsal view; 6 = ventral view; 7 = palp; 8 = I-leg-4-6; 9 = IV-leg-4-6; 10 = genital field. Scale bars = 50 µm.

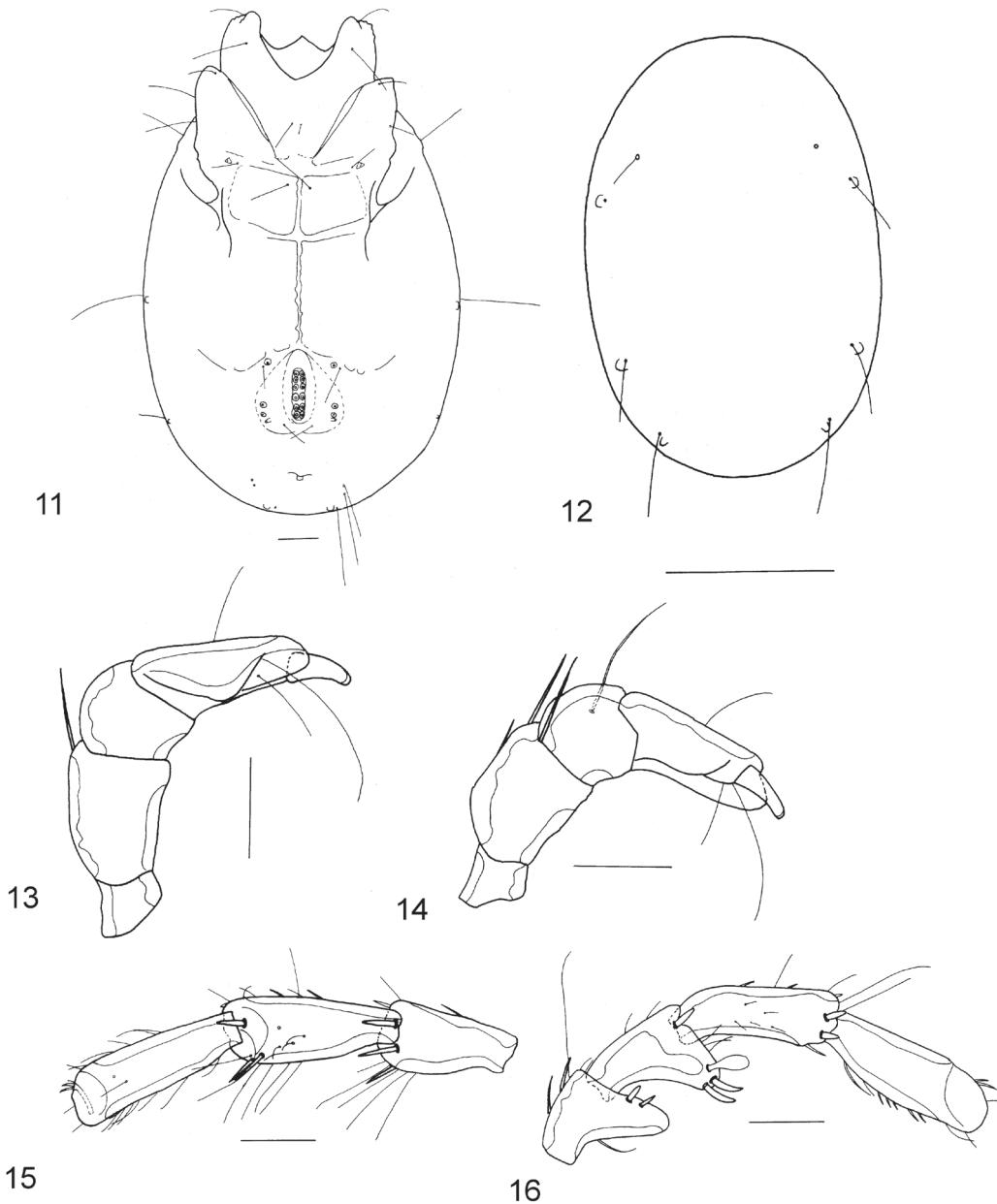
Etymology

Named for its occurrence on Tasmania.

Remarks

This is the second species of the genus, the other one is *M. acares* Harvey, 1988. The new species differs from the latter in the number of acetabula, five acetabula lying in the gonopore and nine acetabula

fused with the ventral shield in *M. acares*. Moreover, the shape of the ventral expansion of III-leg-4 is different in *M. acares* (more angular), and so is the shape of the setae of this extension (compare fig. 25 of Harvey 1988 with fig. 16). Although the new species was collected with a groundwater pump, the presence of eyes and coloration might indicate that it is an epigean species.



Figs 11-15 - *Mellamunda tasmanica* sp. nov., holotype male: 11 = ventral view; 12 = dorsal view; 13 = palp; 14 = palp; 15 = I-leg-4-6; 16 = III-leg-3-6. Scale bars 11, 13-16 = 50 µm, 2 = 200 µm.

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Paving the Way for Standardized and Comparable Subterranean Biodiversity Studies

David C. CULVER ^{(1,*),} Peter TRONTELJ ⁽²⁾, Maja ZAGMAJSTER ⁽²⁾, Tanja PIPAN ⁽³⁾

⁽¹⁾ Department of Environmental Science American University 4400 Massachusetts Ave. NW Washington D.C. 20016 U.S.A.; e-mail: dcculver@american.edu

⁽²⁾ Department of Biology University of Ljubljana Večna pot 111 SI - 1000 Ljbuljana Slovenia

⁽³⁾ Karst Research Institute at ZRC SAZU Titov trg 2 SI-6230 Postojna Slovenia

* corresponding author

ABSTRACT

A series of potential pitfalls (fallacies) in estimating subterranean biodiversity are outlined: (1) provincialism—treating different regions differently, especially with respect to new discoveries and undescribed species; (2) equality of described and undescribed species—ignoring the possibility that undescribed species are not really new species; (3) isotropy—assuming all cave regions of similar size have equally rich faunas; (4) scale invariance—ignoring the effect of area on species richness; and (5) misuse of expert opinion—the over-reliance on experts estimates often without comparable estimates for all areas. Some standard procedures are suggested for subterranean biodiversity studies, and the value of such studies is emphasized.

Key words: biodiversity, species richness, stygobionts, troglobionts

INTRODUCTION

The rapid expansion of analytical techniques (*e.g.*, Colwell, Mao and Chang 2004, Colwell 2009) combined with the possibility of both accumulating and incorporating georeferenced data, has had a profound effect on the study of species richness, especially at regional and global scales. The discovery and identification of hot-spots, originally defined as areas of concentration of endemic species and that face imminent destruction (Myers 1988), but more broadly used as areas of exceptional species richness has been given special attention, in part because of the growing biodiversity crisis. Typically, such analyses have been done on broad-scale monophyletic groups. For example, bird lists for different localities have a long history.

Analyses of subterranean biodiversity patterns have lagged behind not only because of the difficulty in obtaining large quantities of information on species distributions and site locations, but also because the obligate cave-dwelling fauna is spectacularly polyphyletic, with numerous orders and even classes. Because numerous studies indicate that the cave fauna is strongly convergent (trogomorphic in Christiansen's [1962] formulation), the factors that result in colonization and speciation should also be convergent, or at least that is the working hypothesis of subterranean biodiversity studies.

Culver and Holsinger (1992), using some back of the envelope calculations, suggested that there were upwards of 50,000 obligate cave-dwelling species in the world, including both described and undescribed,

discovered and undiscovered species. One of the first, and certainly influential, studies of subterranean biodiversity was that of Sket (1999), who suggested that the Dinaric karst in general and Slovenia in particular was a hotspot of aquatic subterranean biodiversity. Lists of regional faunas (*e.g.* Peck 1998; Trajano and Bichuette 2009) began to appear, and this activity continues up to the present.

The use of spatially defined localities allowed spatial partitioning of species richness (Malard et al 2009), spatial modeling (Christman and Zagmajster 2012), and analysis of variables that potentially explain subterranean species richness (Dole-Olivier et al 2009a; Martin et al 2009). In addition, important strides have been made in sampling strategy itself (Dole-Olivier et al 2009b; Eberhard et al 2009). All of this activity is for the good because it increases the available information on species richness in subterranean habitats, especially caves.

However, despite these many promising trends, we find that there are some unfortunate trends in both inaccurately estimating the likely numbers of species at different sites, and in mis-interpreting regional and global patterns of subterranean biodiversity. The purpose of this note is not to put forward any claim about continental or global patterns of subterranean biodiversity, but rather it is to point out some fallacies in the literature and to point out some best practices in the analysis of biodiversity patterns. We first point out five fallacies of these analyses, then suggest some best practices, and conclude with some general observations.

FALLACIES

The first fallacy is what we have termed the *Fallacy of Provincialism*. By this we mean treating data from one place differently than data from other places. Typically this takes the form of assuming that only in the ‘favored’ region under consideration are there undescribed species and species yet to be discovered. For example, Graening, Fenolio and Slay (2012), writing about the fauna of the caves in Oklahoma and Arkansas, a major part of the Ozark Plateau in the central U.S., argue that Culver et al (2003) are incorrect in not including the Ozark Plateau as a hotspot. Culver et al (2003) reported 77 stygobionts and troglobionts for the region, ranking it well below the Interior Low Plateau (Alabama, Tennessee, Kentucky, etc.), Appalachians (West Virginia, Virginia, Tennessee, etc.), and Edwards Aquifer/Balcones Escarpment (Texas) in species richness. Because Graening et al (2012) found 130 stygobionts and troglobionts in the region, they argue that it was incorrect to assume the Ozarks are a low diversity area, as did Culver et al (2003). The fallacy is that they assume no new species have been described outside of the Ozarks since 2003. This is emphatically not the case, as the example from West Virginia discussed next, demonstrates.

The obligate cave fauna of West Virginia provides an instructive example in this regard. Together with Virginia and the Mammoth Cave region of Kentucky, it has been better studied for longer than any other U.S. cave region. Yet the number of described species is still increasing. In 1976, Holsinger, Baroody, and Culver reported a total of 49 described species of obligate cave dwelling species (Table 1). By 2007 Fong et al reported a total of 88 species, and by 2011, this number had grown to 95 based on new species descriptions by Soto-Adames (2010) and

Shear (2008, 2010). Thus, comparison using 2012 numbers with numbers from other regions based on 2003 data is inappropriate.

Another example is the data on individual cave hotspots. In 2000, Culver and Sket were able to list 20 sites where the combined number of stygobionts and troglobionts was 20 or more. In 2009, 16 additional sites were added to the list (Culver and Pipan 2009). By 2012, Culver and Pipan reported on more extensive data, with 10 sites with more than 25 stygobionts and 6 sites with more than 25 troglobionts. This large change is the result of both better record keeping and new discoveries. The taxonomic (Linnean) shortfall and the biogeographic (Walacean) shortfall is apparently universal in the cave fauna.

The second fallacy is the *Fallacy of Equality of Described and Undescribed Species*. Because of the Linnean shortfall, which is acute for the subterranean fauna in general in all regions, estimates of species richness, especially for single sites, utilize described and undescribed species. Although their lists were not published, Culver and Sket (2000), in their widely cited paper on hotspots of subterranean biodiversity, relied on both described and undescribed species. This is often a necessity because many, and sometimes most species are undescribed, as is the case with the Brazilian cave fauna (Trajano and Bichuette 2009). The problem is not with listing undescribed species, but in using them in numerical calculations without due caution, and perhaps not giving them the same numerical weight as described species. Undescribed species do not all become described species---some languish, seemingly forever in museum drawers; some are described as new species; and some were incorrectly thought to be undescribed new species but in fact were already described.

The checklists of the troglobiotic and stygobiotic fauna of West Virginia that were published in 1976 by

Table 1 - Number of species known at three time intervals in West Virginia caves.

Group	1976 species	1976 undescribed.	1976 total	2007 species	2011	Difference--2011 & 1976
Flatworms	2	2	4	5	5	1
Snails	2	4	6	2	3	-3
Annelida	0	2	2	2	3	1
Amphipoda	7	7	14	16	16	2
Isopoda	5	2	7	7	7	0
Decapoda	1	0	1	1	1	0
Millipedes	6	3	9	7	11	2
Diplura	1	1	2	1	1	-1
Collembola	4	2	6	13	15	9
Coleoptera	11	3	14	17	17	3
Diptera	0	0	0	1	1	1
Acari	1	0	1	3	3	2
Pseudoscorpionida	5	1	6	6	6	0
Araneae	4	0	4	6	6	2
Opiliones	0	0	0	0	1	1
TOTAL	49	27	76	87	96	20

Holsinger et al and again in 2007 by Fong et al, provide an opportunity to track the fate of species that were undescribed in 1976. Holsinger et al listed a total of 27 undescribed species, of which 8 remain unstudied. Of the rest, 13 have been described as new species and 6 turned out not to be new species, but could be assigned to existing species (Table 2). For this one data set, this suggests a “discount rate” for undescribed species of 0.68 (13/19). Of course there are unknown and undescribed species, and when these are added, the increase in the number of described species was 47 in 2011 compared to 1976 (Table 1).

This problem is especially acute in regions where there are relatively few described species such as Brazil where Trajano and Bichuette (2009) list more than 125 species¹ but only 51 are described. Similarly, Guzik et al. (2010) report 407 described obligate cave dwelling species and another 367 undescribed species.

¹ The exact number is impossible to determine because at some entries on their list, they indicate “several” species.

Some mention should be made of the potential impact of different species definitions on species counts. With the growing recognition of the presence of cryptic species (Trontelj et al 2009), the criteria used to delineate these species, such as a rule concerning percent differentiation (Lefébure et al 2006), become critical. A relaxation or the use of alternative criteria, as proposed by Guzik et al (2010) will increase the number of species. They use a combination of unorthodox molecular, phylogenetic and geographic criteria to predict subterranean species in Western Australia. Their procedure might be well suited to identify undescribed species under some species concepts, but at the same time it sheds doubt on their conclusions. This is so because the methodology was not tested against any existing and thus comparable taxonomic array, and because it is likely to produce higher estimates than more traditional approaches used on other continents. These considerations are important mostly at the level of regional, or gamma diversity comparisons. Single cave, or alpha diversity is typically less affected by differences in taxonomic practice, although cases of morphologically cryptic species living together

Table 2 - List of species from West Virginia, listed as undescribed new species by Holsinger et al (1976). Current status from Fong et al (2007), Shear (2008, 2011) and Soto-Adames (2010).

Species	Location	Current Status	Change
<i>Sphalloplana</i>	Harper	<i>S. culveri</i>	new species
<i>Phagocata</i>	Harper	<i>P. angusta</i>	new species
<i>Fontigens 1</i>	Bazzle, Harman, Marthas, Piddling Pit	<i>F. tartarea</i>	old species
<i>Fontigens 2</i>	The Hole, McClung	<i>F. tartarea</i>	old species
<i>Fontigens 3</i>	Hunt	unstudied	unstudied
<i>Laretia</i>	McClung	<i>F. turritella</i>	new species
<i>Stylodrilus</i>	Ct. Street, Tub	<i>S. beattei</i>	new species*
<i>Trichodrilus</i>	Tub	<i>T. culveri</i>	new species*
<i>Apocrangonyx 1</i>	Arbuckle, Parlor, Haynes, Snedegar	<i>Stygobromus pollostus</i>	new species
<i>Apocrangonyx 2</i>	Piddling Pit	<i>Stygobromus nanus</i>	new species
<i>Stygobromus 1</i>	Ditmer	<i>S. biggersi</i>	new species
<i>Stygobromus 2</i>	Coburn	<i>S. spinatus</i>	old species
<i>Stygobromus 3</i>	Patton	<i>S. redactus</i>	new species
<i>Stygonectes 1</i>	Dyers	<i>S. allegheniensis</i> or <i>S. morrisoni</i>	old species
<i>Stygonectes 2</i>	Stillhouse	<i>S. culveri</i>	new species
<i>Caecidotea</i>	Beacon	unstudied	unstudied
<i>Caucasonethes</i>	Indian	unstudied	unstudied
<i>Pseudotremia 1</i>	Hunt	<i>Zygonopus weyeriensis</i>	old species
<i>Pseudotremia 2</i>	Devils Kitchen	unstudied	unstudied
<i>Pseudotremia 3</i>	Organ	<i>Zygonopus weyeriensis</i>	old species
<i>Plusiocampa</i>	Dyepot	unstudied	unstudied
<i>Pseudosinella</i>	Neely Farm	<i>P. testa</i>	new species
<i>Sinella</i>	Mill Run plus many	<i>S. agna</i>	new species
<i>Pseudanophthalmus 1</i>	Mercer Co.	unstudied	unstudied
<i>Pseudanophthalmus 2</i>	Bowden	unstudied	unstudied
<i>Pseudanophthalmus 3</i>	Patton	unstudied	unstudied
<i>Kleptochthonius</i>	Organ	<i>K. hetricki</i>	new species
*in press before pub.			

in a single cave have been reported (*e.g.*, Cobolli Sborodoni et al 1990; Zakšek et al 2009).

Parenthetically we note that there is no discernible difference in species delineation criteria employed by European and American taxonomists. Culver et al (2006) showed difference in species per genus ratios between the two continents, although there is a difference in description of subspecies, which American taxonomists rarely do.

The third fallacy is the *Fallacy of Isotropy*. A simple method to estimate unknown species richness is to use known species counts for a small, well studied area, and then multiply up, using the number of such small areas to cover a region. For example, in their estimate of global species richness of troglobionts and stygobionts on a global scale, Culver and Holsinger (1992) began with an estimate of the number of stygobiotic amphipod species, both described and undescribed (see the *Fallacy of Equality of Described and Undescribed Species*), then used ratios of different groups in Botosaneanu's (1986) **Stygofauna Mundi**, estimate the actual proportion of non-amphipods, and multiply up from there. The problem with this approach is that it is unlikely that the number of amphipod species in the Virginias is representative of the number of amphipod species elsewhere, at least in the U.S. This is especially so because one of the reasons this amphipod fauna was well known was that it was diverse and interesting.

Starting with an especially rich component of the fauna can lead to overestimates, and even absurd results. In her comprehensive study of the copepods of epikarst drips in six Slovenian caves, Pipan (2005) found up to ten species of copepods in one single drip (see Pipan and Culver 2007a). Since populations of epikarst copepods typically have ranges of maximum linear extent on the order of magnitude of 100m (Pipan and Culver 2007b), one might estimate 350 species of epikarst copepods from 35 drips included in Pipan's (2005) study. This absurd result, where the best drip is used to multiply up, is strikingly different from the 37 species actually known in the region.

Yet another example can be taken from Zagmajster et al 's (2008) study of the remarkable troglobiotic beetle fauna of the northwest Balkans, with nearly 300 described species. If we took the most species rich 400 km² quadrat, with 21 species, and multiplied it by the number of quadrats (263), we would obtain the incredible estimate of 5523 troglobiotic beetle species in the region! The errors in this hypothetical example are (1) using the best quadrat and (2) the very process of multiplying up, with the expectation of the same number of beetle species in all quadrats. Zagmajster et al (2008) estimated species richness (including described, undescribed, and unknown species) was about 730 species for the entire region, based on the highest estimate of various species richness estimators. In addition, Zagmajster et al (2010) provide a way of estimating species richness in a gridded

area, essentially by estimating missing diversity for each quadrat (see also Christman and Zagmajster 2012).

This fallacy appears in cases where existing data for karst areas are used to estimate species richness for unsampled karst areas. This is apparently part of the procedure used by Guzik et al (2010) to estimate species richness of unsampled calcrete aquifers. One consequence and indication of the Fallacy of Isotropy is that it results in very large estimates of species numbers and no confidence intervals can be placed on this estimate. Thus, Guzik et al (2010) estimate 4140 species, more than ten times the number of described species. Perhaps they are correct but it is of little comparative value because there are no estimates for other areas done in a similar manner.

The fourth fallacy is the *Fallacy of Scale Invariance*. Gibert and Deharveng (2002) suggested and Malard et al (2009) later confirmed that within a region, that local single-cave species richness (α -diversity) is a small fraction of the total species richness. While comparisons between countries are frequent, if only in a rather casual sense, rarely if ever are differences in area (or more appropriately area of karst) taken into account. Statements about numbers of species in a state or country (*e.g.*, Elliott 2007; Trajano and Bichuette 2009) are of no comparative use unless information on the relationship between species numbers and area is included.

Differences in area could be safely ignored if average species richness for a single cave were a good predictor of regional species richness. Then α -diversity could be used as a surrogate for regional diversity. A way to test this is to see if species accumulation curves (or their analytical equivalent, Mao-Tau curves) for 1,2,3,... randomly chosen caves cross. Dole-Olivier et al (2009b) show that the Mau-Tau curves for the stygofauna of the intensively studied regions that were part of the European Union PASCALIS (Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface) project (Fig. 1) do indeed cross. Culver et al (2006) also found crossing of curves, although in this case crossing occurred near the origin, *i.e.*, at small numbers of caves.

The fifth fallacy is the *Fallacy of Misuse of Expert Opinion*. The recent estimate of Guzik et al (2010) falls into this trap, at least in part. They provide estimates, by taxonomic group by taxonomic experts, but without estimates for other areas they compare their study region to. The previous discussion of the fate of undescribed species from West Virginia (Tables 1 and 2) is a detailed description of the difficulties with expert opinion. Unless multiple experts are used (as is done for example in developing indices of biological integrity [Barbour et al 1999]), or the same expert evaluates multiple regions, it is difficult if not impossible to evaluate different expert opinion by the same set of criteria. For example, what is the frequency of undescribed cryptic species (see Trontelj et al 2009)? Subterranean biology is fortunate that there are a number

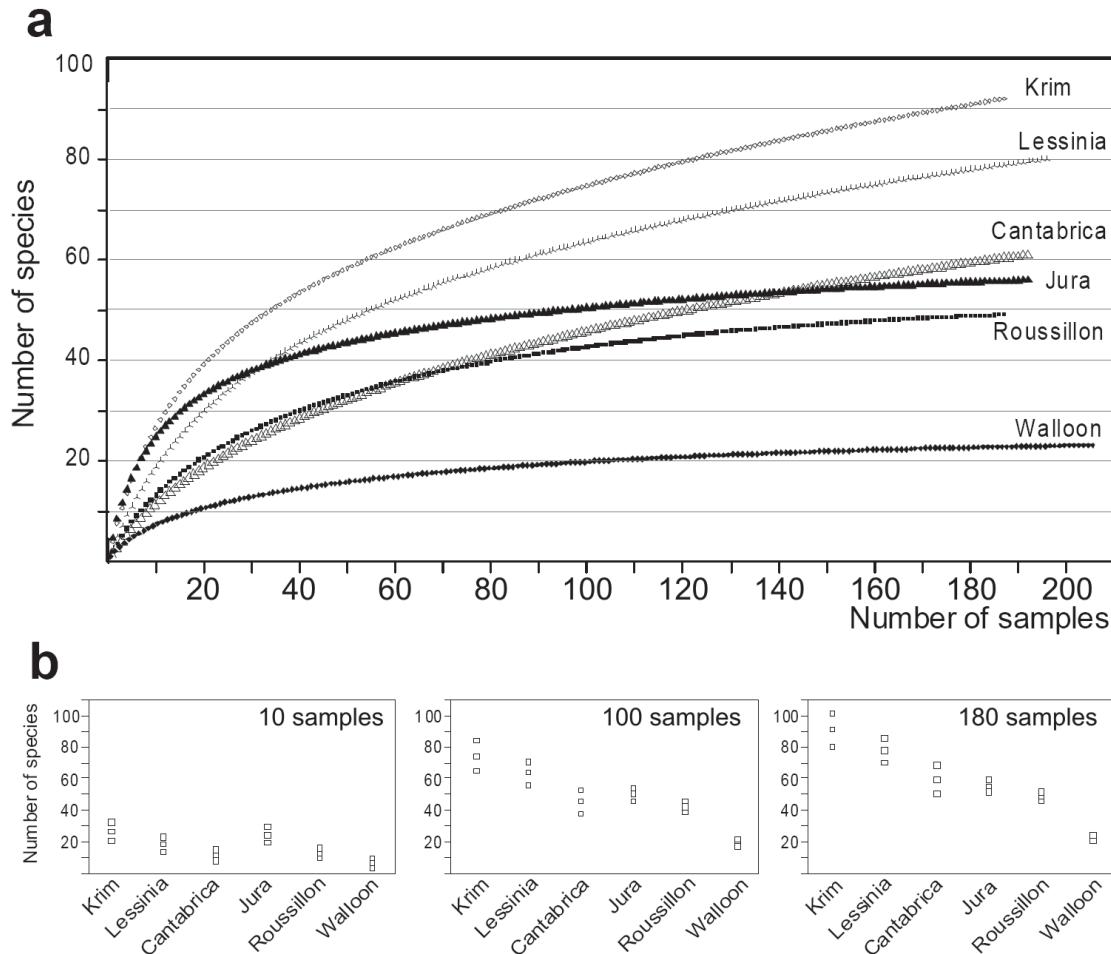


Fig. 1 - Species accumulation curves for stygobionts in the six European PASCALIS regions (top) and 95 percent confidence for the observed number of species (Mao-Tau estimate of Colwell et al [2004]) for three different sampling efforts: 10, 100, and 180 samples (bottom). Sampling regions are Krim Mountain (Slovenia), Lessinian Mountains (Italy), Cantabrica (Spain), Jura Mountains (France), Roussillon (France), and Walloon (Belgium). From Dole-Olivier et al (2009b).

of experts on both particular taxonomic groups and on regional faunas, and as authors we include ourselves in that group. Nevertheless, while expert knowledge is important, there are better and more repeatable ways to estimate species richness, especially those developed to estimate missing species, and widely available in the free software EstimateS (Colwell 2009). If nothing else, they are less prone to subjective interpretations and more easily comparable among regions.

SOME RECOMMENDATIONS

Subterranean biodiversity is often a matter of national and regional pride, and this increases public awareness and often enhances conservation efforts. Poorly documented diversity claims, involving the fallacies we outline above, do make it difficult to put together an accurate picture of global subterranean biodiversity. There has been an explosion of quantitative information about pat-

terns of subterranean species richness, especially at the regional and continental level, yet the broadest scale yet attempted is a European-North American comparison, for terrestrial (Culver et al 2006) and to a very limited extent for aquatic species (Gibert et al 2009). The elucidation of the overall global pattern of subterranean biodiversity is perhaps within reach, but only if data are both comparable and extensive. The following list of recommendations is designed to be the first step in a continuing discussion of how subterranean biodiversity assessment can best be accomplished.

Databases on subterranean biodiversity should, to the greatest possible extent, be based on georeferenced localities. The availability of online georeferenced maps, such as Google Earth[®] make georeferencing of sites much easier than in the past. This enables not only more exact mapping, but also spatial modeling, including spatial autocorrelation (Zagmajster et al 2008), conditional autoregression (Christman and Culver 2001), and kriging (Christman and Zagmajster 2012).

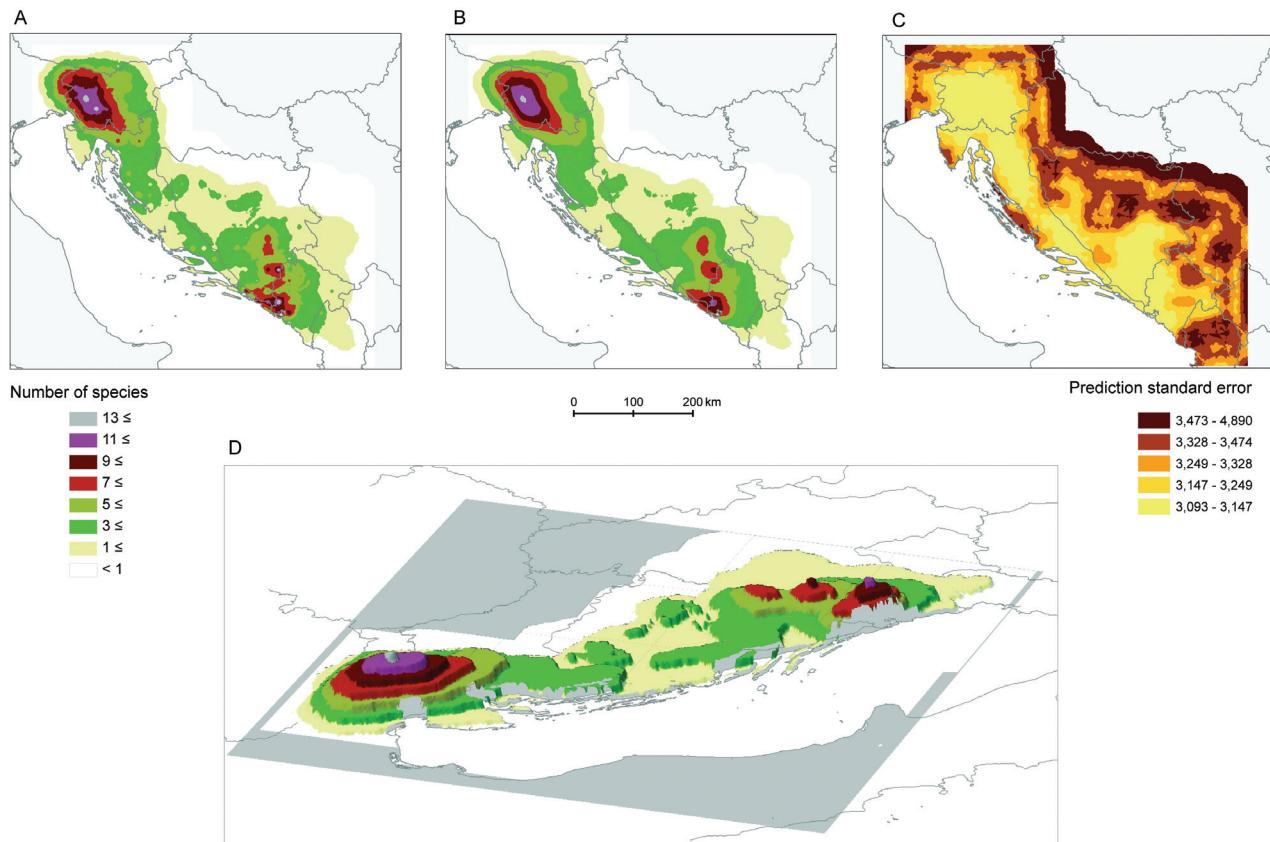


Fig. 2 - Different graphical representations of the same data, number of obligate subterranean beetles (from families Cholevidae and Carabidae) per 20X20 km quadrats in northwestern Balkans. (A) Filled contours based on inverse distance weighting; (B) filled contours based on ordinary kriging; (C) prediction standard error map of ordinary kriging; (D) a three-dimensional presentation of the ordinary kriging predictions. Scale refers to (A), (B) and (C). The first legend refers to (A), (B), and (D), and the second legend to (C). From Christman and Zagmajster (2012).

To the greatest extent possible, the actual data, especially species lists should be made available. This is especially important for large-scale and global comparisons. Without such lists it is impossible to evaluate the claim, especially when exceptional levels of diversity are present. For example, Ozimec and Lučić (2009) report 101 stygobionts and troglobionts in the biodiverse Vjetrenica in Bosnia & Hercegovina, making it the most biologically diverse cave in the world. Yet they published no species list so this claim cannot be evaluated².

For comparative purposes, it is necessary to treat stygobionts and troglobionts separately from non-obligate species. At the same time, they are necessary because differences of opinion exist as to which species are stygobionts and troglobionts. Whatever the criteria used, it should be stated. For example, Fong et al (2007) use a strictly distributional criterion (> 90 percent of the records are subterranean) to define stygobionts and troglobionts in West Virginia, but others (Graening et al 2012)

use morphological criteria as well. Differences in criteria can make for differences in what species are included. Estimates of unknown species should be made using standard estimation techniques, such as Chao2 and bootstrap estimators (e.g. Deharveng et al 2009), which also makes it possible to assign confidence intervals to the estimates. Zagmajster et al (2010) also provide methods for minimizing the effect of different sampling intensities.

For assessment of diversity in a region, there is no substitute for large amounts of inventory data and a thorough geospatial analysis. Christman and Zagmajster (2012) provide a guide to using these techniques. At a minimum, areas need to be gridded, appropriate grid size needs to be determined (Zagmajster et al 2008), and maps need to be made. See Figure 2 for an example of what can be done with good data and good geospatial modeling.

SUMMING UP

While it has been the purpose of this essay to point out some pitfalls in subterranean biodiversity analysis, this

² Sket (2003) does provide a list of 68 stygobionts and troglobionts from the cave.

does not mean that we believe that such analyses are either unimportant or premature. Because of the ubiquitous Linnean shortfall and the Wallacean shortfall in subterranean faunas, there has been a tendency of speleobiologists to avoid any quantitative summary of regional faunas, claiming it is premature. The problem is that both the Linnean shortfall and the Wallacean shortfall are likely to continue indefinitely. Consider the Wallacean shortfall. Even in relatively well studied regions like West Virginia and Slovenia, less than 20 percent of known caves have been investigated biologically, even in a cursory way. Since new caves are discovered every year even in well studied regions, a complete inventory is highly unlikely. The well known shortage of taxonomists suggests that the taxonomic backlog is permanent. For the West Virginia cave fauna, the taxonomic backlog is apparent (Tables 1 and 2).

Rather than focus on what we don't know, we choose to emphasize what is known. There is not accurate count of the number of described species, but it is surprisingly large. Gibert and Culver (2009) summarize the known stygobiotic species richness as follows:

Europe—2000
Asia—561
Africa—335
North America—500
South America—100
Oceania—220

for a total of 3716. Although not tabulated, the number of described troglobionts is several times that. Even though the number of undescribed species is much greater, the patterns of described species, especially when augmented with estimates of missing species can provide valuable insights into subterranean biodiversity patterns (Zagmajster et al 2010).

There is also reason for optimism that even highly incomplete data can provide robust information about the location of high diversity areas. Culver et al (2004) showed that the location of subterranean biodiversity hotspots in Slovenia was basically unchanged from the 1950's, when they compared current information with that available in 1950. During decades of research, absolute numbers of species increased substantially, but relative numerical relationships between areas remained largely unaffected by additional sampling effort. Perhaps the time is right for a large-scale global assessment of subterranean biodiversity based on a standard methodology.

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Literature survey, bibliographic analysis and a taxonomic catalogue of subterranean fauna from Portugal

Ana Sofia P. S. REBOLEIRA ^(1,2,*), Fernando GONÇALVES ⁽¹⁾, Pedro OROMÍ ⁽²⁾

⁽¹⁾ Departamento de Biologia & Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal, e-mail: sreboleira@ua.pt

⁽²⁾ Departamento de Biología Animal, Facultad de Biología, Universidad de La Laguna. 38206 La Laguna. Tenerife. Islas Canarias, Spain

* corresponding author

ABSTRACT

A bibliographic analysis of the hypogean biological studies in Portugal is made, compiling 138 publications related to the subterranean invertebrate fauna, since its beginning in 1870 until November 2012.

A catalogue of hypogean endemic taxa is provided, listing 27 troglobiots and 63 stygobionts, described to be obligate hypogean and endemic from mainland Portugal (Macaronesian archipelagos excluded).

The first impetus on troglobiots studies was provided by the prospections of Barros Machado during 1940's and by an expedition of Lindberg in the spring of 1961; and the major information about stygobiont species was provided by the former Instituto de Zoologia "Dr. Augusto Nobre" from Porto University.

Key words: Bibliography, checklist, troglobiots, stygobionts, Portugal

INTRODUCTION

A literature survey allows a critical assessment of the state of subterranean biology, and is a crucial labor to put order in the knowledge of the hypogean fauna, and to avoid repetition of research due to the difficulties of getting information in old bibliographical sources (Fan 2006).

The subterranean habitat harbours an extremely specialized fauna, being a true laboratory for the study of ecology, natural selection and evolution, central subjects in biology (Culver & Pipan 2009). The study of the subterranean fauna began limited to the perspective of 'human size' caves as the domain of the cave-dwelling fauna (Vandel 1964), but the evolution of knowledge in subterranean biology leads to a broad view of the subterranean habitats as a large variety of places with aphotic conditions, extending their limits far beyond the caves (Pipan & Culver 2012).

The first published register of subterranean fauna from Portugal (Chiroptera excluded) was in the monography "Trehorum oculatorum" where specimens of a ground beetle collected by Schaufuss in portuguese caves were recorded by Putzeys (1870: 18). The specimens were assigned to *Trechus fulvus* Dejean, 1831 in spite of exhibiting a larger and more rounded pronotum than is proper to this species, and neither the caves nor the geographic area of Portugal were specified.

The first hypogean taxon described from Portugal was the troglobiots spider *Nesticus lusitanicus* Fage, 1931, based on specimens collected during a cave survey and exploration by the archaeologist Henri Breuil and the geologist Ernest Fleury, in the beginings of the last century (Gama & Afonso 1994).

The great momentum in the history of Portuguese subterranean biology was given by António de Barros Machado, an eminent arachnologist that began methodical exploration of caves in Portugal in the late 1930's. He performed an intense sampling effort on terrestrial hypogean fauna along the karstic caves of Portugal, and co-authored by his brother Bernardino de Barros Machado described more than 300 caves in the "Inventário das cavernas calcárias de Portugal". In the same decade, Fernando Frade from Lisbon University described *Proasellus lusitanicus* (Frade, 1938) from the Alviela spring in the Estremeno karst massif, paving the way for the study of stygobionts in the following decades by researchers of the former Instituto de Zoologia "Dr. Augusto Nobre" from Porto University.

Most of the biological hypogean studies in Portugal have been done in caves and dark wells. A large cryptic biodiversity still remains to unravel, especially in the mesocavernous shallow substratum (MSS as described by Juberthie et al 1980) and in the anchialine waters of coastal karsts.

More recently several efforts by Reboleira and colleagues, among others, to access subterranean biodiversity in Portugal in karst areas are providing new interesting information (Reboleira et al 2011).

METHODS

The entire bibliography on Portuguese subterranean biology has been revised (excluding that on Macarone-

sian archipelagos and references to Chiroptera) since the first publication in 1870 until November 2012.

The term hypogean is used for the species that live exclusively in subterranean habitats beneath the soil layers, displaying clear adaptations to the subterranean environment, expressed morphologically as convergent evolution, such as integument depigmentation, absence or reduced ocular structures, elongated appendages, and wing reduction in insects. The endogeal fauna occurring inside the soil is excluded of the aim of this catalogue.

RESULTS

As much as 138 scientific publications have been published between 1870 and November 2012, dealing on the hypogean fauna of mainland Portugal, from where 27 troglobiont and 63 stygobiont species have been described so far (see Table 1 and 2 for taxonomic information).

Chronologically, the proportion of higher number of publications is not necessarily the result of higher number of new described taxa (Fig. 1).

The decade of 1960 was the most productive in number of such publications, but the largest number of new hypogean taxa were described in the decades of 1940 and especially 1980 (Fig. 1).

The knowledge of the Portuguese stygofauna increased noticeably from the 40's until the 60's, but the greatest expansion was in the 80's with the description of 19 new species (Fig. 1). The researchers of the former Instituto de Investigação "Dr. Augusto Nobre" from Porto University gave the greatest contribution to the description of new species, being only produced out of this institute 11% of this knowledge. José Maria Braga (25 species) and Odette Afonso (21 species) contributed to 74% of the Portuguese stygobiont species (Fig. 2).

The 1940's were the most productive decade of taxonomical studies on the terrestrial hypogean fauna as a re-

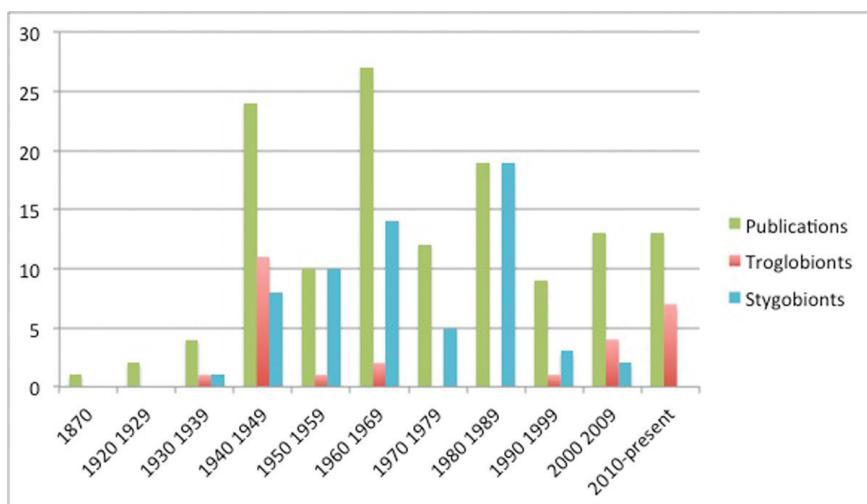


Fig. 1 - Number of publications and described hypogean taxa per decade until August 2012.

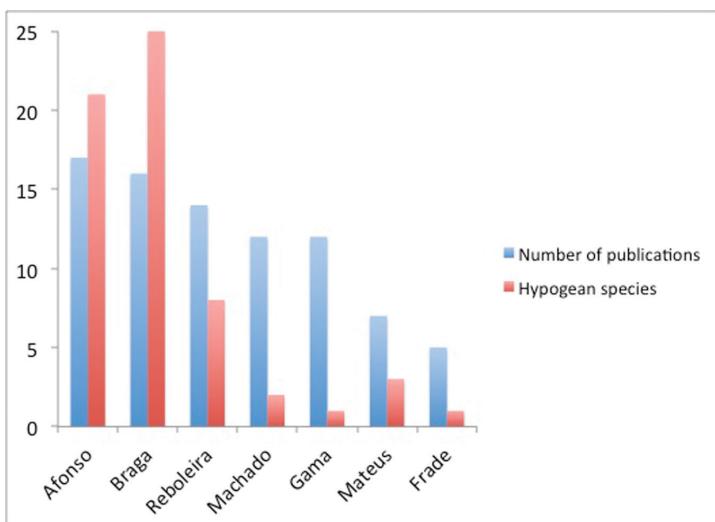


Fig. 2 - Top contributors first authors with five or more publications on hypogean fauna in Portugal and their number of described species until November 2012.

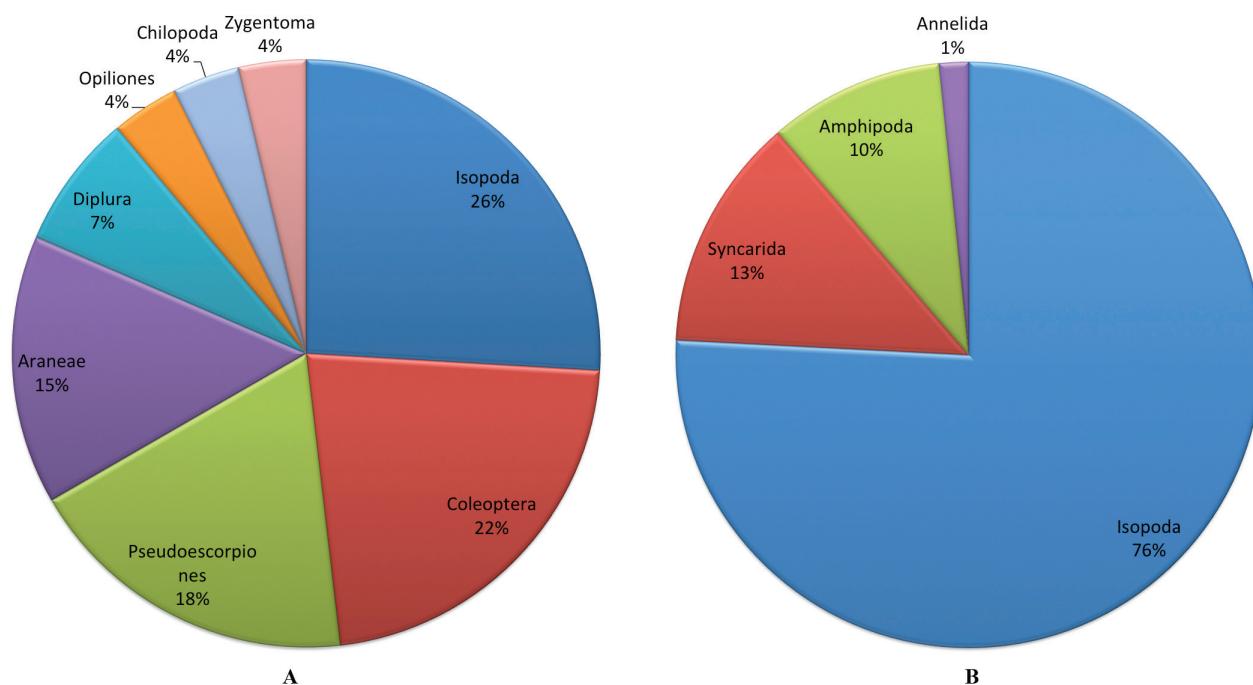


Fig. 3 - Number of hypogean species per higher taxonomic groups, until November 2012. A) Troglobionts and B) Stygobionts.

sult of the great sampling efforts of Barros Machado (Fig. 2), but the activity almost stopped during the 70's and the 80's. The most important contribution in the past, in number of new troglobiotic taxa was afforded by Vandel, who described 6 species of terrestrial isopods (Vandel 1946) (Fig. 3). Isopods are the most diverse group (Fig. 3) in the Portuguese subterranean fauna, characterized by the absence of hypogean species belonging to groups normally with troglobionts in neighbouring Spain, such as Gastropods, Palpigrads, Diplopods, Leptodirine beetles, and other Arthropod groups.

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CATALOGUE OF THE DESCRIBED HYPOGEAN ENDEMIC TAXA FROM PORTUGAL

Table 1 - Troglobiots from Portugal (Macaronesian archipelagos excluded) described until November 2012.

Order	Family	Species
Pseudoscorpiones	Chthoniidae	<i>Chthonius minutus</i> Vachon, 1940
		<i>Chthonius cardosoi</i> Zaragoza, 2012
	Bochicidae	<i>Titanobochica magna</i> Zaragoza & Reboleira, 2010
	Neobisiidae	<i>Roncocreagris blothroides</i> (Beier, 1962)
		<i>Roncocreagris cavernicola</i> (Vachon, 1946)
	Syarinidae	<i>Lusoblothrus aenigmaticus</i> Zaragoza & Reboleira, 2012
Araneae	Dysderidae	<i>Harpactea stalitoides</i> Ribera, 1993
	Leptonetidae	<i>Teloleptoneta synthetica</i> (Machado, 1951)
	Nesticidae	<i>Nesticus lusitanicus</i> Fage, 1931
	Sympytognathidae	<i>Anapistula ataecina</i> Cardoso & Scharff, 2009
Opiliones	Sironidae	<i>Iberosiro distylos</i> Bivort & Giribet, 2004
Lithobiomorpha	Lithobiidae	<i>Lithobius dimorphus</i> Machado, 1946
Isopoda	Porcellionidae	<i>Porcellio cavernicolous</i> Vandel, 1946
	Trichoniscidae	<i>Trichoniscoides broteroii</i> Vandel, 1946
		<i>Trichoniscoides subterraneus</i> Vandel, 1946
		<i>Trichoniscoides meridionalis</i> (Vandel, 1946)
		<i>Trichoniscoides ouremensis</i> (Vandel, 1946)
		<i>Trichoniscoides serrai</i> Cruz, 1993
	Armadillidiidae	<i>Troglarmadillidium machadoi</i> Vandel, 1946
Entomobryomorpha	Onychiuridae	<i>Onychiurus confugiens</i> Gama, 1962
Diplura	Campodeidae	<i>Litocampa mendesi</i> Sandra & Reboleira, 2010
Zygentoma	Nicoletiidae	<i>Squamatinia algarbica</i> Mendes & Reboleira, 2012
Coleoptera	Carabidae	<i>Trechus machadoi</i> Jeannel, 1942

Order	Family	Species
		<i>Trechus gamae</i> Reboleira & Serrano, 2009
		<i>Trechus lunai</i> Reboleira & Serrano, 2009
		<i>Trechus tatai</i> Reboleira & Ortúñoz, 2010
	Staphylinidae	<i>Domene lusitanica</i> Reboleira & Oromí, 2011

Table 2 - Stygobionts from Portugal (Macaronesian archipelagos excluded) described until November 2012.

Order	Family	Species
Tubificida	Tubificidae	<i>Rhyacodrilus lindbergi</i> Hrabe, 1963
Syncarida	Parabathynellidae	<i>Hexabathynella minuta</i> (Noodt & Galhano, 1969)
		<i>Iberobathynella lusitanica</i> (Braga, 1949)
		<i>Iberobathynella barcelensis</i> (Noodt & Galhano, 1969)
		<i>Iberobathynella valbonensis</i> (Galhano, 1970)
		<i>Iberobathynella gracilipes</i> (Braga, 1960)
		<i>Iberobathynella cavadoensis</i> (Noodt & Galhano, 1969)
		<i>Iberobathynella serbani</i> Camacho, 2003
		<i>Iberobathynella pedroi</i> Camacho, 2003
Amphipoda	Melitidae	<i>Pseudoniphargus mateusorum</i> Stock, 1980
		<i>Pseudoniphargus longispinum</i> Stock, 1980
		<i>Pseudoniphargus callaicus</i> Notenboom, 1987
	Niphargidae	<i>Haploglymus bragai</i> Mateus & Mateus 1958
		<i>Metahadzia tavaresi</i> (Mateus & Mateus 1972)
	Bogidiellidae	<i>Bogidiella helenae</i> Mateus & Maciel, 1967
Isopoda	Asellidae	<i>Bragasellus seabrai</i> (Braga, 1943)
		<i>Bragasellus conimbricensis</i> (Braga, 1946)
		<i>Bragasellus frontellum</i> (Braga, 1964)
		<i>Bragasellus incurvatus</i> Afonso, 1984
		<i>Bragasellus pauloae</i> (Braga, 1958)
		<i>Proasellus arthrodilus</i> (Braga, 1945)
		<i>Proasellus assaforensis</i> Afonso, 1988
		<i>Proasellus exiguum</i> Afonso, 1983
		<i>Proasellus lusitanicus</i> (Frade, 1938)
		<i>Proasellus mateusorum</i> Afonso, 1982
		<i>Proasellus nobrei</i> Braga, 1942
		<i>Proasellus rectangulatus</i> Afonso, 1982
		<i>Proasellus rectus</i> Afonso, 1982
		<i>Proasellus spinipes</i> Afonso, 1979
		<i>Proasellus variegatus</i> Afonso, 1982
		<i>Psammellas capitatus</i> Braga, 1968
		<i>Synasellus albicastrensis</i> Braga, 1960
		<i>Synasellus barcelensis</i> Noodt & Galhano, 1969
		<i>Synasellus bragai</i> Afonso, 1987
		<i>Synasellus brigantinus</i> Braga, 1959
		<i>Synasellus capitatus</i> (Braga, 1968)
		<i>Synasellus dissimilis</i> Afonso, 1987
		<i>Synasellus exiguum</i> Braga, 1944
		<i>Synasellus flaviensis</i> Afonso, 1996
		<i>Synasellus fragilis</i> (Braga, 1946)
		<i>Synasellus henrii</i> Afonso, 1987
		<i>Synasellus insignis</i> Afonso, 1984
		<i>Synasellus intermedius</i> Afonso, 1985

Order	Family	Species
		<i>Synasellus lafonensis</i> Braga, 1959
		<i>Synasellus longicauda</i> Braga, 1959
		<i>Synasellus longicornis</i> Afonso, 1978
		<i>Synasellus mariae</i> (Braga, 1942)
		<i>Synasellus mateusi</i> Braga, 1954
		<i>Synasellus meirelesi</i> Braga, 1959
		<i>Synasellus minutus</i> Braga, 1967
		<i>Synasellus nobrei</i> Braga, 1967
		<i>Synasellus pireslimai</i> Braga, 1959
		<i>Synasellus robusticornis</i> Afonso, 1987
		<i>Synasellus serranus</i> Braga, 1967
		<i>Synasellus tirsensis</i> Afonso, 1987
		<i>Synasellus transmontanus</i> Braga, 1959
		<i>Synasellus valpacensis</i> Afonso, 1996
		<i>Synasellus vidaguensis</i> Afonso, 1996
		<i>Synasellus vilacondensis</i> Afonso, 1987
		<i>Synasellus pombalensis</i> Afonso, 1987
		<i>Synasellus favaiensis</i> Eiras, 1974
	Stenasellidae	<i>Stenasellus galhanoae</i> Braga, 1962
		<i>Stenasellus nobrei</i> Braga, 1942

Lazare Botosaneanu ‘Naturalist’ 1927 – 2012



Dr Lazare Botosaneanu (Lazăr Botoșaneanu) died in Amsterdam 19 April 2012, after a short illness. He was almost 85 years old. Lazare Botosaneanu was honorary member of the International Society of Subterranean Biology. On his personal request the funeral card sent out by his wife and two daughters mentioned just the single word ‘naturalist’ to characterize him. During the farewell ceremony at crematorium ‘De Nieuwe Ooster’ in Amsterdam-Watergraafsmeer, a small group of family, friends and colleagues listened to several pieces of classical music selected by him for that occasion. He had emphatically stated not to want flowers or speeches. It was for him clear that in the cycle of life that birth and death are connected, and that his time had come as a natural fact. It’s just how it is in nature, for which he conceived passion at young age. This passion dominated his entire life. ‘Naturalist’ indeed is the best epithet to characterize him.

On the occasion of his 75th birthday, his Romanian colleague Petru Bănărescu wrote about his career and scientific work¹. Lazare Botosaneanu received his aca-

demic training shortly after the Second World War at the Faculty of Biology of the University of Bucharest, the same city where he was born and raised. At a young age he had already showed interest in Zoology. He wrote his first publication –about a new caddisfly species– at the age of 20. As Botosaneanu himself wanted to remark, the prominent Romanian zoologist and man of culture Constantin Motaş had great influence on him. A small portrait of Motaş was one of the few objects adorning his ascetic office in the Amsterdam Museum. Later on, the geneticist and evolutionary biologist Theodosius Dobzhansky and the evolutionary biologist Ernst Mayr greatly influenced his thinking. In 1956, he was appointed as a senior researcher at the Institute of Speleology belonging to the Rumanian Academy of Sciences. Lazare Botosaneanu began his career as an entomologist, and in particular he studied Trichoptera. Until the end of his life he would remain studying this group of insects and most of his publications are dedicated to the Trichoptera and their environment. His colleague and friend Prof. Marcos Gonzalez, of University of Santiago de Compostella (Spain) recently described his contribution to Entomology in an obituary published in the Trichoptera newsletter²

Lazare Botosaneanu’s first contribution to the study of Subterranean Biology took place in 1954, when he co-authored with the Romanian carcinologist Adriana Damian-Georgescu a paper on animals discovered in the drinking water conduits of the city of Bucharest. During the 22 years he worked at the Speleological Institute and thereafter, many more contributions to subterranean biology would follow. His career in Romania came to an abrupt end in 1978. Bănărescu described what happened “..... but, having sent a series of letters to the “Free Europe” broadcast, criticizing the anti-scientific politics of the Ceaușescu government, he was dismissed from the Institute and had to leave Romania in 1978 like his wife and daughters.” He stayed for a short period at the Limnological Station of the Max Planck Institute in Schlitz, Germany, but soon he moved to Amsterdam. At the University of Amsterdam, Prof. Jan Stock managed to get him appointed at a position established for political refugees. In this way he could continue his scientific work as a senior researcher at the Zoological Museum Amsterdam. He got a workplace in the Entomology department of the museum. There he continued working on the systematics, biogeography and ecology of the Tri-

years. *Travaux du Muséum National d’Histoire Naturelle “Grigore Antipa”*, XLIII: 437-456.

¹ Bănărescu, P., 2001. Dr. Lazare Botosaneanu at 75

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² Gonzalez, M.A., 2012. *Braueria* 40.

choptera, but also on subterranean biology, in particular the systematics of certain groups of crustaceans and the biogeography of the aquatic underground fauna. But the Netherlands represented for him also a completely different culture, looser, less structured, more informal.

Early 1979 I met Lazare Botosaneanu for the first time at the office of Jan Stock, not yet being aware of his background and what it means being forced to move to a different society with no ties with family and friends. In Stock's remarkable semi-circular office with views on the Artis Zoo, we prepared an expedition to the Caribbean. It was during the time Jan Stock led a successful scientific program on the biogeography of the stygofauna of the West Indian islands, and Lazare Botosaneanu became strongly involved in that program. This was the beginning of years of intense collaboration with Lazare Botosaneanu, first as a student and later as a junior researcher. We worked intensively together during fieldwork trips to several Caribbean islands and the Venezuelan mainland. Most noteworthy were the two months spent in Haiti exploring subterranean habitats and traveling under difficult circumstances, our first collaboration.

Working together with Lazare Botosaneanu during fieldwork in the Caribbean was exciting and instructive on several fronts. The Jan Stock approach was to work in very small self-organizing teams, and this was new to Lazare Botosaneanu. His earlier experience in the Caribbean came from the rather official Cuban-Romanian Biospeleological expeditions to Cuba³. During these expeditions scientists could concentrate fully on their explorations, logistic and practical support being provided by the Cuban authorities. It was some time before we discovered our complementary skills and were able to operate efficiently as a self-organising team, thereby a generation gap and cultural differences had to be bridged. Lazare Botosaneanu was an extremely disciplined, focused and tenacious field biologist with broad knowledge and interest in natural history in general. In the field, he spent much time observing, aided by a head magnifier, the stygofauna samples and isolating the most vulnerable specimens. Imperturbable, he described each sample station in detail in his field notebook.

Lazare Botosaneanu was a charming and engaging man. Staying at missions in interior Haiti, the mostly French missionaries from Brittany had enjoyable evenings by discussing with him classical music, art, history and politics. Lazare Botosaneanu took pleasure in the cocktail competitions of the clergymen; each missionary believed that his mixture of Barbancourt rum, lime, sugarcane syrup and ice was the ultimate best. He used his charm mostly with success to get help with finding wells, springs and cave entrances, or lodging from the local

people. Lazare Botosaneanu was always eager to learn about local practices and the conditions in which people had to live. His temper could also suddenly turn over; he was sometimes introverted and difficult to access. During long night drives back to our base station he was sitting motionless beside me without saying a word.

Lazare Botosaneanu was a typical observer of Nature and very keen in developing concepts to describe and classify his observations. He did this with meticulous care and harshness for himself and his environment. He was very determined and demanding, both during fieldwork and in his working room at the institute. He needed all these qualities to successfully complete his biggest enterprise in stygobiology, the publication of *Stygofauna Mundi*, the first and so far only faunistic, distributional, and ecological synthesis of the world fauna inhabiting subterranean waters. Actually, he executed this database-like project without aid of modern technologies. The computer was only used by the secretariat that supported him as a type-writing tool. He disliked the use of computer and never did an attempt to learn basic skills. He convinced dozens of authors to contribute, and besides being the editor he took responsibility for several chapters himself as well. He intensively corresponded by handwritten letters in stylograph blue ink or telephone calls with the many authors about the quality and timely delivery of their contributions. In his working room he maintained a strict routine in order to manage all the manuscripts and their subsequent versions. At that time he barely tolerated others in his office. Finally, he managed to convince Brill/Backhuys publishers in Leiden to publish in 1986 the 740 page book in folio format.

After this frantic period Botosaneanu still acted as editor (1985-1992) of the international journal *Stygologia*, devoted to the study of all aspects of subterranean water Biology.

Many of his publications are of a taxonomic nature, both in entomology –his main area of scientific activity– and in stygobiology, his second scientific domain. In stygobiology his taxonomic work mainly focussed on crustaceans, especially anthurid and cirolanid isopods. His descriptions and illustrations of species are detailed and of very high quality with an eye for the variability between individuals, age classes, and sexes. His publications are however not restricted to taxonomy alone, since information and observations on the ecology and evolution of the species are included as well, illustrating Lazare Botosaneanu in the core being a naturalist. His naturalist's perspective is illustrated as well by his synthetic papers on the fauna of natural systems (rivers, karst areas, islands), and on ecological and evolutionary biological issues. Two of them stand out among the rest by its thoroughness: first, his 1991 paper co-authored with John Holsinger, where they dealt with fundamental aspects concerning origin and colonization of the subterranean realm. And second, his 1998 contribution on the role of springs as one

³ Noteworthy is the short note written by Lazare Botosaneanu and Stefan Negrea on the initiation of the Cuban-Romanian biospeleological expeditions to Cuba (1969, 1973) in: Trav. Inst. Spéol. "Émile Racovitza", XLIX: 189-197, Bucarest, 2010.

of the major faunistic pathways connecting the epigean and the subterranean world. Both should deserve the condition of classics of the stygobiological literature.

Lazare Botosaneanu leaves a rich scientific oeuvre of more than 300 publications written in several languages, in the beginning of his career mainly in Romanian and French, later increasingly in English. Beside he published together with others in German, Dutch, Polish, Italian and Spanish; he mastered the Russian language even though he never published in it. He continued to publish into old age. Lazare Botosaneanu was a true polyglot and had a fantastic memory.

A development in the last years of his life that really seized him is the gradual dismantling of the Zoological Museum of the University of Amsterdam. At the time Jan Stock had a leading position in the museum, Lazare Botosaneanu enjoyed a clear and respected position. It was a productive and inspiring time. This gradually changed after Jan Stock's retirement in 1990 and death in 1997. The successors of Jan Stock were not engaged in stygobiology or biospeleology. After his official retirement Lazare Botosaneanu continued to work as an honorary employee but his position was marginalized. He was tolerated rather than respected. Meanwhile, it became clear that the university wanted to dismantle the Zoological Museum and to sell off the monumental buildings. In addition, there was also less appreciation in the university for classical taxonomic work in which he excelled. Lazare Botosaneanu flourished best in a well-structured environment, the developments at the university actually created chaos and where painful to him. Nevertheless he continued his scientific work, characteristic for his tremendous dedication and perseverance.

Lazare Botosaneanu represented a generation of biologists that is almost extinct. By careful observation and profound reflection, and a lifetime lasting fascination for Nature, he developed a profound knowledge and understanding of the natural history of Trichoptera and subterranean fauna and their environments and evolutionary histories. With his dead the Society of Subterranean Biology lost an honorary member who epitomizes stygobiology.

Jos Notenboom, PBL Netherlands Environmental Assessment Agency, Bilthoven, the Netherlands

With the contribution of Damià Jaume, Palma de Mallorca, Spain

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New taxa described in this volume

ARACHNIDA

Acari, Hydrachnidia
Hydryphantidae

Wandesia (Pseudowandesia) minuta sp. nov. Smit : Australia, Victoria, Moleside Creek, Lower Glenelg NP,

Momoniidae

Partidomomonia elongata sp. nov. Smit : Australia, Tasmania, Franklin River, Franklin-Gordon Wild Rivers NP.

Athienemanniidae

Mellamunda tasmanica sp. nov. Smit: Australia, Tasmania, Franklin River, Franklin-Gordon Wild Rivers NP

CRUSTACEA

Isopoda, Microparasellidae

Microcharon boulanouari sp. nov. Coineau, Boutin, Artheau : France, Corbieres, Aude River.

Microcharon ariegensis sp. nov. Coineau, Boutin, Artheau : France, Central Pyrenees, Lachein River.

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