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A new microphthalmic stygobitic *Graptodytes* Seidlitz from Morocco, with a molecular phylogeny of the genus (Coleoptera, Dytiscidae)

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Abstract

We describe *Graptodytes eremitus* **n.sp.** (Coleoptera, Dytiscidae), a depigmented, microphthalmic stygobitic species found in a pool in the deep area of a cave in the High Atlas of Morocco. To establish its phylogenetic position we inferred a molecular phylogeny of the genus *Graptodytes* Seidlitz, using ca. 1.7 Kb of four mitochondrial genes for 18 of the 23 previously known species and subspecies of the genus. *Graptodytes* can be separated in three well supported main lineages, 1) the *G. flavipes* lineage (apex of median lobe narrow in ventral view), 2) the *G. granularis* lineage (apex of median lobe expanded and strongly asymmetrical in ventral view), and 3) the *G. varius* lineage (apex of median lobe expanded but symmetrical in ventral view). The *G. varius* lineage includes the *G. aequalis* and *G. varius* groups, the latter including *G. eremitus* **n.sp.** as sister to *G. delectus* Wollaston (Canary Islands) plus the *G. varius* complex. A molecular clock approach, using a calibration rate of 2.3% divergence/MY for the combined mitochondrial sequence, estimated the origin of the diversification within the genus at ca. 7MY (late Miocene), and the origin of *G. eremitus* **n.sp**. at ca. 2 MY (Pliocene-Pleistocene boundary).

Key words: Coleoptera, Dytiscidae, *Graptodytes*, subterranean medium, new species, diving beetle, molecular phylogeny

Introduction

The genus *Graptodytes* Seidlitz (Coleoptera, Dytiscidae, Hydroporinae) includes 21 known species and two subspecies, with a distribution centred in the Mediterranean basin but including the whole western Palaearctic region, with one species in Uzbekistan (Nilsson 2001, 2003). It belongs to a group of genera including *Metaporus* Guignot, *Stictonectes* Brinck, *Porhydrus* Guignot, *Rhithrodytes* Bameul and the stygobitic *Siettitia* Abeille de Perrin and *Iberoporus* Castro & Delgado (the "*Graptodytes* group", Ribera *et al.* 2002, 2008). The morphology of the group is rather homogeneous, and their affinities have long been recognised: they were all included within the genus *Graptodytes* by Seidlitz (1887), with the exception of the subsequently described stygobitic genera. Within the *Graptodytes* group of genera, *Graptodytes* seems sister to *Metaporus* (Ribera *et al*. 2008), with only two known species (Toledo & Hosseinie 2003). These two genera are morphologically very similar, the main difference being the lack in *Metaporus* of a small, longitudinal impressions on each side of the pronotum characteristic of *Graptodytes* and some other genera of the group.

The *Graptodytes* group of genera includes the only known European stygobitic Dytiscidae, the genera *Siettitia*, with two species in France, and *Iberoporus,* with one species in south Spain (Nilsson 2003). Within *Graptodytes* some species have pale, flattened bodies and are regularly found in interstitial habitats at the edge of rivers or in wells (e.g. *G. fractus* (Sharp), *G. kuchtae* (Breit), *G. aurasius* (Jeannel), see below). In this paper we describe a species known from a single specimen collected in a small pool in the deep area of a cave in Morocco, with morphological characters typical of stygobitic fauna: depigmented, with a wide head,

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TABLE 1. Specimens used in the study, with locality, collector, voucher reference numbers and accession numbers for the sequences.

all	species	voucher	ref MNCN	country	locality	leg	cox1	rrnL-trnL-nad1
1	*G. aequalis* (Zimmermann)	MNCN-AI712	24058	Spain	Córdoba, Sierra de Córdoba, Arroyo de los Arenales, 6.11.2004	A. Castro	HM588263	HM588601
2	*G. aequalis* (Zimmermann)	NHM-MA206		Morocco	Debdou, Meson forestiere, 6.4.1999	I. Ribera	HM588264	AY250910
3	*G. atlantis* (Théry)	MNCN-AI921	24267	Morocco	8, Azrou, Lac Afenourir, 29.4.2000	I. Ribera	HM588265	HM588602
4	*G. atlantis* (Théry)	NHM-IR388		Morocco	8, Azrou, Lac Afenourir, 29.4.2000	I. Ribera	HM588266	AY250911
5	*G. bilineatus* (Sturm)	MNCN-AI608	23954	Sweden	Västerbotten prov., Åmsele, Vindelälven, river lagoon 18/9-05, 64º31'04"N, 19º20'52"E, 18.9.2005	A.N. Nilsson	HM588267	HM588603
6	*G. castilianus* Fery	MNCN-AI1316	24662	Spain	3 ES Navarra 21.7.2004/Pitillas: pond in crossroad/42°25’29“N 1°38‘35“ W/360m	I. Ribera & A. Cieslak	HM588268	HM588604
7	*G. delectus* (Wollaston)	MNCN-AI1092	24438	Tenerife	Chamorga, Bco. Roque Bermejo, 20.7.2006	A. Castro	HM588269	HM588605
8	*G. delectus* (Wollaston)	NHM-IR637		Gran Canaria	S. Nicolas de Tolentino, bco. Guy Guy grande, 14.4.2001	I. Ribera & A. Cieslak	HM588270	AY250913
9	*G. eremitus* sp.n.	IBE-AF33		Morocco	Morocco, Tiqqi 28.7.2008 / cave Doussoulile N 30°44,529’ W9°19,803’	A. Faille et al.	HM588271	HM588606
10	*G. flavipes* (Olivier)	MNCN-AI622	23968	Spain	Córdoba, Sierra de Córdoba, Arroyo de los Arenales, 6.11.2004	A. Castro	HM588272	HM588607
11	*G. flavipes* (Olivier)	NHM-IR40		Spain	Huelva, Almonte, poblado forestal 26.7.1998	I. Ribera	HM588273	AY250914
12	*G. fractus* (Sharp)	MNCN-AI627	23973	Spain	Córdoba, Sierra de Córdoba, Arroyo de los Arenales, 16.4.2005	A. Castro	HM588274	HM588608
13	*G. fractus* (Sharp)	MNCN-AI1200	24546	Greece	01/20.07.2006 - Ípeiros (Ioanniná). Kipi, rivière Voidomatis. N39°51'42.2", E020°47'10.4", 730m	P. & V. Ponel	HM588275	
14	*G. fractus* (Sharp)	NHM-IR238		Spain	Albacete, Rio Argos en las okas, 7.4.2000	A. Millán	HM588276	HM588609
15	*G. fractus* (Sharp)	NHM-IR758		Tunisia	1, Rd. Tabarka-Aïn-Draham, stream 1 km N Aïn-Draham, 23.10.2001	I. Ribera & A. Cieslak	HM588277	HM588610
16	*G. granularis* (Linnaeus)	MNCN-AI609	23955	Sweden	Västerbotten prov., Åmsele, Vindelälven, river lagoon 18/9-05, 64º31'04"N, 19º20'52"E, 18.9.2005	A.N. Nilsson	HM588278	HM588611
17	*G. granularis* (Linnaeus)	NHM-IR784		Austria	Voralberg, Pfäanderstock, Bodensee, 2.7.2001	I. Ribera	HM588279	

continued next page

TABLE 1. (continued)

all	species	voucher	ref MNCN	country	locality	leg	cox1	rrnL-trnL-nad1
18	*G. ignotus* (Mulsant & Rey)	MNCN-DM9	24674	Corsica	Porto-Vecchio, l'Ospedale, 5, 18.9.1999	I. Ribera & A. Cieslak	HM588280	HM588612
19	*G. ignotus* (Mulsant & Rey)	MNCN-AI939	24285	Morocco	58, Tachokchte, Assif Siroua, ca. 1500 m, 19.4.2001	I. Ribera & A. Cieslak	HM588281	
20	*G. ignotus* (Mulsant & Rey)	MNCN-AI876	24222	Sardinia	5, Sassari, Mt Limbara, entre Valliciola et le sommet. N 40º51'27.5" E9º9'25.0" 1028m, 20.5.2006	P. & V. Ponel	HM588282	
21	*G. ignotus* (Mulsant & Rey)	MNCN-AI908	24254	Morocco	27 Morocco 24.4.2000 / Oued Âit-Baha, Tioulit / 29º52'59"N 9º00'65"W / 1150m	I. Ribera	HM588283	
22	*G. ignotus* (Mulsant & Rey)	MNCN-AI623	23969	Spain	Córdoba, Sierra de Córdoba, Arroyo de los Arenales, 6.11.2004	A. Castro	HM588284	
23	*G. ignotus* (Mulsant & Rey)	IBE-AF124		Morocco	[87] Morocco, Tetuan 11.4.2009 / rd N Souk-Khemis-des-Anjra / N35º43'17.1" W5º31'21.3" 178m	I. Ribera & A. Cieslak	HM588285	
24	*G. ignotus* (Mulsant & Rey)	NHM-MA251		Spain	Cadiz, Ventorillo de las Canillas, Afl. R. Hozgarganta 27.7.1998	I. Ribera	HM588286	HM588613
25	*G. ignotus* (Mulsant & Rey)	NHM-IR531		Spain	Girona, Estanys de Capmany, 3.2001	P. Aguilera	HM588287	AY250915
26	*G. kuchtae* (Breit)	MNCN-AI177	23523	Mallorca	5, Ternelles, Torrent de Ternelles, 72m, N 39º53'37.2" E3º00'14.9", 4.10.2004	I. Ribera & A. Cieslak	HM588288	HM588614
27	*G. kuchtae* (Breit)	NHM-IR500		Mallorca	3, Embalse Cuber, residual ponds below dam, 11.11.2000	I. Ribera & A. Cieslak	HM588289	AY250916
28	*G. pictus* (Fabricius)	MNCN-AI660	24006	Poland	1 POL. Zachodniopomorsky/16.8.04 Dygowo: garden pond/N54º07'59" E15º42'49"/25m	I. Ribera & A. Cieslak	HM588290	HM588615
29	*G. pietrii* Normand	MNCN-HI11	24776	Algeria	V50,8.6.2006 Algeria, Raml-Souk (puit) / N 36° 47.494' E 008° 31.326' / 101.19 m, Bouzid leg.	S. Bouzid	HM588291	HM588617
30	*G. pietrii* Normand	MNCN-DM37	24702	Tunisia	3,Rd. Beja-Teboursouk, Oued 3 km NW Teboursouk, 23.10.2001	I. Ribera & A. Cieslak	HM588292	HM588616
31	*G. sedilloti* (Régimbart)	NHM-IR585		Cyprus	3.2001	K. Miller	HM588294	HM588619
32	*G. sedilloti phrygius* Guignot	MNCN-AI111	23457	Chios	WGS 84 683/4267 Marmaro marsh, 19.4.2004	G.N. Foster	HM588293	HM588618

continued next page

TABLE 1. (continued)

all	species	voucher	ref MNCN	country	locality	leg	cox1	rrnL-trnL-nad1
33	*G. siculus* Fery	MNCN-AH162		Sicily	8 Sicilia, Parco dei Nebrodi/ Stream Trail Lago Urio - Plla. dell'Obolo/ 1085m 33N 448991 E, 4193859 N/ 13.06.07	P. Abellán & F. Picazo	HM588295	HM588620
34	*G. varius* (Aubé)	IBE-PA264		Spain	2 ESP. Granada 4.1.03/Albuñuelas, r. Saleres	I. Ribera & A. Cieslak	HM588296	
35	*G. varius* (Aubé)	MNCN-AH160		Sicily	8 Sicilia, Parco dei Nebrodi/ Stream Trail Lago Urio - Plla. dell'Obolo/ 1085m 33N 448991 E, 4193859 N/ 13.06.07	P. Abellán & F. Picazo	HM588297	HM588622
36	*G. varius* (Aubé)	MNCN-AH185		Sicily	18 Sicilia, Parco delle Madonie/ Stream in Piano Battaglia/ 1150m 33N 412095 E, 4194166 N/ 15.06.07	P. Abellán & F. Picazo	HM588298	
37	*G. varius* (Aubé)	MNCN-AI1158	24504	Sardinia	4, Sassari, Mt Limbara, Madonna della Neve, ruisseau près chapelle. N 40º51'09.6" E 9º10'10.4", 1242m, 20.5.2006	P. & V. Ponel	HM588299	
38	*G. varius* (Aubé)	MNCN-HI16	24781	Algeria	V67,24.8.2006 Algeria, Aïn Damous / N 36° 25.350' E 007° 51.367' / 523.34 m	S. Bouzid	HM588300	HM588621
39	*G. varius* (Aubé)	MNCN-DM38	24703	Tunisia	3,Rd. Beja-Teboursouk, Oued 3 km NW Teboursouk, 23.10.2001	I. Ribera & A. Cieslak	HM588301	
40	*G. varius* (Aubé)	NHM-IR532		Spain	Girona, Estanys de Capmany, 3.2001	P. Aguilera	HM588302	HM588623
41	*G. veterator* (Zimmermann)	MNCN-AH161		Sicily	8 Sicilia, Parco dei Nebrodi/ Stream Trail Lago Urio - Plla. dell'Obolo/ 1085m 33N 448991 E, 4193859 N/ 13.06.07	P. Abellán & F. Picazo	HM588304	HM588625
42	*G. veterator* (Zimmermann)	MNCN-AI1207	24553	Greece	01/26.07.2006 - Makedonía (Kastoria). Fotini, rive N-E du lac de Kastoriá. N40°33'00.5", E021°19'06.3", 633 m.	P. & V. Ponel	HM588305	
43	*G. veterator behningi* Zaitzev	MNCN-AI774	24120	Turkey	TR03 Düzce 23.4.2006, Rd. to Kartalkaya from Çaydurt, pools in mountain pass, 1700m N40º40'20" E31º47'05"	I. Ribera	HM588303	HM588624
44	*Metaporus meridionalis* (Aubé)	MNCN-AI917	24263	Morocco	8, Azrou, Lac Afenourir, 29.4.2000	I. Ribera	HM588306	
45	*Metaporus meridionalis* (Aubé)	NHM-IR34		Spain	Albacete, Robledo, Ojos de Villaverde, 7.9.1997	I. Ribera	HM588307	AY250919

microphthalmic, and with long sensory setae (see e.g. Castro & Delgado 2001; Miller *et al*. 2009). We study the phylogenetic relationships of this new species with a molecular phylogeny of the genus, including all known species but five.

Material and methods

Taxon sampling, DNA extraction and sequencing. Specimens were preserved in absolute ethanol in the field (Table 1). Extractions of single specimens were non-destructive, using a standard phenol-chloroform method or the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany). Vouchers and DNA samples are kept in the collections of the Museo Nacional de Ciencias Naturales, Madrid (MNCN), the Institute of Evolutionary Biology, Barcelona (IBE) and the Muséum National d'Histoire Naturelle, Paris (MNHN).

We included multiple examples of most species of the genus *Graptodytes*, with the exception of *G. aurasius*, *G. bussleri* Fery, *G bremondi* Guignot, *G. parisii* Gridelli and *G. snizeki* Hendrich (Table 1). Of these, the latter three are known only from the types, and the former two have a very restricted distribution in north Algeria and Israel respectively (Nilsson 2003). Trees were rooted in *Metaporus meridionalis* (Aubé), which is sister to the genus *Graptodytes* (Ribera *et al*. 2008).

We amplified fragments of four mitochondrial genes: 3' end of *cox1*; 5' end of *rrnL*; full *trnL* and 5' end of *nad1* (see Table 2 for primers used, and Ribera *et al*. 2002 for general PCR conditions), with a total of ca. 1600 bp. Sequences were assembled and edited using Sequencher TM 4.1.4 (Gene Codes, Inc., Ann Arbor, MI). New sequences have been deposited in GenBank (NCBI) with Accession Numbers HM588263-HM588307 and HM588601-HM588625 (Table 1).

TABLE 2. Primers used in the study. F, forward; R, reverse.

Gene	Name	Sense	Sequence	Reference
cox1	Jerry (M202)	F	CAACATTTATTTTGATTTTTTGG	Simon *et al*. (1994)
	Pat (M70)	R	TCCA(A)TGCACTAATCTGCCATATTA	Simon *et al*. (1994)
	Chy	F	T(A/T)GTAGCCCA(T/C)TTTCATTA(T/C)GT	Ribera *et al*. (2010)
	Tom	R	AC(A/G)TAATGAAA(A/G)TGGGCTAC(T/A)A	Ribera *et al*. (2010)
rrnL-nad1	16saR (M14)	F	CGCCTGTTTA(A/T)CAAAAACAT	Simon *et al*. (1994)
	16Sa	R	ATGTTTTTGTTAAACAGGCG	Simon *et al*. (1994)
	16Sb	R	CCGGTCTGAACTCAGATCATGT	Simon *et al*. (1994)
	ND1A (M223)	R	GGTCCCTTACGAATTTGAATATATCCT	Simon *et al*. (1994)

Phylogenetic analyses. We aligned the sequences using the program MAFFT online v.6 and the Q-INS-i algorithm (Katoh & Toh 2008), a progressive pair-wise method with secondary refinement. Bayesian analyses were conducted on a combined data matrix with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001), which runs two independent, simultaneous analyses, using three partitions corresponding to the *cox1*, *rrnL*+*trnL* and *nad1* fragments and with a GTR+I+G evolutionary model. MrBayes ran for 18x10^6 generations using default values, saving trees at each 500 generations. "Burn-in" values were established after visual examination of a plot of the standard deviation of the split frequencies between two simultaneous runs. We also used Maximum Likelihood as implemented in the on-line version of RAxML (which includes an estimation of bootstrap node support, Stamatakis *et al*. 2008), using GTR+G as the evolutionary model and the same gene partitions used in MrBayes.

Estimation of the ages of diversification. To calibrate the tree we use molecular clock-methods, with a combined rate for the mitochondrial genes of 2.3% per MY (i.e. the standard arthropod rate, Brower 1994), shown to be similar to rates obtained based on fossil and biogeographic calibrations in different groups of Coleoptera (see e.g. Ribera *et al*. 2010; Papadopoulou *et al*. 2009). To obtain an ultrametric tree we used

Bayesian probabilities as implemented in Beast 1.4.7 (Drummond & Rambaut 2007) with a matrix including only specimens with the complete sequence for both mitochondrial genes (Table 1). Well supported nodes according to the results with the combined data were constrained to be monophyletic (see below), and a GTR+I+G model was enforced with an uncorrelated lognormal relaxed clock and a Yule process speciation model. Priors and other parameters were left with default values, with the exception of the prior of the evolutionary rate, which was set to a normal distribution with mean of 0.0115 substitutions/site/MY and a standard deviation of 0.001. The results of two independent runs were merged with Tracer v1.4 and TreeAnnotator v1.4.7 (Drummond & Rambaut 2007).

Results and discussion

Graptodytes eremitus n.sp.

(Figs 1–6, 9–11)

Type locality. Cave Doussoulile, Tiqqi, SW Morocco (N 30°44,529’ W9°19,803’).

Type material. Holotype male, “Morocco, Tiqqi 28.VII.2008 / cave Doussoulile N 30°44,529’ W9°19,803’ / leg J.M. Bichain, A. Faille, B. Junger & M. Perreau” plus holotype label. Specimen and DNA voucher number IBE-AF33, MNHN Wit Tamdoun expedition code WT-NP51. DNA aliquotes preserved in the DNA and tissue collections of the MNCN and IBE; voucher specimen, with aedeagus mounted in a transparent card, preserved in the MNHN.

Diagnosis. The morphology of the aedeagus clearly places the new species in the genus *Graptodytes*, despite the lack of longitudinal impressions in the pronotum. The large size (ca. 3 mm), parallel-sided shape, uniformly pale brown colour, small eyes and shape of the median lobe allow an easy separation of *G. eremitus* n.sp. from all related species of the genus.

Description. Habitus as in Figs 1–4. Length 3.05 mm, maximum width 1.30 mm. Body outline elongated, parallel-sided, flattened. Colour uniformly light brown, cuticle translucent (Figs 3–4). Body surface with a very fine, hardly visible dense microreticulation.

Head very large, wide, regularly round, glabrous. Eyes reduced, flat; ommatidia well defined on periphery of eye but hardly distinguishable in central part; maximum diameter of approximately eight ommatidia and 1/4 of length of head (vs. 12–15 ommatidia and half length of head in species of the *G. varius* group) (Figs 6–8). Eyes surrounded first by a smooth and flat area and then a ring of stronger punctures with some setae. Antennae long and slender.

Pronotum finely bordered; wider in anterior part, slightly wider than elytra; posterior part only slightly narrower than base of elytra. Angle between pronotum and elytra obtuse. Sides of pronotum with some very long, fine white setae. Surface with very sparse, stronger setiferous punctures with whitish short fine setae; with an irregular row of setiferous punctures on lateral, anterior and posterior margins. Submarginal lateral marks on pronotum almost imperceptible, forming very shallow depressions externally, although an internal mark is clearly visible by transparency (Fig. 3).

Elytra elongated, parallel-sided, uniformly pale brown, with traces of irregular serial rows of setiferous punctures, inter-strial spaces slightly raised. Margins with rows of very long, fine whitish setae (Fig. 5).

Legs long and slender, protarsal claws of male simple, without apparent modifications. Ventral side of anterior and middle femora with two rows of very robust setae. Tibia with long, whitish sensory setae.

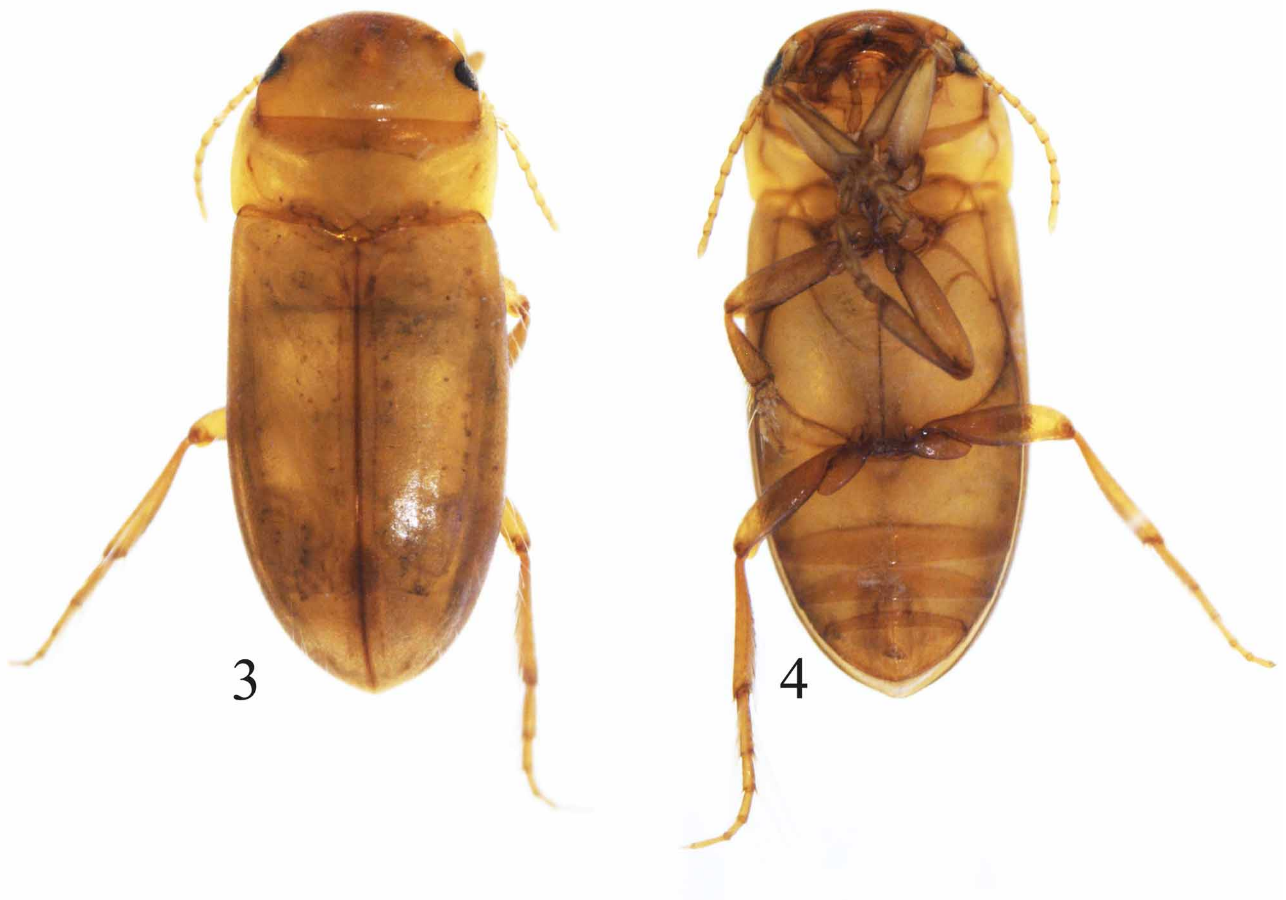
Ventral surface covered with long, sparse fine setae (denser than on upper surface). Medial part of posterior margin of metacoxa deeply incised (Fig. 4).

Aedeagus as in Figs 9–11, apex of median lobe flat in lateral view, very slightly asymmetrical and with a medial emargination in ventral view; parameres as in Fig. 11.

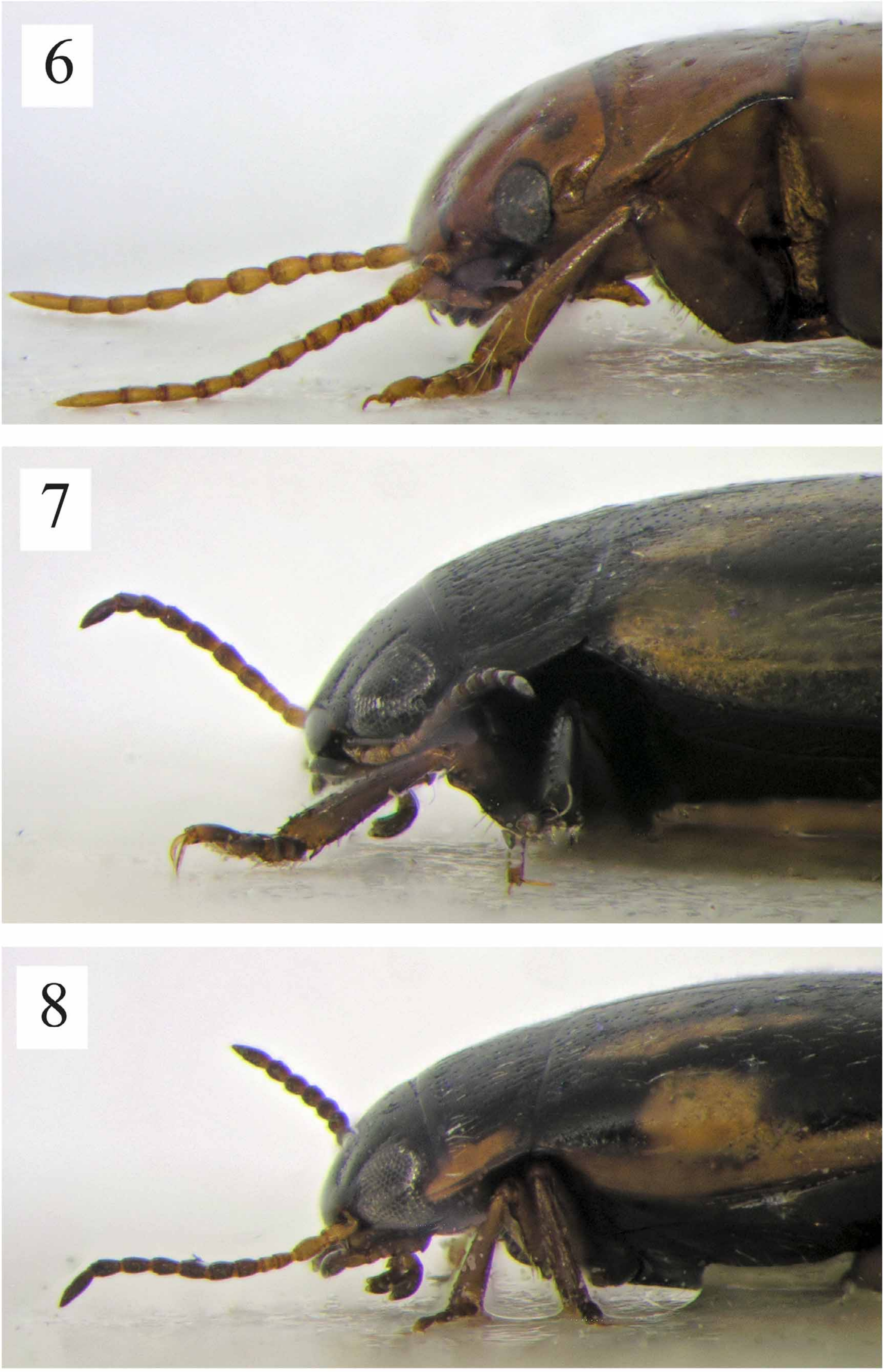
Habitat. The single known specimen was found in a small pool (a siphon) in the deep part of the Doussoulile cave (at ca. 70 meters below the level of the entrance). The pool was on rock with some fine sediment, with clear water, and the specimen was actively swimming. Other fauna collected in the same cave were Collembola, Oniscidae and Amphipoda (Crustacea), Acari, and some spiders and Coleoptera (*Antisphodrus* sp., *Porotachys bisulcatus* Nicolay and trogloxene Staphylinidae).

Etymology. The hermit *Graptodytes*, from the Latin *eremita*, and this from the Greek *eremites*, from *eremos* (desert), in reference to the loneliness of the single known specimen in the deep area of a cave in the (relative) vicinity of the Sahara desert.

FIGURES 1–2. Habitus of *Graptodytes eremitus* **n. sp.** (holotype, after DNA extraction, specimen mounted dry); 1, dorsal view (photo: J. Fresneda); 2, lateral view (photo: J. Gómez-Zurita).



FIGURES 3–5. *Graptodytes eremitus* **n. sp.** (holotype, previous to DNA extraction, submersed in ethanol); 3, dorsal view; 4, ventral view; 5, detail of the marginal setae of the elytra. Note the membranous wings, the genitalia and the longitudinal impressions at both sides of the pronotum, visible through the translucent cuticle (photos: I. Ribera).



FIGURES 6–8. Detail of the lateral view of the left eye of 6, *G. eremitus* **n. sp.**; 7, *G. atlantis*; 8, *G. ignotus* (photos: J. Fresneda).

FIGURES 9–11. Genitalia of *G. eremitus* **n. sp.** (holotype); 9, median lobe, ventral view; 10, median lobe, lateral view; 11, right paramere (photos: J. Fresneda).

A molecular phylogeny of the genus *Graptodytes*

MrBayes runs converged at ca. 13x10^6 generations, with values of the split frequency below 0.005. The last 5x10^6 generations (i.e. 10,000 trees) were used to compile the consensus topology and estimate the parameters.

The results of the two phylogenetic reconstructions (Bayesian probabilities and Maximum Likelihood) were very similar, with differences only in the level of support of some nodes and in the topology of some of the relationships among terminal taxa in the *Graptodytes varius* complex (see below). The genus was divided in three major lineages, which can be characterised by the general shape of the male genitalia:

1) The single species *G. flavipes* (Olivier), sister to the rest of the species of the genus, with median lobe narrow and with an acute apex in ventral view (e.g. Fery 1995: Fig. 14)

2) The *G. granularis* lineage. It includes species with a strongly asymmetrical expansion at the apex of the median lobe in ventral view: *G. sedilloti* (Régimbart), *G. granularis* (Linnaeus), *G. pictus* (Fabricius), *G. bilineatus* (Sturm) and *G. veterator* (Zimmermann). Differences between Sicilian, Greek and Turkish specimens of *G. veterator* were substantial, with the Turkish sister to the Sicilian and Greek specimens (Figs 12–13, Table 1). Specimens from the Middle East were described as a distinct subspecies, *G. veterator behningi* Zaitzev, based on differences in elytral pattern (Zaitzev 1927). On the contrary, haplotypes of *G. granularis* and *G. pictus* appear intermixed and with deep divergences within *G. granularis*, despite the clear morphological differences among individuals (e.g. Nilsson & Holmen 1995).

3) The *G. varius* lineage. It includes species with an expanded but symmetrical (or only slightly asymmetrical) apex of the median lobe in ventral view. The basal split separates two groups, the *G. aequalis* group in the sense of Fery (1995) and what can be called the *G. varius* group. The *G. aequalis* group is formed by four closely related species, *G. aequalis* (Zimmermann), *G. pietrii* Normand, *G. castilianus* Fery and *G. siculus* Fery, the latter three with modified male metatrochanters and the apex of the male parameres with a digital expansion of characteristic shape (Fery 1995). In both analyses (RAxML and MrBayes) the three European species (*G. aequalis* in S Iberia and West Morocco, *G. castilianus* in central Iberia, and *G. siculus* in Sicily) were sister or nested within the East Maghrebian *G. pietrii* (East Argelia and Tunisia), suggesting an

East Maghrebian origin of the group (Fig. 12). The *G. varius* lineage has *G. atlantis* (Théry) from central Morocco, *G. eremitus* n.sp. also from central Morocco, *G. delectus* (Wollaston) from the Canaries and the complex of *G. varius* (Aubé), including *G. ignotus* (Mulsant & Rey), *G. kuchtae* and *G. fractus*. Within the latter species complex there does not seem to be a clear phylogenetic structure as measured with the mitochondrial markers used here, which is in agreement with the difficulties in establishing clear morphological boundaries between the species (e.g. Guignot 1959: 103). According to our results, the *G. varius* group would have its origin in the western Maghreb, in Morocco.

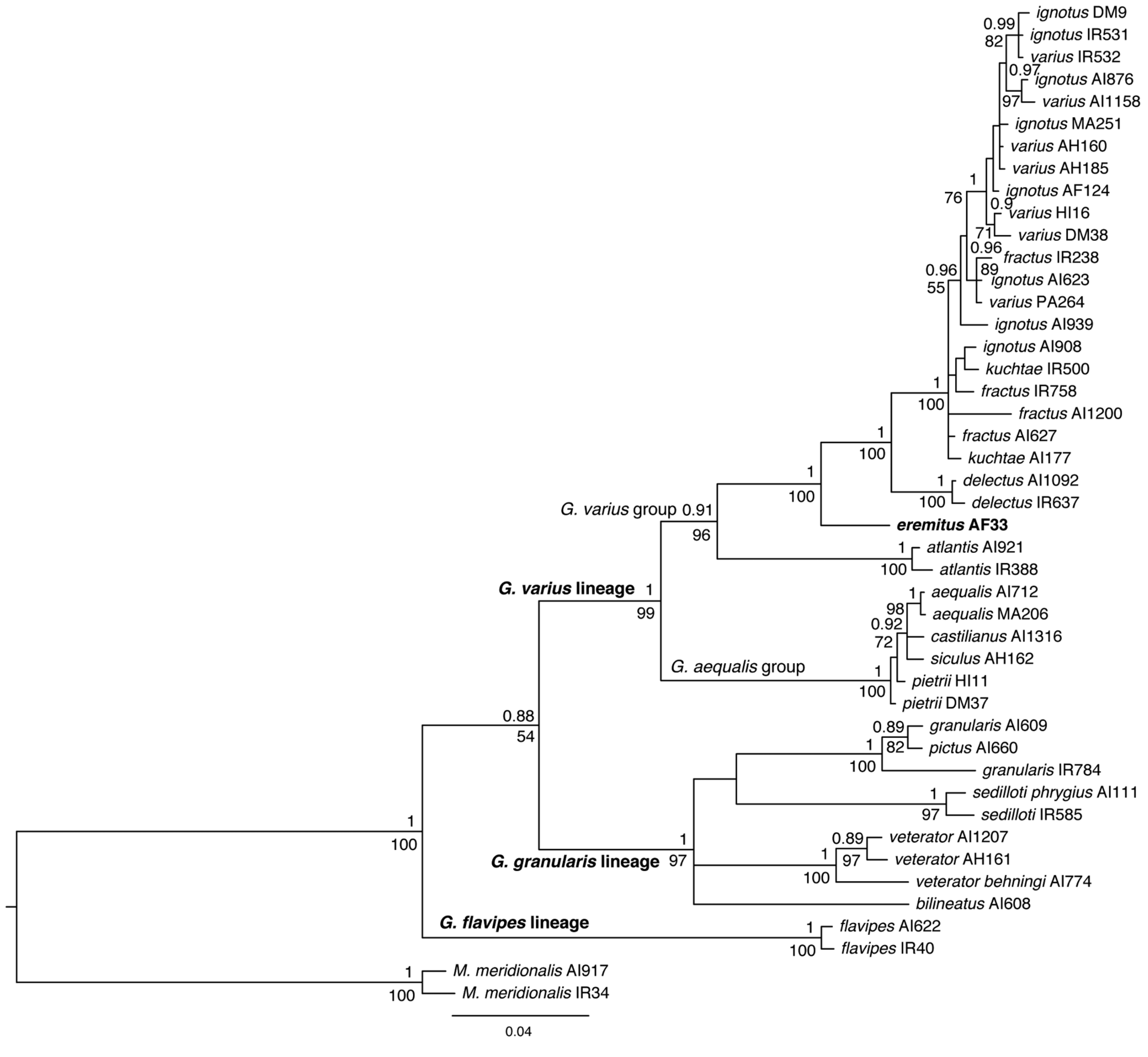


FIGURE 12. Phylogram obtained with MrBayes. Numbers above nodes, posterior probabilities (if above 0.5); below nodes, bootstrap support values obtained in RAxML (if above 50%). See Table 1 for codes and locality data of the specimens.

The phylogenetic relationships of some of the five species of *Graptodytes* for which there are no molecular data can be tentatively hypothesized according to their morphological characters. Thus, *G. bremondi* and *G. aurasius* (both from North Africa) clearly belong to the *G. varius* complex, both for their external morphology and the shape of the median lobe (Guignot 1959). *Graptodytes snizekin* clearly belongs to the *G. granularis* lineage (Hendrich 1993), but *G. bussleri*, despite being apparently close to *G. sedilloti*, has a symmetrical apex of the median lobe (Fery 1994) thus its inclusion in the *G. granularis* lineage is doubtful. Finally, the affinities of *G. parisii* (from the Tassili in Argelia) are unknown, as only the original description is available and no specimens other than the female holotype are known, although Guignot (1959) suggested it is an isolated species, which may even be related to different genera within the *Graptodytes* group.

According to the temporal reconstruction of the diversification of *Graptodytes* obtained with a standard mitochondrial rate of 2.3% divergence per MY, the most recent common ancestor of the current species of the genus has to be dated at ca. 7±2 MY, in the late Miocene, with the separation of the two major lineages dating back to ca. 6±1.5 MY (i.e. Messinian). The origin of *G. eremitus* n.sp. was estimated to be at ca. 2±0.6 MY, in the lower Pleistocene. All groups of closely related species (*G. granularis* and *G. pictus*, and those of the *G. aequalis* and *G. varius* groups) are of more recent, middle to late Pleistocene origin, in agreement with estimations for other species of Mediterranean Dytiscidae with narrow distributions (Ribera & Vogler 2004).

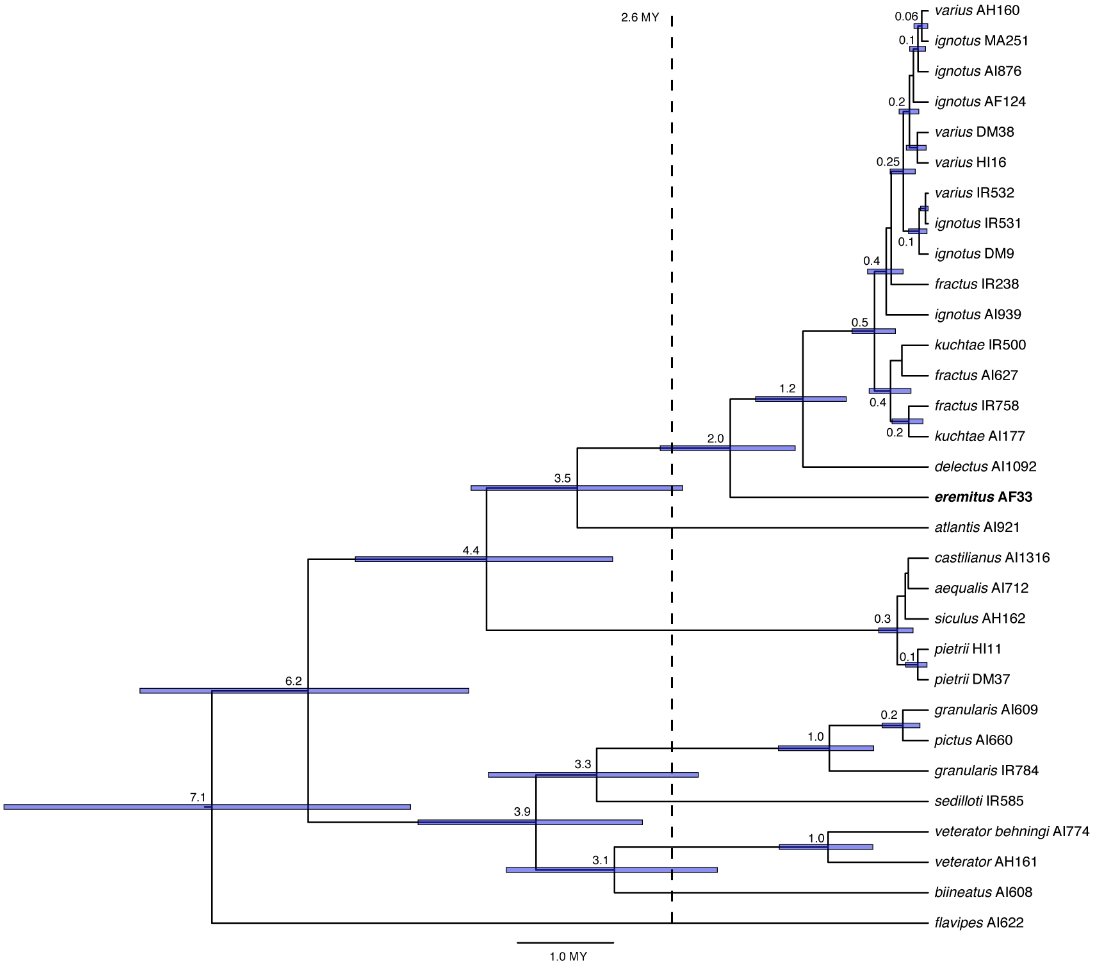


FIGURE 13. Ultrametric tree obtained with Beast using the combined sequence, excluding outgroups and specimens with incomplete data (see Table 1). Numbers in nodes, age estimate (MY) using a rate of 2.3% MY as prior; bars, 95% confidence intervals. The dashed line marks the Pliocene-Pleistocene boundary. See Table 1 for codes and locality data of the specimens.

Origin of the stygobitic or interstitial species

The *Graptodytes* group of genera includes the only known European stygobitic species of water beetles, *Siettitia balsetensis* Abeille de Perrin, *S. avenionensis* Guignot and *Iberoporus cermenius* Castro & Delgado. The two genera are closely related, and closely related also to the genus *Rhithrodytes*, which includes some species with interstitial habits (e.g. *R. agnus* Foster) (Régimbart 1905; Zimmermann 1932; Bameul 1989). In the genus *Graptodytes*, some species are known to have interstitial habits, being regularly found in wells (e.g. *G. kuchtae* in Mallorca in company of stygobitic crustacea, D. Jaume personal communication 2009), or, occasionally, in caves (e.g. *G. varius* in the Wit Tamdoun cave at a depth of about 2km, A. Faille leg.). Some species have a flattened body and are usually depigmented (*G. fractus*), or have the typical parallel-sided body shape of stygobitic species (*G. aurasius*). The latter has, in the words of its descriptor, "étonnamment l'aspect du *Sietittia balsetensis*" (Jeannel 1907: 18), but with well developed eyes and incomplete longitudinal impressions on the sides of the pronotum. The species *M. meridionalis* can also be found deep in the gravel of the edge of rivers or streams, although it is also regularly found in well vegetated isolated ponds, not linked to running water.

It is interesting to note the repeated development of interstitial or stygobitic habits in the same lineage, apparently without any common external trigger and at different geological times. This scenario would be in agreement with the "habitat shift" hypothesis for the origin of the subterranean fauna (Howarth 1980), i.e. a colonization of the subterranean medium not driven by climatic change or the unfavourable conditions of the epigean habitat, in contrast to what has been hypothesized for the large radiation of stygobiontic dytiscids in the calcretes of SW Australia (Leys *et al.* 2003). With the possible exception of the two species of *Siettitia* there are no known instances of speciation within the subterranean or interstitial medium in the *Graptodytes* group of genera, but our knowledge of the stygobitic Coleoptera of the Iberian peninsula and the Maghreb is too incomplete to draw any firm conclusions in this respect.

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