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# Biodiversity patterns of cavernicolous ground-beetles and their conservation status in the Azores, with the description of a new species: *Trechus isabelae* n. sp. (Coleoptera: Carabidae: Trechinae)

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## Abstract

Diversity patterns of cave and epigean Trechinae (Coleoptera, Carabidae) from the Azores (Portugal) are reported based on recently standardized sampling protocols in different habitats of this geologically young and isolated volcanic archipelago. A total of 10 species are studied, including *Trechus isabelae* **n. sp.**, collected in a volcanic pit on São Jorge, one of the nine islands of the Azores. This new *Trechus* species represents the eighth species of Trechinae described from the underground environment of the Azores. An identification key for the Azorean species of *Trechus* is provided along with additional information per species on their distribution and conservation status in the archipelago. Possible reasons for the different degrees of adaptation to the conditions of the underground environment exhibited by Trechinae are also discussed.

Key words: Cave beetles, Trechus, Thalassophilus, taxonomy, ecology, conservation, Azores

## Introduction

The Azores, an archipelago comprising nine islands in the North Atlantic, are rich in lava tubes and volcanic pits that are inhabited by an interesting troglobitic fauna (see Borges & Oromí 1991; 1994; in press; Pereira *et al.* in press). The recent age of most of these islands (Borges & Brown 1999) may explain the very few cases of polytypic genera among the Azorean endemic insects, one being the genus *Trechus* which displays one the of most interesting cases of diversification in this archipelago (see also Borges *et al.* 2005a; Amorim 2005). During the past two decades, intense biospeleological, entomological and ecological studies have been carried out in the Azores (see Borges *et al.* 2004; 2005b; 2005c; 2006; Borges & Oromí in press; Amorim 2005), resulting in a total of nine new species of Trechinae: *Thalassophilus azoricus* Oromí & Borges, 1991 from São Miguel; *Trechus terceiranus* Machado, 1988 and *Trechus terrabravensis* Borges, Serrano & Amorim, 2004 from Terceira; *Trechus jorgensis* Oromí & Borges, 1991 and *Trechus isabelae* **n. sp.** from São Jorge; *Trechus picoensis* Machado, 1988, *Trechus montanheirorum* Oromí & Borges, 1991 and *Trechus pereirai* Borges, Ser-

rano & Amorim, 2004 from Pico; and *Trechus oromii* Borges, Serrano & Amorim, 2004 from Faial. Only two species of Azorean *Trechus* are epigean: *T. terrabravensis* living in the native forest of Terceira, and *Trechus torretassoi* Jeannel from São Miguel. The latter was the first *Trechus* species to be described from the archipelago (Jeannel 1937), but the fact that, despite all collecting efforts, it has not been recorded since 1985 (see: Gillerfors 1986), may indicate that it has become extinct (see: Borges *et al.* 2004; Amorim 2005).

The purposes of the work are: i) to describe a new species of *Trechus*; ii) to review biodiversity patterns within islands and their habitats; and iii) to provide an updated key for the identification of *Trechus* species from the Azores.

A phylogeographic study of the Azorean *Trechus* based on mitochondrial DNA sequence has been performed and will be presented elsewhere (see also Amorim 2005).

# Trechus isabelae Borges & Serrano, new species

(Figs. 1-3)

**Type material:** Holotype, male, Azores, São Jorge, Algar do Morro Pelado (= Algar do Montoso), 5–11.VII.2004. (P.A.V. Borges & F. Pereira leg.). Paratypes 1 female, same locality as holotype, 7–11.V.2004 (P.A.V. Borges & F. Pereira leg.); 2 males and 1 female, same locality as holotype, 5–11.VII.2004 (P.A.V. Borges & F. Pereira leg.); The Holotype and two paratypes (1 male, 1 female) are deposited at the University of the Azores, Terceira ("Arruda Furtado" Collection); one paratype (female) is deposited at the Faculty of Sciences of the University of Lisbon, and the other one (male) at the University of La Laguna, Dep. of Animal Biology.

Additional material: One male was collected in the same locality (7–11.V.2004) for molecular analysis.

**Etymology.** The species is named after Isabel Amorim who has discovered several new species of *Trechus* in the Azores.

**Diagnosis.** Large species (4.8–5.38 mm) (Fig. 1), dark brown pigmentation, with some lighter parts; appendages less pigmented (light brown), tibiae and tarsomeres with the same colour patterns as the femora; pronotum as dark as elytra. Eyes slightly convex;  $3^{rd}$  antennomere longer than  $2^{nd}$ ; pronotum rather cordiform and transverse, with lateral furrows larger than in *T. terrabravensis*; wingless.

Description. Length of holotype: 5.18 mm. Length of female specimens: 4.80-5.38 mm. Large size, convex and ovate. Head almost 1.30x as wide as long (females: 1.29-1.42), 0.78x narrower than pronotum (females: 0.74–0.78); eyes well-developed (0.025 mm), moderately convex, diameter of eyes as long as temples; temples with microchaetae. Frons slightly convex, the frontal furrows deeply curved and rounded. Anterior margin of labrum very concave. Maxillary palpi long and slender. Chaetotaxy: two pairs of supraocular setae; four setae in clypeus (outer ones larger); six setae on labrum, the outer ones being the largest. Antennae 2.99 mm long (females: 2.74–2.99 mm), reaching only the first quarter of elytra; the 3rd antennomere 1.32 x longer than 2nd (females, 1.11x-1.14x); 1st antennomere 2.0x longer than wide (females, 1.88x - 2.0x),  $2^{nd}$ antennomere 2.33x longer than wide (females: 2.60x - 2.67x), 6th-8th antennomeres 2.5x longer than wide (females: 2.67x - 3.0x), 9th–10th antennomeres 2.31x longer than wide (females: 2.14x - 2.8x), 11th antennomere 2.92x longer than wide (females: 2.86x - 3.6x). Pronotum convex, cordiform, highly transverse, 1.31xas broad as long (females: 1.30x-1.34x); side margins curved till hind angle; anterior angles obtuse; hind angles obtuse, well marked and protruding; basal border emarginated; lateral furrows larger than in T. terrabravensis; basal fovea well marked. Chaetotaxy: the anterior seta is located in the second quarter and the posterior seta located in the hind angle. Elytra ovate with rounded shoulders, 1.64x wider than pronotum (females: 1.50x-1.70x) and 1.42x as long as broad (females: 1.29x-1.50x); apex rounded, lateral furrows canaliculated; striae all well marked and interstriae convex. Recurrent striae less curved than in T. terrabravensis, but well connected to the 5th. Chaetotaxy: umbilical series typical of Trechus; preapical seta nearer

the suture than to the apex; the first discal seta in the 3rd stria is located in the anterior quarter and the second discal seta approximately in the center. Aedeagus with apex of median lobe bent down (lateral view) (Fig. 2a); Left and right parametes with 4 setae each (Fig. 2b).

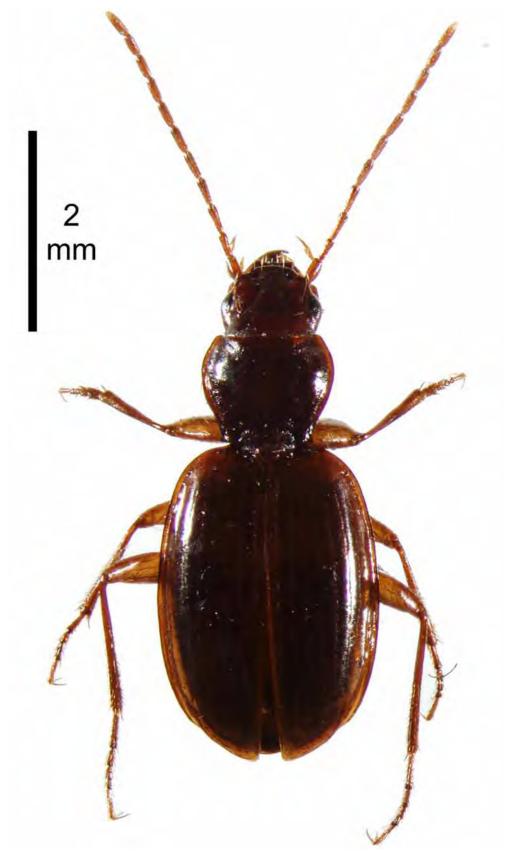
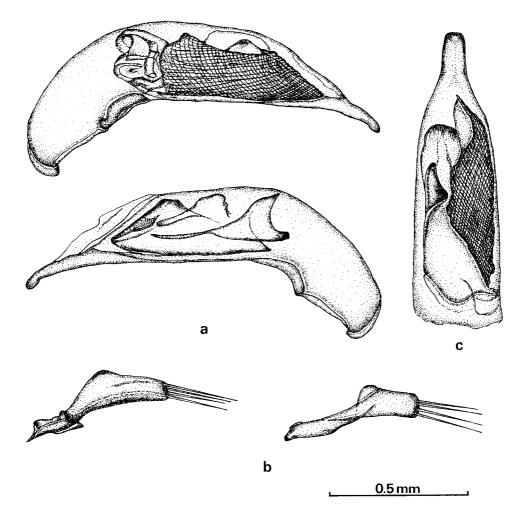


FIGURE 1. Aspect of the male of Trechus isabelae n. sp. (Photo M. Ibáñez & H. López).



**FIGURE 2.** Aedeagus of *Trechus isabelae* **n. sp.** a) median lobe, lateral view; b) left and right parametes c) median lobe, dorsal view. Scale: 0.5 mm.

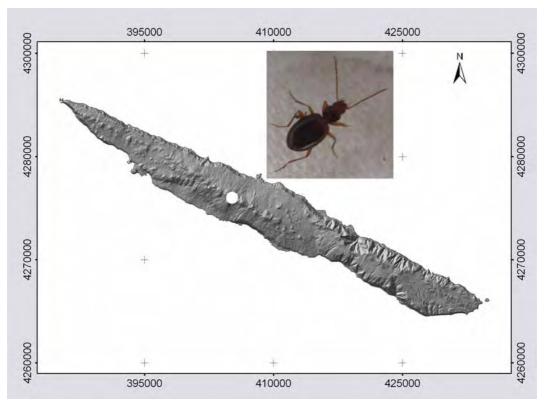
**Ecological notes.** The specimens were all sampled inside Algar do Morro Pelado, a volcanic pit of great dimensions (140 m deep) located at 1000 m a.s.l. (Fig. 3). The surrounding area consists of natural grassland and is under the European NATURA 2000 network protection. The cave was visited in May and July 2004 during two biospeleological expeditions organized by GESPEA and "Os Montanheiros". The pit has two large superimposed chambers. The lower and larger chamber has the darkest conditions (with 150 m x 70 m and about 50 m height) and is 140 m deep. The upper chamber is 30–40 m deep and has permanent access to sunlight with some darker parts. Due to logistics, only the upper chamber was sampled using 20 traps baited with fresh cow liver. All specimens were sampled alive in traps located deep inside the chamber, in very humid spots and protected from direct sunlight. The habitat is similar to that of *Trechus jorgensis* in the volcanic pit Algar das Bocas do Fogo (also in São Jorge, but at 385 m a.s.l.) and *Trechus terceiranus* in Algar do Carvão (Terceira).

**Taxonomic notes.** The new species, as well as *T. montanheirorum*, presents the median lobe of aedeagus (lateral view) with a similar form (*cf.* Fig. 2a with Fig. 6 in Oromi & Borges 1991). However, particular features of the head and pronotum length and width, form and length of eyes and the location of the preapical setae of elytron, easily separate the two species (Table 1). Besides length of body and some features of the eyes, pronotum and apical angle of elytron, the form of median lobe in lateral view (mainly the apex bent down) separates also the new species from all the others occurring in Azores. The general morphological aspect of *T. isabelae* **n. sp.** resembles also the epigean *Trechus terrabravensis* (endemic to Terceira island),

but is larger and easily distinguishable by the larger size of the  $3^{rd}$  antennomere, which is longer than the  $2^{nd}$ . Taking into account the comparison of aedeagus and all analysed characters, the new species seems to have a closer relationship with *T. montanheirorum* (see also Amorim 2005), a species that occurs in a different but adjacent island (Pico).

	T. isabelae	T. montanheirorum
Colour	Pigmented, dark brown, some lighter parts	Pigmented, reddish-brown to dark brown
Length (body)	4.80–5.38 mm	4.27–4.88 mm
Head	1.30x as wide as long	1.20x as wide as long
Eyes	Well developed, convex, as long as temples	Well developed, convex, shorter than temples
Antennae	Male 3rd segment 1.32 x as long as 2nd	Male 3rd segment 1.12 x as long as 2nd
Pronotum	Index W/L= $1.31$	Index W/L= 1.26
Preapical seta	Closer to the suture than to the apex	Closer to the apex than to the suture
Aedeagus	Apex slightly curved downwards in profiles	Apex curved downwards in profiles

TABLE 1. Morphological differences between Trechus isabelae n. sp. and Trechus montanheirorum.



**FIGURE 3.** Location in the island of São Jorge (digital map of João Tavares adapted from Pereira *et al.* in press) and general aspect of *Trechus isabelae* **n. sp.** (Photo P.A.V. Borges).

## Key to the Azorean species of *Trechus* (modified from Borges et al. 2004)

1	Apical angle of elytra rounded	2
	Apical angle of elytra pointed or sharp angled	
2	Larger species, length $> 3.5$ mm; lateral margins of pronotum markedly sinuated before the basis, deg	ree
	of pigmentation variable	3

-	Length 3.0-3.2 mm; lateral margins of pronotum hardly sinuated before basis; clearly dark pigmented
	species torretassoi Jeannel
3	3 <sup>rd</sup> antennomere subequal to 2 <sup>nd</sup>
-	3 <sup>rd</sup> antennomere longer than 2 <sup>nd</sup>
4	Pronotum cordiform and highly transverse (index $W/L = 1.35$ ), with lateral furrow very narrow (epigean
	species)terrabravensis Borges, Serrano & Amorim
-	Pronotum less transverse (index $W/L = 1.26$ ); lateral furrow larger (beetles occurring in entrance of caves)
	montanheirorum Oromí & Borges
5	Body depigmented with a convex and ovate aspect
-	Body pigmentation dark brown, with some lighter parts; appendages less pigmented (light brown); body
	more depressed isabelae new species
6	Aedeagus with apex of lamella slightly blunt; known from Picopicoensis Machado
-	Aedeagus with apex of median lobe deeply curved upwards; known from Faial
	oromii Borges, Serrano & Amorim
7	Apical angle of elytra sharp (sometimes notched), forming a small protruding point; total length 3.6-4.3
	mm terceiranus Machado
-	Apical angle of elytra sharp (forming a right angle); total length < 3.5 mm
8	Slightly pigmented on elytra and pronotal disc; aedeagus in lateral view with apex of median lobe more or
	less straight and pointed; left paramere with 4 setae pereirai Borges, Serrano & Amorim
-	Depigmented; aedeagus in lateral view with apex of the median lobe sharpened and slightly curved
	upwards; left paramere with 5 setae jorgensis Oromí & Borges

# **Distribution and conservation remarks**

All the Azorean Trechinae species belonging to the genera *Thalassophilus* and *Trechus* are single island endemics. These species are among the rarest Azorean endemic arthropods, since they occur on only one island and in very specific habitats: i) eight of them are only found in caves, and ii) the two epigean species are found in the hyper-humid native forest. However, the 10 species differ in their patterns of within island occurrence. Figure 4 shows that the species-range-size distribution follows a clear cut pattern with three wide-spread and seven clearly restricted species.

The lack of population studies and demographic monitoring makes it difficult to characterize the conservation status of the Azorean Trechinae endemic species. Despite this limitation, there is recent information from standardized studies performed in native forests (Borges *et al.* 2005b; 2006) and in caves (Amorim 2005; Borges & Pereira, unpublished data):

1) A five year time-series sampling data for the most common species, *Trechus terceiranus*, at the Algar do Carvão volcanic pit (pitfall traps baited with fresh cow and/or pig liver that was kept in a special container inside the trap, allowing for the capture of live specimens that could later be returned to the cave environment); Figure 5 shows a more or less stable population throughout the sampled years (2001–2006) (Borges & Pereira, unpublished data).

2) Amorim (2005) sampled 49 lava tubes and volcanic pits in seven islands with standardized methods (during the same period of time and with similar effort per cave) and found 329 specimens of *T. terceiranus*, 216 of *T. picoensis*, 24 of *T. montanheirorum*, 15 of *T. oromii*, 7 of *T. pereirai*, and 2 of *T. jorgensis*.

3) During the BALA project (Borges *et al.* 2005b; 2006), a total of 247 specimens of *Trechus terrabravensis* were sampled.

Thus, the two species from São Miguel, the epigean *Trechus torretassoi* and the cave-dwelling *Thalasso-philus azoricus*, have not been collected in recent years. Both species seem to be quite rare, the latter known

from a single cave (see: Oromí & Borges 1991; Borges & Oromí 1994). The recent land-use changes in part of the historical localities of *Trechus torretassoi*, and the failure to find this species during intensive sampling done for the BALA project may indicate that this species either has become extinct, or is on the verge of becoming extinct, or its distribution is extremely restricted.

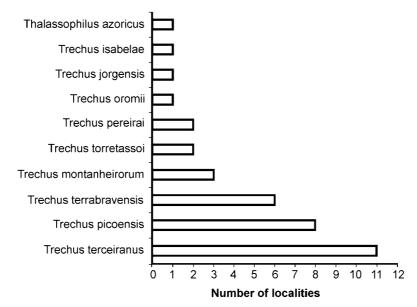
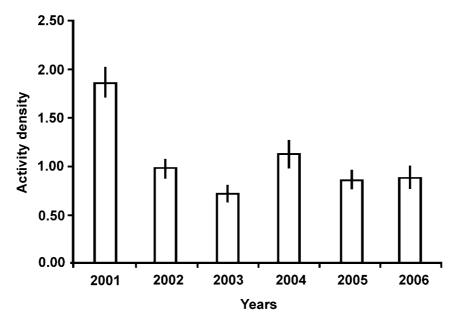


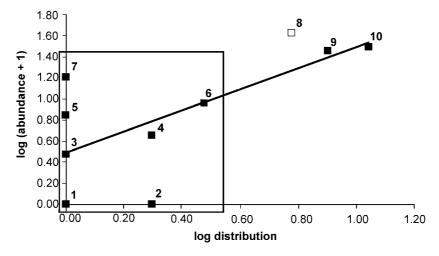
FIGURE 4. Number of caves and sites for each of the Azorean Trechinae.



**FIGURE 5.** Mean activity-density (+ s.e.) of *Trechus terceiranus* Machado (Coleoptera, Carabidae) in Algar do Carvão (Terceira island) between 2001 and 2006 (until September). Activity-density was measured as the number of specimens per trap per sampling night.

The patterns of distribution and abundance above described could be easily plotted and clearly show a positive relationship between mean abundance and spatial occupancy (r = 0.69; log abundance = 0.48 + 0.99 log distribution; p = 0.028) (Fig. 6). The observed pattern indicates that all species within the square (Fig. 6) are at a two-fold risk of extinction both because of their restricted distribution and because of their low abundances. Only *T. terrabravensis*, *T. picoensis* and *T. terceiranus* (species 8, 9 and 10, respectively in Fig. 6) seem to be common and abundant species not at risk of extinction. However, Gaston *et al.* (2006) showed that

*T. terrabravensis* is an outlier in the abundance-occupancy relationship (see open square in Fig. 6), probably due to the fact that it is a restricted, specialized endemic species. Moreover, Borges *et al.* (2006) showed that *T. terrabravensis* is limited exclusively to specific sites within the laurel forest and has been located in fewer sites than expected according to the observations based on its mean local abundance (Gaston *et al.* 2006). Therefore, we should also consider *T. terrabravensis* as a vulnerable species.



**FIGURE 6.** Relationship between the logarithm of the mean abundance +1 and the logarithm of distribution for the 10 Azorean species of Trechinae. The species within the quadrate are the most restricted and less abundant. The species marked with an open square is *Trechus terrabravensis* (see text for more details); 1—*Thalassophilus azoricus*, 2—*Trechus torretassoi*, 3—*Trechus jorgensis*, 4—*Trechus pereirai*, 5—*Trechus isabelae*, 6—*Trechus montanheirorum*, 7—*Trechus oromii*, 8—*Trechus terrabravensis*, 9—*Trechus picoensis*, 10—*Trechus terceiranus*.

## Discussion

Ground-beetles adapted to underground environments are among the most unusual species found in the Azores. This family is only represented by two genera in the underground: Thalassophilus (1 sp.) in São Miguel and *Trechus* in the central island group, both belonging to the Trechinae subfamily. The only species of Thalassophilus is confined to a single cave on São Miguel island (Gruta de Água de Pau) and is apparently a relict species, since no epigean congeneric relatives are known in the archipelago (Oromí & Borges 1991). A different pattern is observed in Madeira and the Canary Islands. In those archipelagos there are respectively two (Th. coecus and Th. pieperi) and one (Th. subterraneus) hypogean Thalassophilus species, and the epigean Th. whitei which is still common in both archipelagos and seems to be closely related to the three hypogean species (Erber 1990; Machado 1990). Remarkably, Th. azoricus is only found in one cave, despite that many other caves from the central, geologically young part of São Miguel have apparently good conditions to harbour this species. Those are the typical recent lava tubes (and some pits) recently formed in superficial young lavas and lying a few meters below the surface, which allow roots, soil and water to reach easily the caves. Cave-dwelling fauna is commonly found in such lava tubes on other islands in the Azores and the Canaries. In contrast to that, Gruta de Água de Pau is an older lava tube opening at the base of a sea cliff, and therefore lying beneath a thick layer of lavas originating from earlier eruptions unrelated to the one that created the cave. No roots and very little organic matter coming from the surface reach this deep lava tube, and consequently such caves in volcanic terrains are usually very poor in or even devoid of any fauna. Surprisingly, after several biospeleological studies carried out in São Miguel (Oromí et al. 1990; Borges & Oromí 1991; Oromí & Borges 1991; Amorim 2005; Ashmole et al. unpublished data), the only troglobitic ground beetle ever found was collected in Gruta de Água de Pau, and hardly any other adapted arthropod has been

found in the younger caves on the island where a richer fauna would be expected. The absence of cave adapted fauna in caves located in the younger part of São Miguel could be explained by recent extinctions caused by extensive land-use changes on the surface (see also Borges *et al.* in press).

With this contribution, the total number of *Trechus* spp. known from the Azores has been increased to nine. *Trechus* species can be found on five of the islands, and seven of the nine species are confined to caves. It is noteworthy mentioning that various species of *Trechus* beetles restricted to the cave environment can be found on two of the Azorean islands: 3 spp. on Pico and 2 spp. on São Jorge. The occurrence of multiple species on the same island could be explained by independent colonization events of the underground environment and also of the islands (see also Amorim 2005). Comparing the different ecological patterns of the Azorean *Trechus* to those of the Madeiran and Canarian archipelagos yields remarkable patterns. In the Madeiran and Canarian archipelagos this genus has radiated to 21 and 14 epigean species, respectively, but only two species from the Canaries are exclusive cave-dwellers (Borges & Oromí 1991). This pattern could also be partly explained by the extinction of most of the epigean *Trechus* species in the Azores.

All Azorean Trechus species are clearly neo-endemics and the degree of troglomorphy varies among the cave-dwelling species: T. isabelae n. sp. from São Jorge and T. montanheirorum from Pico have similar characteristics (see Table 1), and are less adapted to the cave environment, hardly showing eye reduction and body depigmentation. Trechus montanheirorum is only found at the entrance of caves and T. isabelae n. sp. has been found in the uppermost part of a humid volcanic pit with permanent twilight, though protected from direct sunlight. We believe that both species represent early stages of cave adaptation. The remaining five species are better adapted to the cave environment with conspicuous eye reduction and depigmentation, and are usually found deeper inside the caves; however, they are far from being highly troglomorphic species. Concerning *Thalassophilus azoricus*, it is the only eyeless ground beetle in the Azores showing a higher degree of troglomorphism than any of the cave-adapted Azorean Trechus. This could be explained by the older age of São Miguel (4 Ma) compared to that of the other Azorean islands where hypogean Trechus occur (from 0.25 Ma - Pico to 3 Ma - Terceira). A parallel case is observed in the Canary Islands, where the three hypogean Trechinae occurring in Tenerife (12 Ma) are completely eyeless, while those from the younger islands of La Palma (2 Ma) and El Hierro (1 Ma) still have reduced eyes (Machado 1988; 1990). Thalassophilus azoricus is also more troglomorphic than the three hypogean species of the genus found in Madeira and the Canary Islands, which still have small eyes. The only island of the Canaries where Th. subterraneus occurs (La Palma, 2 Ma) is also younger than São Miguel, but this is not the case for Madeira (5 Ma). Therefore, we cannot always apply a simple correlation between the age of the island and the degree of troglomorphism for the Thalassophilus beetles found on the Macaronesian islands.

An alternative hypothesis for the high degree of cave adaptations of *Th. azoricus* is that the limited resources in Gruta de Água de Pau could have promoted selection towards a higher degree of troglomorphy in this species than in the other Azorean Trechinae living in caves rich in nutrients (i.e. volcanic pits and shallow younger lava tubes). A similar situation can be found on Tenerife (Canary Islands) in the genera *Wolltinerfia* (Carabidae), *Domene* (Staphylinidae), *Loboptera* (Blattellidae) among others. These genera have eye-reduced hypogean species in the energy rich underground environment of the older Anaga region of Tenerife, while vicariant eyeless, more troglomorphic species occur in the poorer underground of the younger central part of the island (Oromí & Martín 1992). The molecular clock using DNA sequences has partially confirmed this hypothesis for the genera *Loboptera* (see: Izquierdo *et al.* unpublished data) and *Wolltinerfia* (see: Moya *et al.* 2005, unpublished data). Such molecular approach should also be useful for clarifying this question concerning the Azorean Trechinae, but it would be necessary to include the closest epigean relative of *Th. azoricus*, which is unknown and may even be extinct.

The male genitalia of *T. isabelae* **n. sp.** presents a general appearance resembling the aedeagus of *T. montanheirorum* from Pico, the epigean *T. terrabravensis* from Terceira and the epigean *T. torretassoi* from São Miguel, and even the aedeagus of some species from Madeira (e.g. *T. custos* and *T. umbricola*). In a previous work (Borges *et al.* 2004), based on the external morphology and shape of the aedeagus, the authors suggested the possible occurrence of at least two separate invasions of the Azores by epigean *Trechus* colonizers. However, despite of the presence of two clear morphological types of the aedeagus, recent molecular data (Amorim 2005) indicate that the Azorean *Trechus* are monophyletic.

The local radiation of the genus *Trechus* has been much higher than any other ground-beetle genus in the Azores. Apart from the nine species described so far, one may hypothesize from one to three more surfacedwelling *Trechus* species, which represent the epigean ancestors of the cave-dwelling species found in Faial, Pico and São Jorge islands, which either have not yet been discovered or became extinct. The reasoning for the assumption of the present or past existence of three extra epigean species of *Trechus* in the Azores is as follows: cave adapted species are not able to survive outside the underground in an exposed environment. Therefore, they should not be able to colonize other islands (Oromí *et al.* 1991; Contreras-Díaz *et al.* 2007) and the evolution of hypogean species must have occurred independently on each of the islands where the cave adapted beetles are found nowadays.

It is highly probable that other islands within the archipelago of the Azores may also have experienced the evolution of endemic species of *Trechus*. Namely, Santa Maria and Graciosa may harbour unique species of *Trechus* due to: i) their proximity to other islands that were colonized by *Trechus* beetles, ii) the fact that they are older islands than Pico, Faial and São Jorge, and iii) the fact that their natural ecological conditions ought to be suitable up to recent times for *Trechus* to thrive in. Nevertheless, and despite all collecting efforts on Santa Maria and Graciosa (BALA expeditions (Amorim 2005) and recent expedition to Graciosa, (Pereira *et al.* in press)), no Trechinae were ever collected on those islands.

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