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Trirhacus helenae sp. n., a new cave-dwelling planthopper from Croatia (Hemiptera: Fulgoromorpha: Cixiidae)

Hannelore Hoch*

Museum für Naturkunde – Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Invalidenstr. 43, 10115 Berlin, Germany

Abstract

A new obligatorily cavernicolous (troglobitic) species of the cixiid tribe Cixiini is described from Croatia and assigned to the Palearctic genus *Trirhacus* Fieber. Information on its ecology is given. This is the first record of a troglobitic planthopper from Croatia and Dinarides, and only the 2nd cavernicolous cixiid species from the Mediterranean region. *T. helenae* sp. n. is closely related to the epigean *T. dubiosus* Wagner suggesting cave-adaptation via an adaptive shift and parapatric speciation.

Introduction

A recent survey of caves and subterreanean habitats in Croatia by members of the Croatian Biospeleological Society, Zagreb, revealed the existence of a hitertho unknown cave-dwelling species of the planthopper family Cixiidae. This is the first record of a troglobitic planthopper not only from Croatia, but from the whole of the Dinarides Region, and the 2nd cavernicolous cixiid species from the Mediterranean region. Outside Europe, cavernicolous Cixiidae are known from many regions, e.g., Hawaii (Fennah 1973a, Hoch & Howarth 1999), Galápagos (Hoch & Izquierdo 1996), Australia (Hoch & Howarth 1989a, b), New Zealand (Fennah 1975), Mexico (Fennah 1973b, Hoch 1988), Argentina (Remes Lenicov 1992), Brazil (Hoch & Ferreira 2012), Madagascar (Synave 1953) and Reunion (Hoch et al. 2003). In Europe, cave-dwelling Cixiidae are known from the Makaronesian Islands (Azores: Hoch 1991; Canary Islands: Remane & Hoch 1988, Hoch & Asche 1992) and Sicily (D'Urso & Grasso 2009). The new cixiid species from Croatia is assigned to the genus *Trirhacus* Fieber, 1875. It is partially troglomorphic and presumably restricted to life underground (see Ecology and Discussion).

Material and methods

Collecting, preservation, permanent storage. The specimen was detected by visual search, collected by hand, and transferred immediately to 96 % ethanol. For permanent storage, after dissection and examination, the genitalia were transferred to a polyethylene vial, and associated with the specimen vial.

Morphological examination techniques, visualization. Measurements and examinations of external body features were made from the specimen in alcohol, without further manipulation.

To prepare male genitalia for dissection, the genital capsule was removed from the specimen, and then macerated in 10 % KOH (24 h) at room temperature, washed in water, transferred to glycerine for storage, or to glycerine-jelly for drawings. Examinations and drawings were made using a Leitz stereomicroscope with *camera lucida* attachment.

Habitus photographs. Photographs in Fig. 1 were obtained from combined image-stacks taken with the aid of a Leica MZ 16 with a camera (Canon EOS 450 D) attachment and Combine ZP software, and subsequently processed with Adobe Photoshop CS3.

Taxonomy

According to the key to the European genera of Cixiidae provided by Holzinger (2002) the species from

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* E-mail: hannelore.hoch@mfn-berlin.de
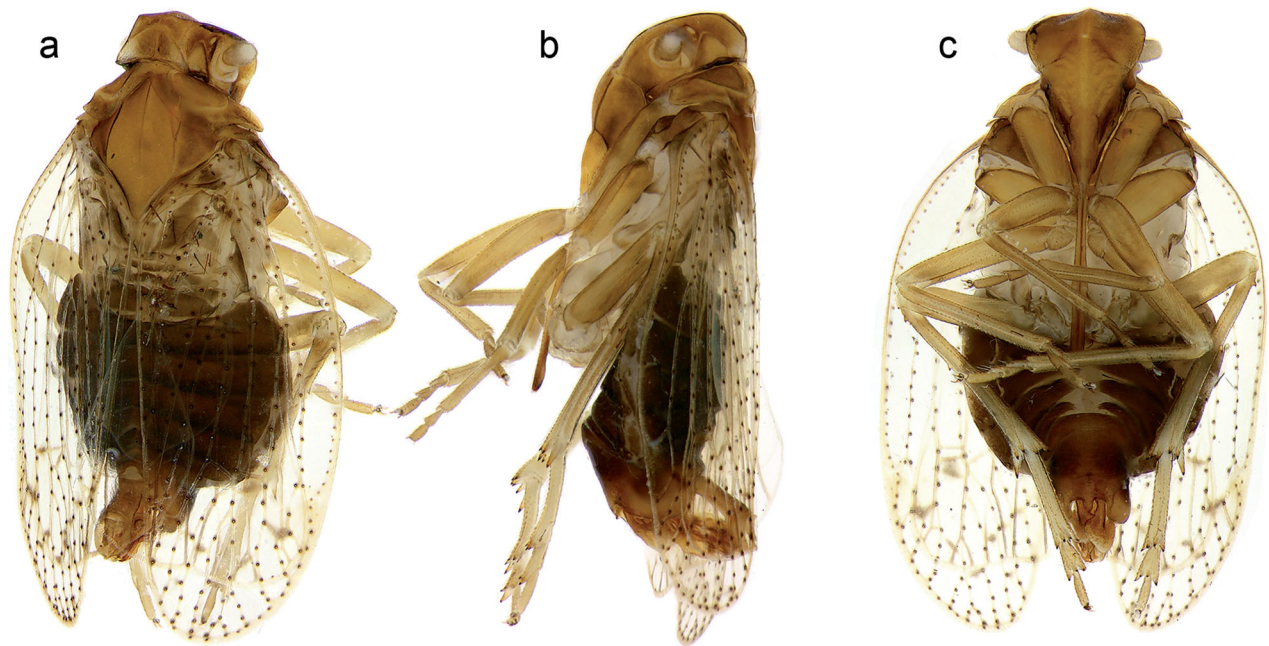
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Figure 1. *Trirhacus helenae* sp. n., habitus male; **a.** dorsal aspect; **b.** same, lateral aspect; **c.** ventral aspect.

Croatia belongs to the genus *Trirhacus* Fieber, 1875 which is recognizable by the following characters: "Fore wings ovoid, thus specimen oval-shaped. Abdomen of females truncate, without wax plate. Phallosoma ventrally has a rigid bifurcate processs. Apical part of aedeagus with a large subapical process on the left side. Anal segment in males short and stout, apical process asymmetrical, bearing two large lobes" (Holzinger 2002: 388). Other diagnostic characters which are, however, not unique to *Trirhacus* are the tricarinate mesonotum, hind tibia with 1–3 lateral spines, tegmina with setiferous tubercles on veins and on the distal margin between veins, reduction of the hind wings between costa and cubitus, and presence of a distinct incision of the apical margin of the hind wings at the end of cubital veins, median carina of postclypeus distinct (Holzinger 2002: 373, 377).

All four species currently accommodated in *Trirhacus* are distributed on the Balkan Peninsula: *T. setulosus* Fieber, 1876 (Greece and perhaps former Yugoslavia: Nast 1972), *T. peloponnesiacus* Holzinger, 2002 (Greece), *T. dubiosus* Wagner, 1959 (Croatia), and *T. biokovensis* Dlabola, 1971 (Croatia).

Among these, the cavernicolous species from Croatia – according to the information given by Holzinger (2002) – is similar to *T. dubiosus* Wagner in the general configuration of the male genitalia, however, differs in external morphology and in several characters of the male genitalia, especially the aedeagus. It is therefore assumed to represent a separate reproductively isolated entity and is described as a new species.

Trirhacus helenae sp. n.

Figures 1–3

Description

Body length (anterior margin of head to distal tip of anal segment 3.7 mm; anterior margin of head to distal margin of tegmen 4.2 mm).

Colouration. Head dorsally sordid light brown except vertex laterally with whitish triangles; antennae whitish; frons sordid light brown; post- and anteclypeus light yellow; head laterally generally sordid light brown, with yellowish portions except a small, dark bulge at posterior margin of head, just lateroventrally of vertex, in dorsal aspect creating the impression of a small, narrow compound eye. Area of (reduced) compound eye unpigmented. Pronotum sordid light brown. Mesonotum light yellow between lateral carinae, lateral portions sordid light brown. Dorsal portions of thorax covered by tegmina whitish, with sordid brown portions; abdominal tergites and sternites uniformly dark brown. Tegmina translucent, very light yellowish, without any conspicuous colour pattern; veins light yellowish, not contrasting with area of tegmen. Legs light yellow.

Head. Posterior compartments of vertex forming a wide triangle (2.18 × wider than medially long), with a faint median longitudinal carina; anterior compartment of vertex separated from frons by a blunt transverse carina. Frons 0.6 × as long as maximally wide and 0.4 × as long as post- and anteclypeus together. Frons laterally smooth, lateral margins ridged and directed laterally. Frons and clypeus with a faint and blunt median carina. Frontoclypeal suture highly
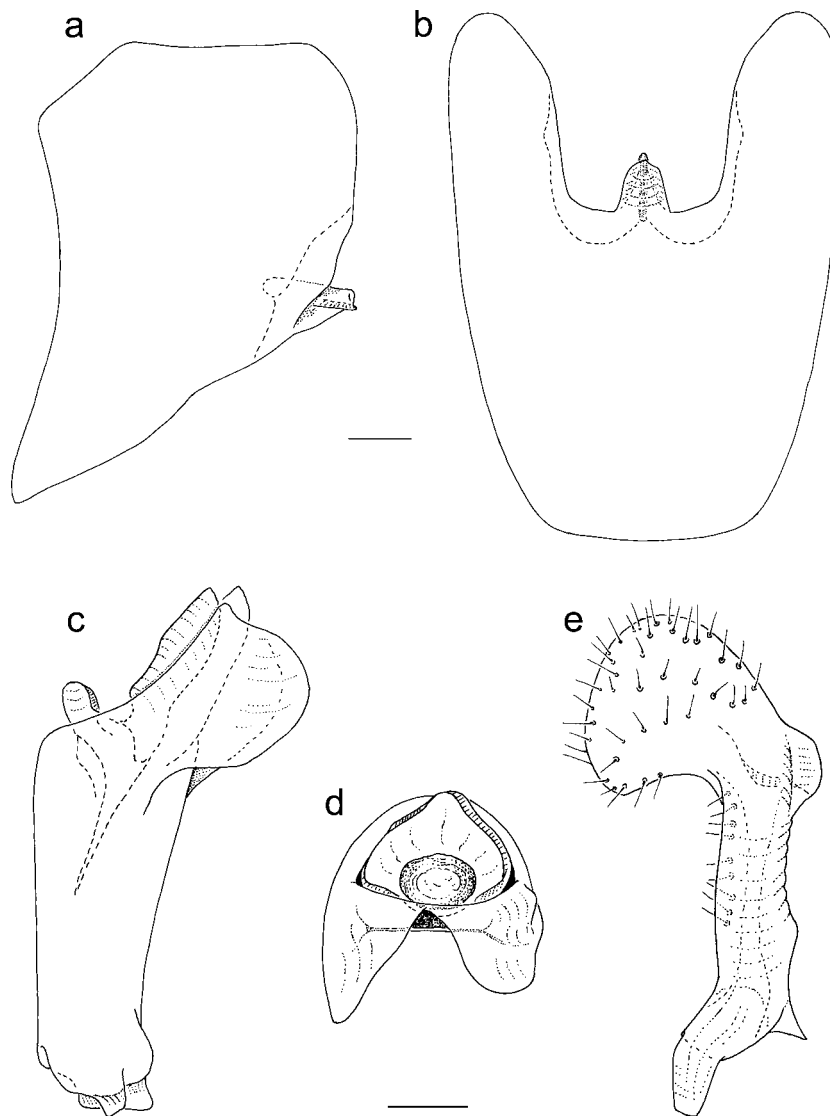

Figure 2. *Trirhacus helenae* sp. n., male genitalia; **a.** genital segment, left lateral aspect; **b.** same, ventral aspect; **c.** anal segment, left lateral aspect; **d.** same, strict caudal aspect; **e.** left paramere, left lateral aspect. Scale bar 0.1 mm.

vaulted towards frons, medially forming an almost 90° angle. Compound eyes absent, their former position recognizable as a shallowly vaulted area; lateral and median frontal ocelli present, small. First antennal joint (scape) short, ring-like, second antennal joint (pedicel) subcylindrical, ca. 1.5 × longer than its diameter. Rostrum well surpassing hind coxae.

Thorax. Pronotum short, ca. 2.2 × wider than vertex posteriorly, caudal margin medially deeply incised; tricarinate with median carina short, lateral carinae each enclosing a discoidal area. Tegulae minute, leaving base of tegmen exposed.

Mesonotum tricarinate, slightly wider than medially long.

Tegmina ovoid, in repose slightly surpassing tip of anal segment. All veins beset with conspicuous dark brown tubercles, also between veins along distal margin of tegmen. Pterostigma inconspicuous, minute, at ca. $\frac{3}{4}$ of total length of tegmen. Apices of anterior and posterior subapical cells at same level; intercubital transverse veinlet joining posterior margin of tegmen distally of claval junction.

Wings well developed, in repose distally only slightly surpassed by tegmina; distal margin with a distinct incision at level of cubital veins.

Legs. Metatibia laterally with two small rigid spines, distally with 6 teeth (external one = largest). First and second metatarsal joints distally with 4 spines.

Male genitalia. Genital segment bilaterally symmetrical, in lateral aspect subtrapezoidal, caudal margin truncate, straight, forming a nearly 90° angle with dorsal margin; laterodorsal angles broadly rounded; medioventral process in ventral aspect tongue-shaped, distally subtriangular, with a distinct median carina on its dorsal surface.

Anal segment in dorsal aspect 2.5× longer than wide at base; lateral margins ± parallel, only slightly converging near base, caudal margin rounded, ventrally bilaterally asymmetrical, produced into two compressed, rounded lobes: lobe on right side longer and more
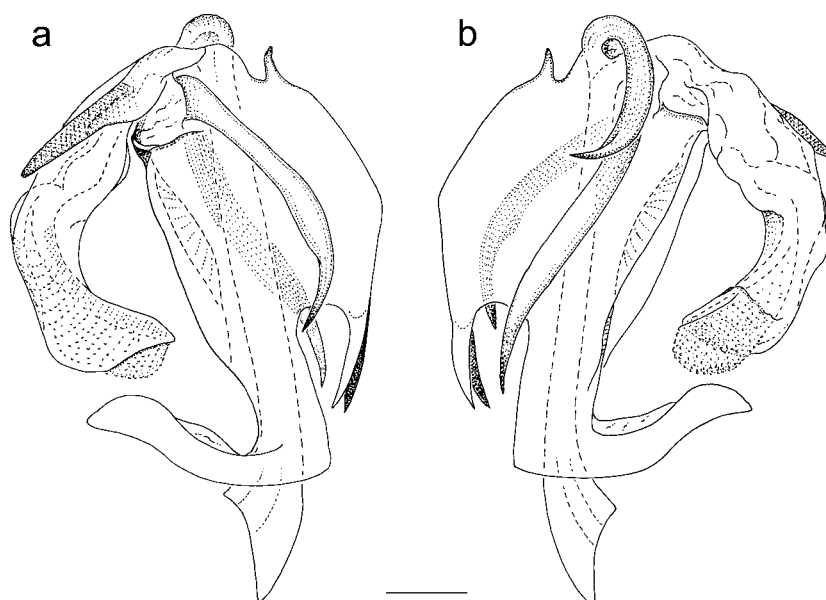
Figure 3. *Trirhacus helenae* sp. n., male genitalia: aedeagus; **a.** left lateral aspect; **b.** same, right lateral aspect. Scale bar 0.1 mm.

strongly produced ventrally than on left side; epiproct disc-shaped, distally rounded, caudally not exceeding paraproct.

Parameres symmetrical; narrow, subtubular in proximal two thirds, expanding dorsally into a bluntly triangular, medially concave dilation, ventrally into a small, compressed velum which is mediodorsally produced into a narrow ledge.

Aedeagus. Shaft dorsally with a longitudinal furrow, ventrally in distal two thirds expanding into a strongly compressed dilation which is ventrobasally adorned with a rigid, sturdy, bifurcate spinose process directed basally, and a short, slender, subacute spine near aedeagal joint. Shaft with 3 movable, subapical spinose processes, one arising left laterally (slender, terete, irregularly tapering, in repose curved ventrobasally), and two arising right laterally (both terete, regularly tapering, one short, in repose strongly curved ventrolaterally, the other ca. twice as long, in repose gently curved ventrobasally).

Flagellum elongate, nearly attaining base of shaft, tubular throughout, only slightly expanding near apex, in repose bent dorsally and to right side. Flagellum with a single rigid spinose process, arising from a wide base left laterally near aedeagal joint, rapidly tapering, tip subacute. Phallotreme apically, in repose exposed ventrobasally; ejaculatory duct in distal half of flagellum rugose.

Diagnosis. Small cixiid with conspicuous coloration (head and thorax light, tegmina and wings translucent, abdomen dark brown) and reduced compound eyes. Anal segment elongate, distally with two asymmetrical rounded lobes, aedeagus ventrally with compressed dilation which is ventrobasally produced into a bifurcate process and near aedeagal joint with a short, slender spine; shaft of aedeagus with 3 movable subapical

spines (one on left, two on right side), flagellum of aedeagus with one rigid spine near base on left side. Among the other *Trirhacus* species most similar to *T. dubiosus* in male genital configuration, but differs from *T. dubiosus* in the following characters of the head (reduction of compound eyes, vs compound eyes well developed in *T. dubiosus*) and the male genitalia (ventral dilation of aedeagus shaft with short, slender spine near aedeagal joint, vs smooth in *T. dubiosus*; movable subapical shaft spines – as in *T. dubiosus*, one arising left laterally and two arising right laterally – in relative length and direction differing from those drawn by Holzinger for that species (2002: 395). *T. helenae* is slightly smaller than the only known specimen of *T. dubiosus*: 4.2 vs 4.8 mm.

Etymology. The species is named in honour of Helena Bilandzija, biospeleologist and explorer.

Distribution and ecology. Špilja kod Nerezinog dola is a small cave, only 35 meters in length. Its 6 × 2.5 metre entrance, which is enclosed with a combination of dry-stone wall and wood, is located about 200 m above sea level (Miculinić et al, 2010). Like many caves in Croatia, not so long ago its entrance chamber was used as a pen for sheep and goats. The cave is formed within Jurassic-Cretaceous dolomites with layers of carbonates. The fact that the main tunnel is oval in shape suggests the cave was formed in phreatic conditions. It is now completely dry.

Known from a single cave, Špilja kod Nerezinog dola, on Mljet island, Croatia (Figs 4–5).

Temperature within the cave is from 12.6 – 13.2 °C, and relative humidity is around 99.5%. Found exclusively in the deepest part of the cave, where the epikarst (soil layers) is very thin and many roots penetrate
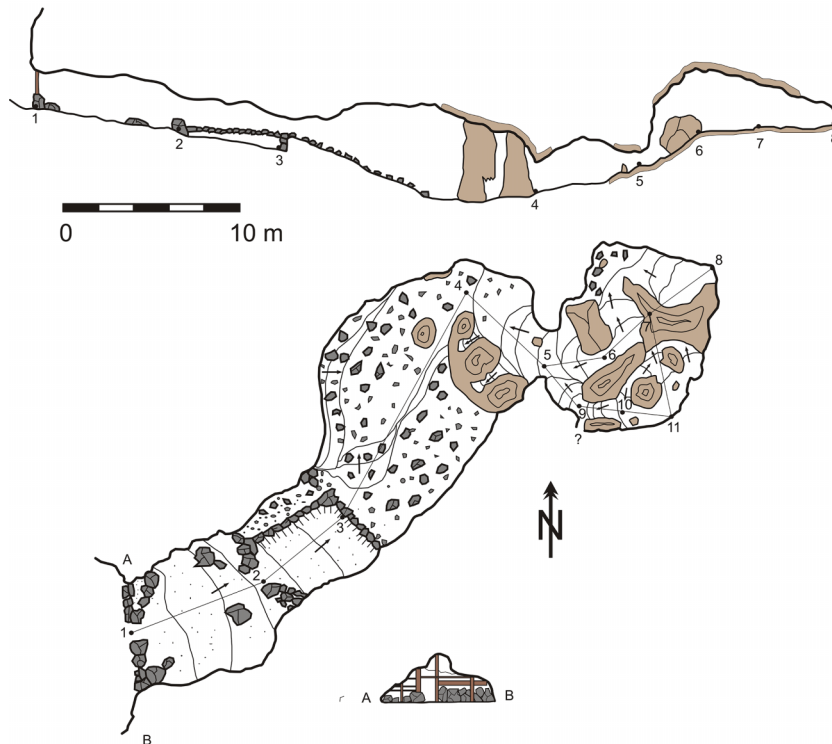
Figure 4. Map of Špilja kod Nerezinog dola, Mljet Island, Croatia.

Discussion

into the underground chamber providing food and a suitable habitat.

Material examined. Holotype ♂: CROATIA, Mljet Isl., Mljet National Park, Špilja kod Nerezinog dola, Ropa, 11.XII.2011, B. Jalžić leg., Croation Natural History Museum (CNHM), Zagreb; Zhem (Hemiptera Collection of CNHM) inv. no. 6603 (Zhem 6603).

Figure 5. Interior of Špilja kod Nerezinog, with speleologist Hrvoje Cvitanovic preparing a topographical map (Photo by Branko Jalzic; used with permission).

Trirhacus helenae sp. n. – a true troglobite?

T. helenae displays several troglomorphic characters such as the absence of compound eyes, and the light pigmentation of the head and thorax. The tegmina and wings are comparatively short, but not significantly reduced in relation to epigean *Trirhacus* species. Although nothing is known about the behaviour and biology of *T. helenae*, it is likely that the species is presumably blind, but perhaps capable of maintaining some flight. On the basis of morphological (absence of compound eyes) and habitat (high relative humidity) parameters, it is assumed that *T. helenae* is restricted to a subterranean environment, and likely to complete its life cycle underground (Howarth 1983). It is thus ecologically classified as an obligate cavernicole, or troglobiont (Sket 2008).

Trirhacus helenae sp. n. – relict or explorer?

To explain the evolution of terrestrial troglobites, two alternative hypothesis have been provided, commonly known as the climatic relict hypothesis (*CRH*) (e.g., Barr 1968) and the adaptive shift hypothesis (*ASH*)

(Howarth 1981, Howarth & Hoch 2005). While the *CRH*, mainly developed on the basis of observations in temperate regions, postulates the extinction or extirpation of surface populations due to drastic climatic changes (e.g, glaciation) with subsequent adaptation of "surviving" troglophilic populations in underground environments, the *ASH* assumes the exploitation of a novel food resource to be the driving force for cave adaptation (Howarth 1981). On geologically young, oceanic islands in the tropics, like e.g., Hawaii, closely related epigean and troglobitic cixiid planthopper species of the genus *Oliarus* occur parapatrically on three islands (Hoch & Howarth 1999). At least 6 lineages have colonized lava tubes where they feed on the roots of the native tree *Metrosideros polymorpha* (Myrtaceae), a rich and otherwise largely unutilized food resource. One of these lineages, the blind and flightless *Oliarus polyphemus* on the Big Island of Hawaii, has even undergone rapid speciation subsequent to initial cave adaptation (Hoch & Howarth 1993), revolutionizing our perception of cave adaptation as a dead-end of evolution (Wessel et al. 2013).

In Europe, which experienced several glacial cycles during the Pleistocene (e.g., Williams et al. 1998), cave adaptation in terrestrial organisms is seemingly best explained under the assumptions of the CRH. In Cixiidae, the only troglobitic species hitherto known from the Mediterranean, *Ibleicixius duniae*, apparently does not have any closely related species in the epigean fauna (D'Urso & Grasso 2009), and in the Canary Islands, no epigean relatives could be identified for several lineages which have colonized subterranean environments (Hoch & Asche 1993). In another planthopper family, the Meenoplidae, represented on the Canary Islands with four obligate cavernicolous species (Remane & Hoch 1988, Hoch & Asche 1993, Hoch et al. 2012), epigean species are entirely missing on these islands (Hoch et al. 2012). In the Azores, however, two non-relictual troglobitic species have been recognized (Hoch 1991): *Cixius cavazoricus* Hoch from Fajal, and *Cixius azopicavus* Hoch from Pico. For both species, close epigean relatives are still extant on the surface: on Sao Jorge, Terceira, and Pico, however, not from Faial (Hoch 1991: 8). Accordingly, *C. cavazoricus* from Fajal is regarded a "rélicte insulaire" (Martín et al. 1989), i.e. a cavernicolous species with close epigean relatives not on the same, but on neighbouring islands (Hoch 1991). A similar situation is observed for *Trirhacus helenae* sp. n.: close epigean relatives are extant, however, have not been recorded on Mljet, but on mainland Croatia. Thus, also *Trirhacus helenae* sp. n. today is a "rélicte insulaire". The close phylogenetic relationship of *T. helenae* and *T. dubiosus* (as suggested by the high similarity of male genital morphology) and their quasi-parapatric distribution suggests that the evolution of a cavernicolous species may be due to an adaptive shift rather than to the extinction of surface populations with subsequent adaptation to the subterranean environment, indicating that adaptive shifts may be a more general

pattern underlying cave adaptation also in temperate regions. It is, however, evident that obtaining temporal information from a combination of geologic history of the region and molecular clock estimates will be critical to achieve a better understanding of divergence events between surface and cavernicolous sister-taxa.

Acknowledgements

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