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LOCAL ENDEMIC AND THREATENED FRESHWATER HYDROBIIDS OF WESTERN GREECE: ELUCIDATION OF ANATOMY AND NEW RECORDS

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Abstract: *Islamia trichoniana* Radoman, *Pseudoislamia balcanica* Radoman and *Trichonia trichonica* Radoman are local endemics and threatened hydrobiids living in Lake Trichonis, Western Greece. The rough initial description of their reproductive organs (i.e. penis, bursa copulatrix and receptacula seminis) is supplemented with new data. The radulae of *I. trichoniana* and *P. balcanica* are described in detail for the first time. New records of the three species are given and preliminary data about their feeding regime are provided.

Keywords: *Islamia trichoniana*, *Pseudoislamia balcanica*, *Trichonia trichonica*, freshwater periphytic diatoms, Lake Trichonis

INTRODUCTION

The Mediterranean Basin Biodiversity Hotspot (MBBH) is well known for its globally important freshwater biodiversity; the freshwater bodies of Greece are part of its Balkan sub-region supporting some of the most diverse and heavily threatened ecosystems (Darwall et al. 2014). However, the freshwater fauna of Greece, especially Truncatelloidea, remains poorly inventoried; the distributional information on endemic and threatened species is often out of date or unavailable (Radea et al. 2013, Darwall et al. 2014).

Lake Trichonis (Etoloakarnania, Western Greece) is the largest lake in Greece. Its littoral or sublittoral zones hold several rare endemic and/or threatened Truncatelloidea (Schütt 1980, Radoman 1983), including the hydrobiids *Islamia trichoniana* Radoman, 1978, *Pseudoislamia balcanica* Radoman, 1978 and *Trichonia trichonica* Radoman, 1973 (Figs 1–6). These gastropods share their type locality: "the stony northeast bank near the place Mirtia" of Lake Trichonis (Radoman 1973, 1978, 1983). In the IUCN Red List of Threatened Species, both the valvatiformes *I. trichoniana* and *P. balcanica* are classified as Critically Endangered B1ab(iii) (Hauffe et al. 2011a and Hauffe et al. 2011c, respectively), whereas *T. trichonia* is Critically Endangered B2ab(i,iii) (Hauffe et al. 2011b).

The shell morphology and soft body anatomy of *I. trichoniana* and *P. balcanica* have been described by Radoman (1973, 1978, 1983). Nevertheless, some significant diagnostic characters, namely the radula and the reproductive organs, remain unknown or have not been described in detail. The shell morphology of *T. trichonica*, and the reproductive organs of the genus' type species [namely *T. kephalovrissonia* = *Radomaniola kephalovrissonia* (Radoman, 1983) sensu Falniowski et al. (2012)], have been described by Radoman (1973, 1983) and Schütt (1980). Later, Szarowska (2006) provided images and drawings of the protoconch, radula and reproductive organs of the genus *Trichonia*.

Data on the distribution of these species have been provided by the above authors as well as by

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Figs 1–6. Shells of *Islamia trichoniana*, *Pseudoislamia balcanica* and *Trichonia trichonica*: 1 – *I. trichoniana* from Lake Trichonis, 2 – *P. balcanica* from a stream on N. shore of lake Trichonia, 3 – *P. balcanica* from the springs of Aghia Sophia, 4–5 – *T. trichonica* from Neromana, 6 – *T. trichonica* from Lake Trichonis, an immature specimen of *Dreissena blanci* Westerlund, 1890 is attached to the hydrobiid shell. Bars equal 1 mm

Reischütz & Reischütz (2003), Albrecht et al. (2006, 2009), Bank (2006), Frogley & Preece (2007), Szarowska & Falniowski (2004, 2011) and Radea et al. (2013).

It is known that in brackish and marine systems the Hydrobiidae are regarded as deposit feeders and/or herbivores feeding on periphytic algae (e.g. Lopez & Levinton 1978, Koutsoubas et al. 2000, Evagelopoulos et al. 2009), either scraping sediment particles including diatom frustules or handling large diatoms as individual food particles (Forbes & Lopez 1986, Hagerthey et al. 2002). There are no detailed data on the diet of the freshwater Hydrobiidae based on diatoms, though diatoms are among the dominant members of the littoral microalgae communities constituting the phototrophic biofilms in both lotic and lentic freshwater habitats (Molino & Wetherbee 2008, Sheath & Wehr 2015).

The aim of this study was to expand the knowledge about the soft body anatomy and distribution of the threatened local endemics *I. trichoniana, P. balcanica*, and *T. trichonica* and to provide preliminary data on their food items.

MATERIAL AND METHODS

Etoloakarnania has an extensive aquatic system in Western Greece including natural and artificial lakes, rivers, lagoons, and springs. Seven localities in Lake Trichonis and adjacent springs and streams were selected for sampling (Table 1) in the spring and autumn of 2014, 2015 and 2016. The snails were hand-collected from stones, gravel, mosses and dead leaves, and were fixed 'unrelaxed' in 70% ethanol for safer preservation of radulae and reproductive organs. Before dissection, the shells were removed by soaking in Pereny's solution. Radulae were cleaned with KOH solution (5 g l^{-1}) at room temperature, rinsed in distilled water and air-dried before being mounted on stubs and spray-coated in gold-palladium for Scanning Electron Microscopy (SEM Jeol JSM–35 operating at 25 kV).

Table 1. Sampling localities of *I. trichoniana*, *P. balcanica* and *T. trichonica* in Etoloakarnania, Greece, n – new records, c – re-discovery

Sampling localities	Coordinates		*I. trichoniana*	*P. balcanica*	*T. trichonica*
N.W. shore of Lake Trichonis	38°35'11.41"N	21°30'55.55"E	n	n	–
N. shore of Lake Trichonis	38°36'01.27"N	21°34'06.29"E	–	–	n
N. shore of Lake Trichonis	38°36'01.02"N	21°34'10.04"E	–	–	–
Stream, close to N. shore of Lake Trichonis	38°35'58.36"N	21°33'41.05"E	–	n	–
Spring in Aghia Sophia	38°34'57.71"N	21°38'57.11"E	–	c	–
Cave spring in Varasova	38°20'36.98"N	21°35'55.71"E	–	n	–
Spring in Neromanna	38°37'02.70"N	21°35'41.95"E	–	–	n

Digital pictures of reproductive organs were taken using a Canon EOS 1000D camera attached to a stereomicroscope Stemi 2000-C, Zeiss. During this procedure, the specimens were submerged in water in order to avoid distortion of important taxonomic features that could be caused by the long-term tissue preservation buffers.

The digestive system contents were analysed by isolating both the intestinal faecal pellets and the stomach of the hydrobiids examined. For detailed examination, faecal pellets and stomach of four specimens fixed in ethanol (one *I. trichoniana*, one *T. trichonica,* and two *P. balcanica,* one from Lake Trichonis and one from Aghia Sophia springs) were extracted and treated separately on microscope slides. A few drops of 50% hydrochloric acid (HCl) were added to eliminate any ingested carbonates (limestone, shell fragments). Then, a few drops of hydrogen peroxide H_20_2 (30%) were added to oxidise the organic matter (stomach walls, algal cell contents and any organic debris). The slides were left in Petri dishes for two days in direct sunlight (for faster organic oxidation); from time to time hydrogen peroxide was added to avoid drying. Digital pictures of diatoms were taken using an AmScope MU503 Real-Time Live Video Microscope Digital Camera attached to a Carl Zeiss Axiolab E re Microscope.

Anatomical terminology follows that of Hershler & Ponder (1998).

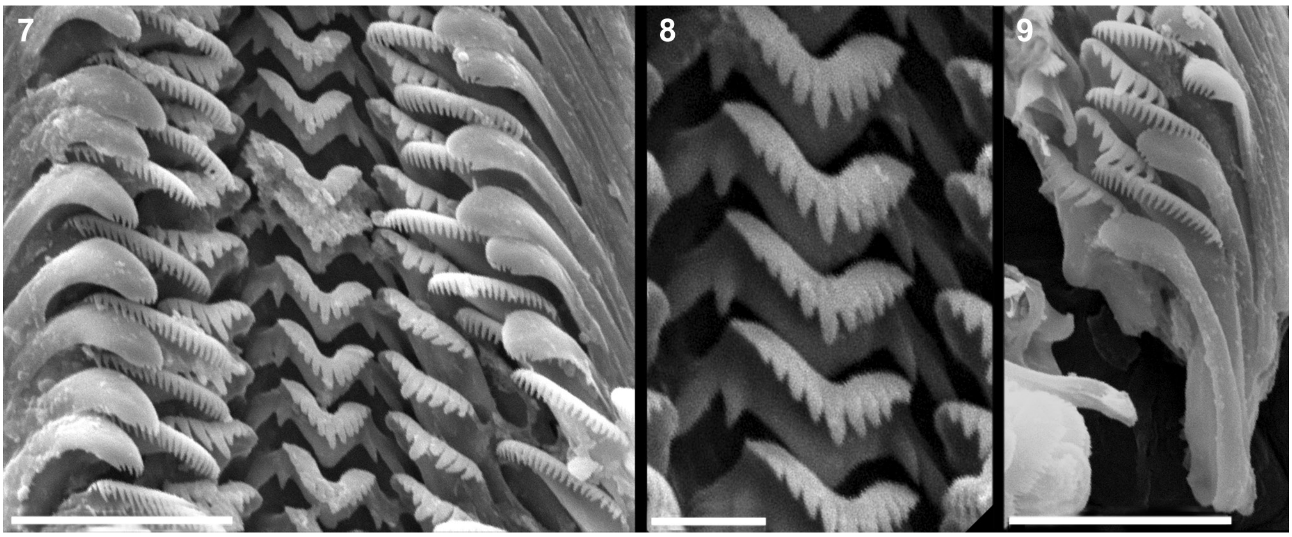
RESULTS

RADULA, REPRODUCTIVE ORGANS AND NEW RECORDS

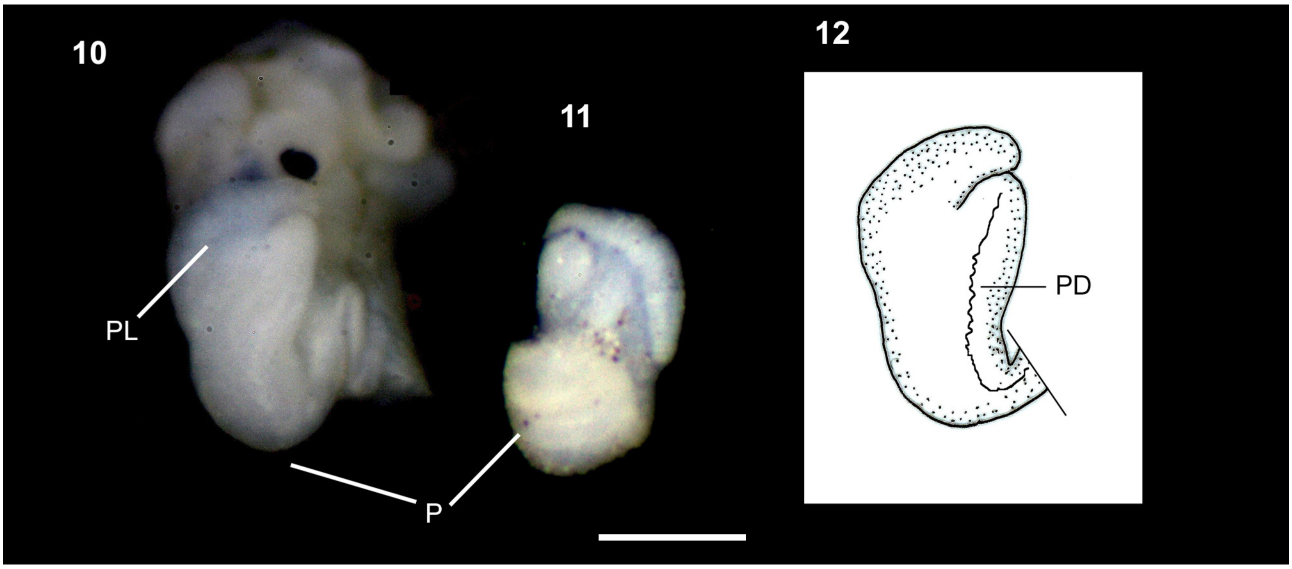
Islamia trichoniana Radoman, 1978

Shell valvatiform (Fig. 1), as described by Radoman (1978).

Radula (two specimens examined) taenioglossate (Figs 7–9). Central tooth trapezoidal, its dorsal edge strongly concave; one pair of medium-sized basal cusps (bc2), basal tongue broadly V-shaped and about equal to lateral margin; tooth base excavation more than 50% of tooth height; median cusp narrow hoe-shaped, protruding, followed by shorter narrow



Figs 7–9. Radula of *Islamia trichoniana*: 7 – radula portion, 8 – central teeth, 9 – lateral, inner and outer marginal teeth. Bars equals 20 μm



Figs 10–12. Penis of *Islamia trichoniana*: 10–11 – dorsal and ventral view, 12 – drawing (P – penis, PD – penial duct, PL – penial lobe). Bar equals 250 μm

cusps in decreasing order of size; formula of central tooth (Fig. 8):

$$\frac{4(5)\text{–C–}5(6)}{1\text{–}1}$$

lateral tooth face higher than wide (Fig. 9), basal tongue well developed; outer margin with concave end; cutting edge much shorter than outer wing; central cusp longer than lateral cusps, formula of lateral tooth: 3(4)–C-5, internal cusps shorter and stouter than the external ones; inner marginal tooth with ca. 25 long cusps of almost equal size (Fig. 9); outer marginal tooth with ca. 23 cusps.

Penis (Figs 10–12). Penis whitish, large in relation to head, long, wide, dorso-ventrally flattened, bifid due to well-developed penial lobe on its left side and penis proper on the right side; penial lobe with bent pointed tip covering penis apex; border of penial lobe with continuous refringent band; muscular pleat in the middle of penial lobe on ventral side of penis not protruding at the left side of penis (Fig. 11); penis apex with a small papilla visible ventrally; penial duct undulating, located at outer (right) penis edge, opening at apex of penis proper.

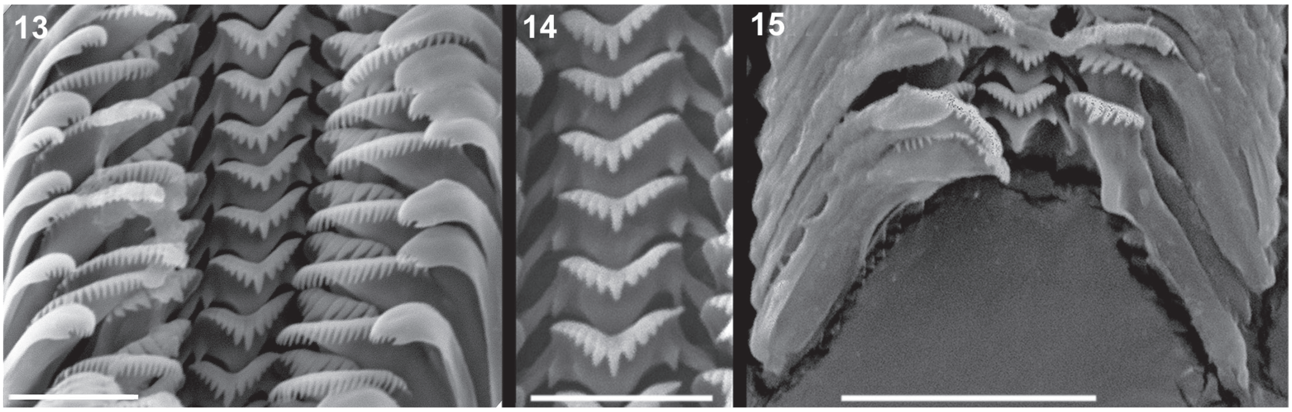
Female reproductive organs. No female specimen was collected during the present study and, consequently, the characters of female reproductive organs remain unknown.

New records. N.W. shore of Lake Trichonis between the villages Kainourgio and Kato Tragana, 38°35'11.41"N, 21°30'55.55"E, two adults and one immature specimen on aquatic plants, October 2014, leg. Radea, Bazos, Danielides.

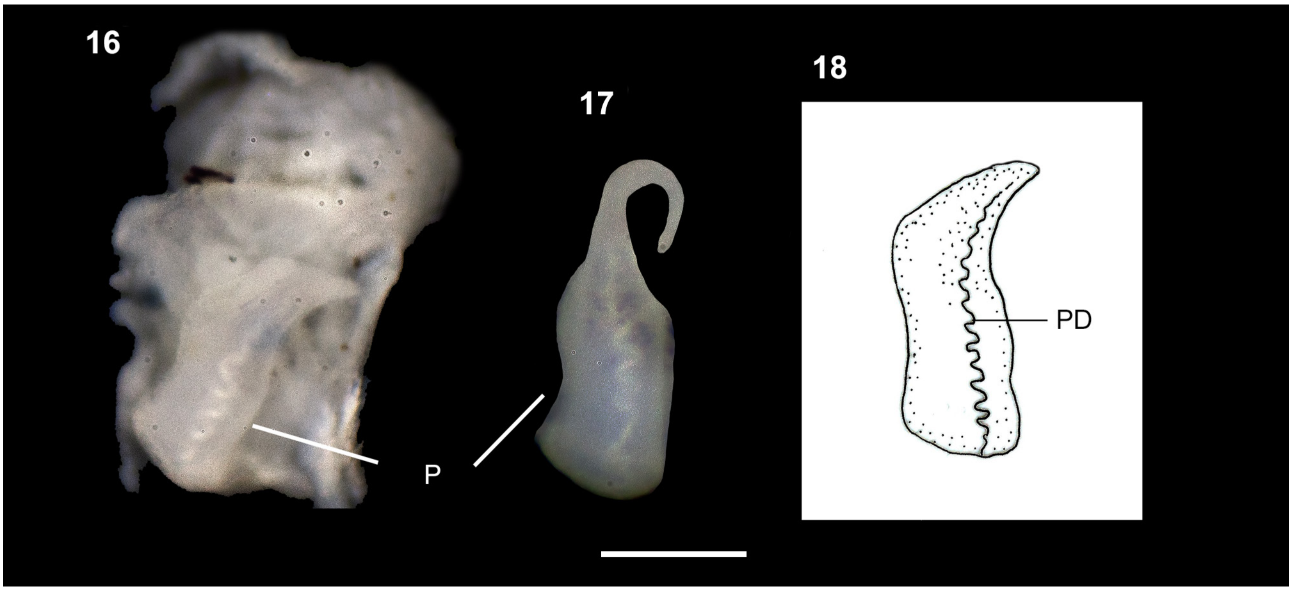
Pseudoislamia balcanica Radoman, 1978

Shell valvatiform (Figs 2–3), as described by Radoman (1978).

Radula (four specimens examined) taenioglossate (Figs 13–15). Central tooth trapezoidal, its dorsal edge concave; one pair of medium-sized basal cusps



Figs 13–15. Radula of *Pseudoislamia balcanica*: 13 – radula portion, 14 – central teeth, 15 – lateral and inner marginal teeth. Bar equals 10 μm



Figs 16–18. Penis of *Pseudoislamia balcanica*: 16 – penis in situ, 17–18 – photo and drawing of penis (P – penis, PD – penial duct). Bar equals 250 μm

(bc2), basal tongue narrowly V-shaped and about equal to lateral margin; tooth base excavation 25–50% of tooth height; median cusp narrow, pointed, protruding, followed on each side by shorter narrow pointed cusps in decreasing order of size; formula of central tooth (Fig. 14):

$$\frac{5\text{–C–}4(5)}{1\text{–}1}$$

lateral tooth face higher than wide (Fig. 15), basal tongue well developed; outer margin with concave end; cutting edge much shorter than outer wing; central cusp longer than lateral cusps; formula of lateral tooth: 3(4)–C–5; inner marginal tooth with ca. 27 long cusps of almost equal size (Figs 13, 15); outer marginal tooth with ca. 23 cusps (Figs 13, 15).

Penis (Figs 16–18). Penis whitish, large in relation to head, long, wide, dorso-ventrally flattened, bent back upon itself; distal section pointed at the tip and bent to the right like a hook; inner (left) edge bearing wide, blunt, unpigmented outgrowth almost in the middle of total penis length; outer (right) penis edge with a small grey-black pigmented area; penial duct strongly undulating, located almost centrally, clearly visible in fresh specimens.

Female reproductive organs (Figs 19–21, 23, 24). Bursa copulatrix semi-globular, medium-sized, located posteriorly to albumen gland; proximal (rs_2) seminal receptacle globular with a pink pearly sheen when filled with spermatozoa (Figs 19, 20), slightly smaller than bursa and lying against it; distal (rs_1) seminal receptacle elongate, much smaller than rs_2.

New records. Lake Trichonis, N.W. shore between the villages Kainourgio and Kato Tragana, 38°35'11.41"N 21°30'55.55"E, five specimens on aquatic plants, October 2014, leg. Radea, Bazos, Danielides. Stream, N. shore of Lake Trichonis, 38°35'58.36"N 21°33'41.05"E, 12 specimens on stones, October 2014, leg. Radea, Gioutlakis, Defiggou. Spring near village Varasova, 38°20'36.98"N 21°35'55.71"E, one specimen on stone, May 2016, leg. Bakolitsas.

Trichonia trichonica Radoman, 1973

Shell conical (Figs 4–6) as in Radoman (1973) and Szarowska (2006).

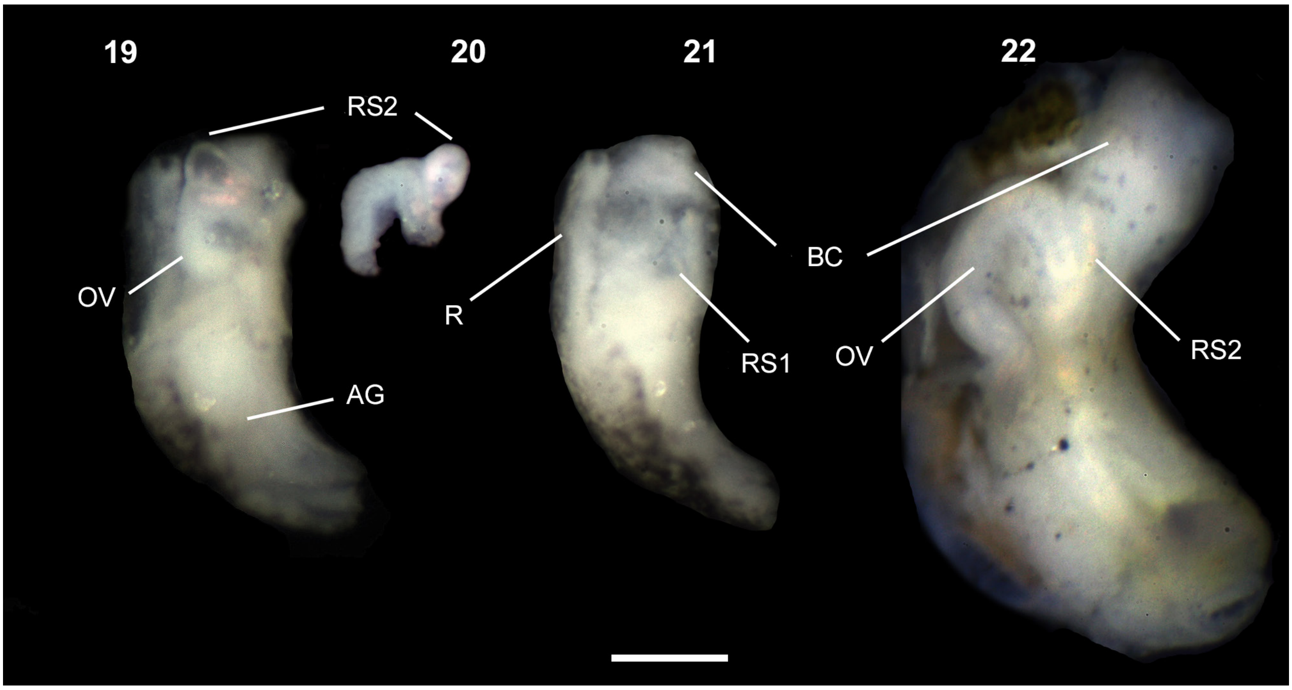
Radula and penis. Described earlier (see Introduction).

Female reproductive organs (Figs 22, 25). Bursa copulatrix globular-pyriform, large-sized, located posteriorly to albumen gland; renal oviduct well developed; proximal (rs_2) seminal receptacle elongate with a pink pearly sheen, lying against bursa copulatrix; distal (rs_1) seminal receptacle smaller than rs_2.

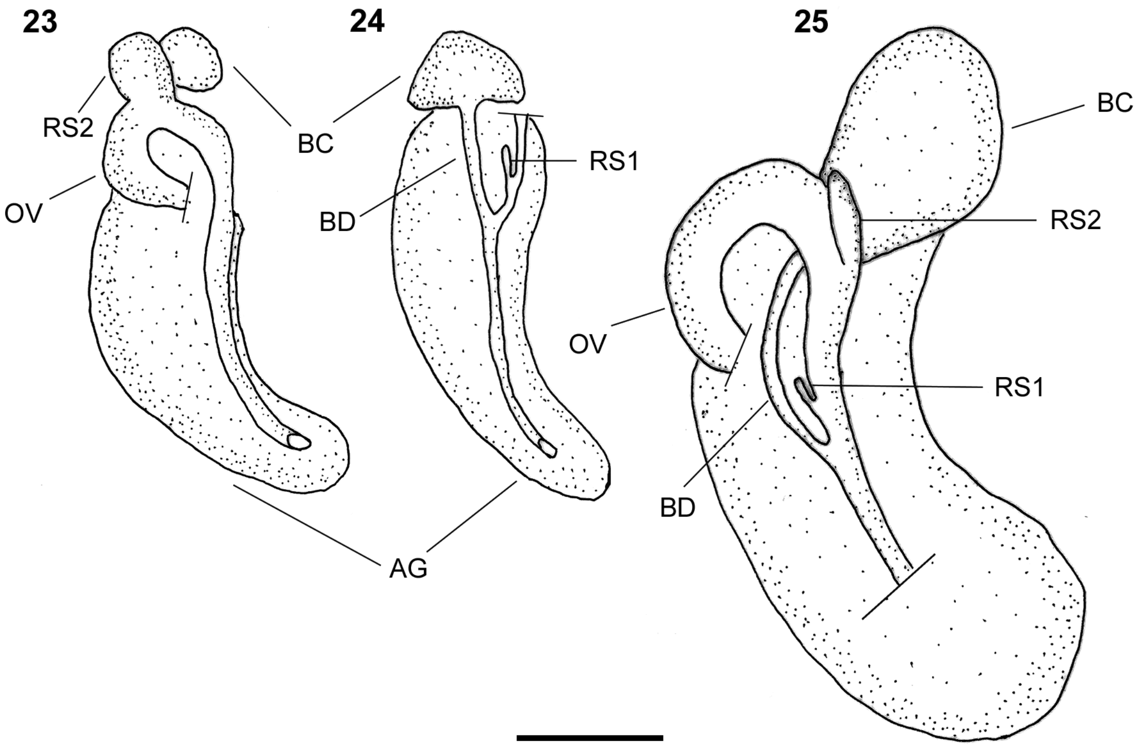
New records. Lake Trichonis, 38°36'01.27"N 21°34'06.29"E, nine specimens on aquatic plants, October 2014, leg. Radea, Bazos. Spring, Neromanna, 38°37'2.70"N 21°35'41.95"E, two specimens on stones, December 2015, leg. Bakolitsas.

DIATOM FOOD ITEMS IN STOMACH CONTENTS AND FAECAL PELLETS

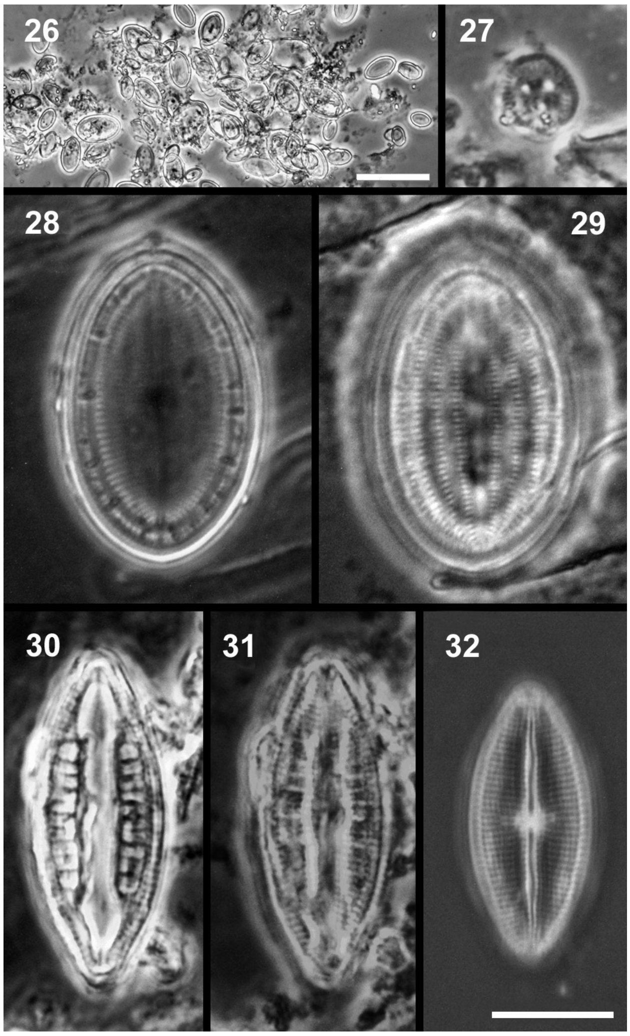
The diatom *Cocconeis placentula* Ehrenberg sensu Jahn et al. (2009) (Figs 26, 28, 29) was abundantly ingested by *P. balcanica* (Aghia Sophia) and in smaller quantities by *T. trichonica* (Lake Trichonis). A few valves of *Cyclotella trichonidea* var. *parva* Economou-Amilli (Fig. 27) and *Mastogloia elliptica* (Agardh) Cleve (Figs 30, 31) were found in the digestive system of *T. trichonica* (Lake Trichonis). No diatom remains were observed in the digestive system of *I. trichoniana* and *P. balcanica* from Lake Trichonis.



Figs 19–22. Female reproductive organs of: 19–21 – *Pseudoislamia balcanica*: 19 – general view, 20 – seminal receptacle (rs_2 sensu RADOMAN 1983) and part of oviduct loop, 21 – female reproductive organs without oviduct loop; 22 – *Trichonia trichonica* (AG – albumen gland, BC – bursa copulatrix, OV – oviduct loop, R – rectum, RS1 – distal seminal receptacle, RS2 – proximal seminal receptacle). Bar equals 250 μm



Figs 23–25. Drawings of female reproductive organs of: 23–24 – *Pseudoislamia balcanica*, 25 – *Trichonia trichonica* (AG – albumen gland, BC – bursa copulatrix, BD – bursal duct, OV – oviduct loop, R – rectum, RS1 – distal seminal receptacle, RS2 – proximal seminal receptacle). Bar equals 250 μm



Figs 26–32. Valves of diatoms found in the digestive system of *Psudoislamia balcanica* and *Trichonia trichonica* after treatment: 26 – general view of an aggregation of *Cocconeis* valves from the digestive system of *P. balcanica* (Aghia Sophia), 27 – *Cyclotella trichonidea* var. *parva* from the digestive system of *T. trichonica* (Lake Trichonis), 28–29 – raphe and rapheless valves, respectively, of *Cocconeis placentula* var. *euglypta* from the digestive system of *T. trichonica* (Lake Trichonis), 30 – *Mastogloia elliptica* showing the partectal ring from the digestive system of *T. trichonica* (Lake Trichonis), 31– *Mastogloia elliptica* showing the transapical striae from the digestive system of *T. trichonica* (Lake Trichonis), 32 – an intact valve of *Mastogloia elliptica* found in periphytic samples from Lake Trichonis better showing the details in valve morphology. Bar equals 100 μm in Fig. 26 and 10 μm in Figs 27–32

DISCUSSION

ANATOMY AND DISTRIBUTION

Our knowledge about the internal morphology of the three endemic species *I. trichoniana*, *P. balcanica* and *T. trichonica* is mainly based on the short descriptions and drawings of RADOMAN (1973, 1978, 1983). The detailed description of both the unknown radulae and the poorly known reproductive organs of *I. trichoniana* and *P. balcanica* presented here supplement the species descriptions.

Islamia trichoniana

The genus *Islamia* is characterised by the presence of either one or two pairs of basal cusps on the central radular tooth (RADOMAN 1983, BODON et al. 2001). *I. trichoniana* is included among those having a single pair of basal cusps on the central tooth; this character state is also observed in the European *I. atteni*, *I. gaiteri*, *I. globulus*, *I. valvataeformis* and *Islamia* sp. (BODON et al. 1995, 1996, 2001, ARCONADA & RAMOS 2006). In the former Rissooidea, the single pair of basal cusps was assumed to be plesiomorphic (PONDER 1985).

The reproductive organs of *I. trichoniana* were not described by RADOMAN (1978: 25, 1983: 127). He only mentioned that the penis of this species was similar to that of *Islamia graeca* Radoman, 1973, a species described from a lake (L. Amvrakia) near Lake Trichonis. However, this is not confirmed by our findings. The penis of *I. graeca* bears “small, short branches and a large fold on the ventral side” according to RADOMAN (1983: 125, fig. 70C). On the contrary, the penis of *I. trichoniana* has no short branches and it bears a large penial lobe overgrowing the penis apex proper; the penial duct is clearly visible and the muscular pleat (“fold” according to Radoman) is of medium size. The muscular pleat on the ventral side of penis and the well-developed penial lobe show that *I. trichoniana* belongs to the “oriental” group of *Islamia* species which live in the Balkan Peninsula, Turkey and part of Italy (BODON et al. 1995).

Regnier et al. (2009) considered *I. trichoniana* to be extinct from Lake Trichonis. Despite the efforts made to find live specimens (Reischütz & Reischütz 2003, Szarowska 2006, Albrecht et al. 2009, Radea et al. 2013), only empty shells were recorded in the lake. In 2003, Reischütz & Reischütz found live specimens in Aghia Sophia, a spring close to Lake Trichonis. During the present survey, *I. trichoniana* was re-found in Lake Trichonis but not in its second known locality in Aghia Sophia.

Pseudoislamia balcanica

The central radular tooth of *P. balcanica* bears a single pair of basal cusps (similarly as in *I. trichoniana*). The cusps of the central and lateral teeth are narrower and more pointed compared to those of *I. trichoniana* (present study) and *T. trichonica* (Szarowska 2006: 112, fig. 130).

The male and female reproductive organs differ from those described and illustrated by Radoman (1978: 23–24, fig. 1, 1983: 83–84, fig. 44); namely, the pointed penial apex, the lower position of the outgrowth, the presence of a grey-black pigmented area on the dorsal side of penis, as well as the larger size and the semi-globular shape of bursa copulatrix are notable differences.

During the present study, *P. balcanica* was found in two new localities, a stream close to Pantanassa on the N. shore of Lake Trichonis and a spring 20 km farther (in Varasova). Additionally, it was re-found in the springs of Aghia Sophia, where it had been recorded for the first time by Radea et al. (2013). The number of the species' localities is now four. These records corroborate the earlier suggestion (Radea et al. 2013) that *P. balcanica* is more widespread than previously thought and that the species inhabits both lentic and lotic waters.

Trichonia trichonica

The shape of the female reproductive organs of *T. trichonica* is quite similar to those of the type species of *Trichonia,* i.e. *T. kephalovrissonia* (Radoman 1973: 22, 1983: 82). The bursa copulatrix was found to be even larger than that illustrated for the type (Radoman 1983: 84, fig. 43).

It is remarkable that in the majority of specimens from Lake Trichonis the reproductive organs were misshapen: the males had dwarf penes and the female reproductive organs were damaged by trematodes. There is a distinct interaction between pollutants and trematode parasitism in freshwater gastropods; pollution can modulate levels of parasitic infections by suppressing host immunity (Hock & Poulin 2012). Lake Trichonis receives pollutants from various sources, especially from intensive agricultural practices, urban sewage, stock grazing and small industries (Bertahas et al. 2006).

In the last three decades, *T. trichonica* was found alive in Lake Trichonis three times, in 1985 (Szarowska 2006), 2009 and 2012 (Radea et al. 2013). During 24 years the species was considered to be extinct from the lake (Regnier et al. 2009). In the present study, *T. trichonica* was collected in a new locality (Neromanna), a spring 3 km NE of Trichonis as well as from a new site in Lake Trichonis, west of that reported in Radea et al. (2013). As in the case of *P. balcanica,* the distribution of this species is wider than previously thought.

INGESTED DIATOMS AND MORPHOLOGY OF RADULAR CUSPS

The main ecological characters of the diatom taxa found in the stomach and the faecal pellets of the hydrobiids examined are presented below.

Cyclotella trichonidea var. *parva* Economou-Amilli

C. trichonidea var. *parva* is an endemic (Economou-Amilli 1979, 1982) planktonic and periphytic (Economou-Amilli & Tafas 2000) diatom described from Lake Trichonis. In periphytic samples it was observed to be loosely attached to the substratum (Louvrou & Economou-Amilli, unpublished), thus belonging to the low profile ecological guild sensu Passy (2007).

Mastogloia elliptica (Agardh) Cleve

Mastogloia elliptica is regarded as new for Lake Trichonis, and new for Greece. In Lake Trichonis, it was found living on the substratum (Fig. 32) with no obvious extracellular polysaccharide structures and restricted motility, thus belonging to the low profile ecological guild.

Cocconeis placentula Ehrenberg sensu Jahn et al. (2009)

C. placentula is a common periphytic diatom of Lake Trichonis that abundantly colonises all kinds of substrata; it has been found as epizoic on the gastropod *Potamopyrgus antipodarum* (J. E. Gray, 1843), epiphytic on aquatic plants (*Myriophyllum spicatum* L. and *Nasturtium officinale* R. Br. (Radea et al. 2008) and on abiotic substrata (Louvrou & Economou-Amilli, unpublished). The species is included in the low profile ecological guild since it is prostrate, i.e. adheres firmly to the substratum through the entire raphe valve surface (Passy 2007).

Aquatic gastropods with taenioglossate radula which are adapted to 'rasping' and 'scraping' the biofilms are able to feed on specific growth forms of microalgae (crustose, prostrate, gelatinous, and some of the stalked and short filamentous forms) (STEINMAN 1996, VENKATESAN et al. 2016).

P. balcanica, with its narrow and pointed cusps on the central and lateral teeth (Figs 21–23) might be able to feed on organic debris and protists' cells by rasping and by piercing and tearing fleshy algae (HAWKINS et al. 1989, PADILLA 2004).

T. trichonica has wide and blunt radular cusps on the central and lateral teeth (SZAROWSKA 2006: p. 112, fig. 130), a structure offering more surfaces in contact with the substratum when feeding. This radula type is eminently effective for rasping and removing loose material from the substratum (PADILLA 1985, 2004).

Our findings on the diatom food items (Figs 27–31) and their mode of attachment to the substratum are in accordance with the above views on the function mode of the radula of *P. balcanica* and *T. trichonica*.

BIODIVERSITY OF LAKE TRICHONIS

In the "Project Aqua" Trichonis was characterised as an area of high potential research value because of its high number of endemic algae, molluscs and fishes (LUTHER & RZOSKA 1971). It was designated as Special Area for Conservation (SAC) and, finally, included in the European Ecological Network Natura 2000 due to the priority habitat of calcareous fens.

This lake is known as a hotspot of freshwater biodiversity in Greece, particularly of molluscs. It is remarkable that the index of gastropod endemism estimated for the Lake Trichonis Basin is close to such values for Lake Baikal, Russia, and Lake Biwa, Japan, and is only exceeded by Lake Ohrid, Macedonia/Albania, and the ancient lakes of Sulawesi, Indonesia (ALBRECHT et al. 2009).

The continuous presence of the local endemic hydrobiids *I. trichoniana*, *P. balcanica* and *T. trichonica*, together with the presence of diatoms regarded as endemic (*C. trichonidea* and *C. trichonidea* var. *parva*) is a valuable indication of the biodiversity preservation in Lake Trichonis.

During the last thirty years, Lake Trichonis has shown a general stability of its physicochemical parameters and has retained its oligo- to mesotrophic character. However, the presence of certain zooplanktonic species which are typical of eutrophic lakes could reflect a possible alteration of its trophic status (DOULKA & KEHAYIAS 2008, KEHAYIAS & DOULKA 2014). Being mainly hydrology-dependent, the trophic status of Trichonis is unpredictable. Consequently, effective management targeting both elimination of nutrient pollution loads and controlled water extraction is necessary (ALBRECHT et al. 2006, BERTAHAS et al. 2006) in order to protect the lake's biodiversity.

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