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CONTRIBUTION TO THE MORPHOLOGY OF THE BULGARIAN STYGOBIONT TRUNCATELLOIDEA (CAENOGASTROPODA)

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ABSTRACT: The paper deals with morphology of the representatives of an entirely stygobiont, molecularly distinct and ancient clade of Bulgarian Truncatelloidea. Ten nominal species of seven genera (two of them new: *Devetakiola* Georgiev and *Stoyanovia* Georgiev) are analysed; six of them (except four *Pontobelgrandiella* species) are re-described. The knowledge of subterranean stygobiont gastropods is still limited; cave-dwelling species are mostly known only from empty shells found on the surface. The study includes all the Bulgarian stygobiont truncatelloideans known not only from empty shells. Their shells, soft part pigmentation, female reproductive organs and penes are presented. The results are compared with fragmentary data from the literature.

KEY WORDS: Hydrobiidae, stygobiont, new genus, shell, pigmentation, reproductive organs, Bulgaria

INTRODUCTION

The knowledge of subterranean, stygobiont gastropods is still limited. Especially cave-dwelling species are usually known only from empty shells found on the surface. Many species and genera of subterranean truncatelloideans have recently been described from Bulgaria, as a result of the regular exploration of the cave fauna (BERON 2007). RADOMAN (1978) described the genus *Pontobelgrandiella*, designating *P. nitida* (Angelov, 1972) as the type species; its shell, soft part morphology and anatomy were described by RADOMAN (1983) and SZAROWSKA (2006). Later, several nominal species of *Pontobelgrandiella* (often as *Belgrandiella* Wagner, 1927) were described from Bulgaria (GEORGIEV 2011a, 2013), based exclusively on the shell and penial morphology. This study deals with ten species of seven genera (*Pontobelgrandiella* has been presented elsewhere: RYSIEWSKA et al. 2016); the paper deals with all the nominal taxa of stygobiont gastropods known from Bulgaria and available not only as empty shells. Many of these taxa are known from one site each: *Balkanica yankovi* Georgiev, 2011, *Balkanospeum schniebsae* (Georgiev, 2011), *Devetakia krushunica* Georgiev et Glöer, 2011 and *Devetakia mandrica* Georgiev, 2012 (GEORGIEV 2011b, GEORGIEV & GLÖER 2011, GEORGIEV 2012). Some are known from two sites: *"Bythiospeum" stoyanovi* Georgiev, 2013, *"Bythiospeum" devetakium* Georgiev et Glöer, 2013 and *Cavernisa zaschevi* (Angelov, 1959) (GEORGIEV 2013, GEORGIEV & GLÖER 2013); only *Pontobelgrandiella* is widely distributed (RYSIEWSKA et al. 2016). There are also records of a new species of *Bythiospeum* Bourguignat, 1882, but the occur-

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rence of the representatives of this genus in Bulgaria has never been confirmed. Several representatives of the Moitessieriidae, like *Paladilhiopsis* Pavlovic, 1913, or *Iglica* Wagner, 1927, are erroneously assigned to *Bythiospeum* (e.g. SLAPNIK 1995). Moreover, nearly any truncatelloid with a tiny, turriform shell, found in a spring or cave, may be described as "*Bythiospeum*", despite the fact that it definitely does not belong to the family Moitessieriidae, and represents Hydrobiidae/Sadlerianinae (e.g. FALNIOWSKI et al. 2014). The small number of specimens available – some nominal species represented by single empty shells – coupled with the rudimentary knowledge of their anatomy, biology, distribution, etc., resulted in dozens of nominal species, whose distinctness and phylogenetic relationships are doubtful.

Our molecular study of the obligatory stygobiont Bulgarian truncatelloids revealed a monophyletic, highly supported lineage, including five distinct clades of presumably generic level (OSIKOWSKI et al. in press). The stygobiont snails live in populations composed of extremely small number of specimens, and are not easy to collect. Thus many species are known as empty shells only, or a few fixed specimens. The aim of this paper is to re-describe the truncatelloidean taxa inhabiting caves of Bulgaria.

MATERIAL AND METHODS

SAMPLE COLLECTION AND FIXATION

Snails were collected from 13 cave localities in Bulgaria either by hand or with a sieve (Fig. 1, Table 1). The snails were washed in 80% ethanol and left to stand in it for about 12 hours. The ethanol was then changed twice during 24 hours.

MORPHOLOGICAL TECHNIQUES

The shells were cleaned with an ultrasonic cleaner, and photographed with a CANON EOS 50D digital camera. The snails were dissected and their penes photographed under a NIKON SMZ18 stereoscopic microscope with dark field and phase contrast, and a CANON EOS 50D digital camera. The female reproductive organs were drawn with a NIKON drawing apparatus. Original descriptions were used for comparisons (RADOMAN 1983, GEORGIEV 2011b, 2012, 2013, GEORGIEV & GLÖER 2011, 2013).

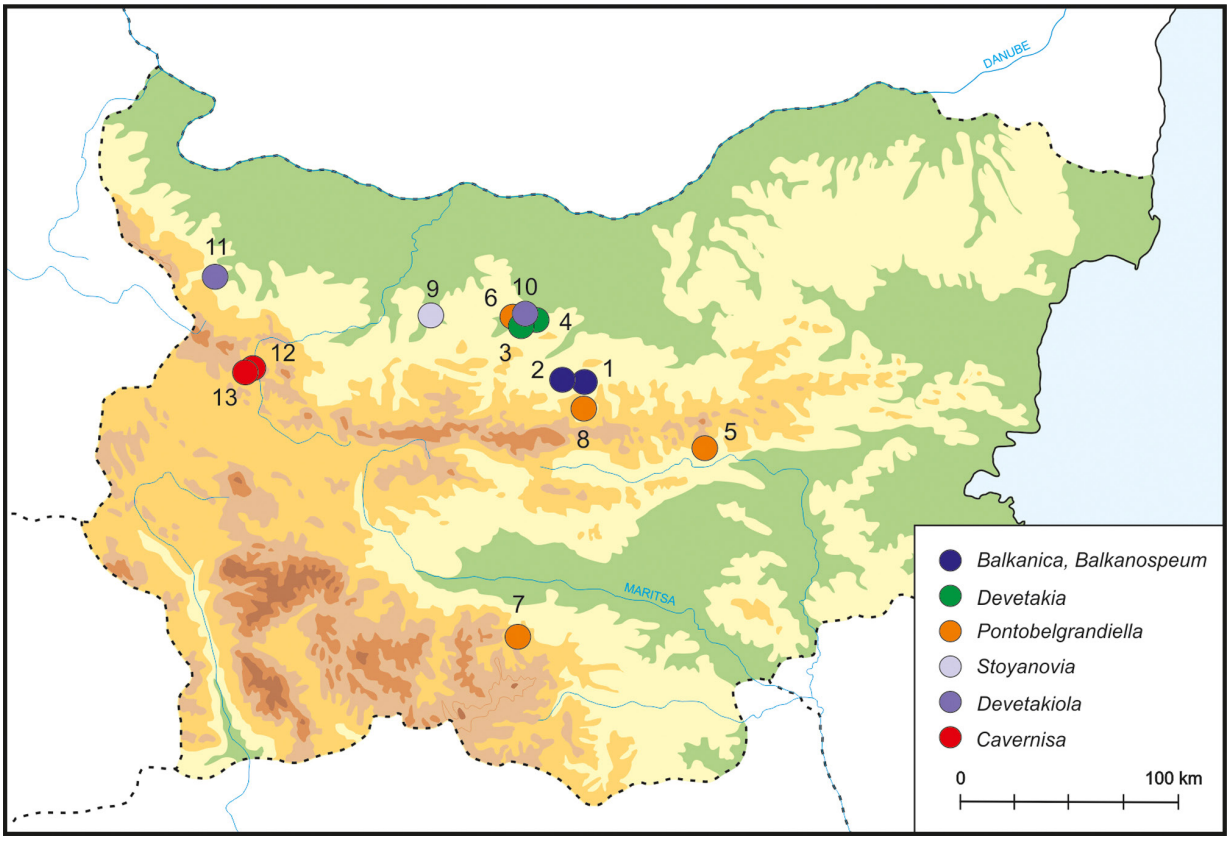


Fig. 1. Localities of the studied cave gastropods in Bulgaria (see: Table 1)

Table 1. Sampling localities of the studied populations with their geographical coordinates

ID	Locality	Coordinates		Original name
	Balkanica			
1	Bulgaria – Sulari and Yantra villages, Izvora (Padaloto, Yantra) cave, Stara Planina Mts	42°57'23"N	25°18'52"E	*Balkanica yankovi*
	Balkanospeum			
2	Bulgaria – Zdravkovets village, Machanov Trap cave, Stara Planina Mts	42°57'59"N	25°13'30"E	*Balkanospeum schniebsae*
	Devetakia			
3	Bulgaria – Chavdartsi village, Mandrata cave, N. foothills of Stara Planina Mts	43°14'32"N	24°58'09"E	*Devetakia mandrica*
4	Bulgaria – Krushuna village, Urushka Maara cave, N. foothills of Stara Planina Mts	43°14'27"N	24°57'49"E	*Devetakia krushunica*
	Pontobelgrandiella			
5	Bulgaria – Tvarditsa town, spring, Stara Planina Mts	42°42'20"N	25°53'52"E	*Belgrandiella angelovi*
6	Bulgaria – Devetashka cave, stream at the cave entrance, N. foothills of Stara Planina Mts	43°14'03"N	24°53'04"E	*Belgrandiella pandurskii*
7	Bulgaria – Chardaka, water source, W Rhodopes Mts	41°53'13"N	24°53'07"E	*Belgrandiella dobrostanica*
8	Bulgaria – Shipka Pass, water source, Stara Planina Mts	42°49'02"N	25°19'28"E	*Belgrandiella angelovi*
	Stoyanovia			
9	Bulgaria – Bezhanovo village, Parnitsite cave, N. foothills of Stara Planina Mts	43°13'44"N	24°23'19"E	*Bythiospeum stoyanovi*
	Devetakiola			
10	Bulgaria – Alexandrovo, Brashlyanskata cave, N. foothills of Stara Planina Mts	43°14'14"N	24°56'12"E	*Bythiospeum devetakium*
11	Bulgaria – Prevala village, spring near Vreloto cave, N. foothills of Stara Planina Mts	43°28'10"N	22°51'40"E	*Bythiospeum devetakium*
	Cavernisa			
12	Bulgaria – Tserovo village, Vodnata cave	43°00'25"N	23°20'33"E	*Cavernisa zaschevi*
13	Bulgaria – Tserovo village, Yamata cave	43°00'05"N	23°19'40"E	*Cavernisa zaschevi*

RESULTS AND DISCUSSION

Balkanica Georgiev, 2011

Balkanica yankovi Georgiev, 2011

Material: Sulari and Yantra villages, Izvora (Padaloto, Yantra) cave, Stara Planina Mts (locality 1).
Shell (Figs 2–8) very small (1.3 mm high), broad and ovate-conical with a moderately high spire formed by 3.5 relatively fast growing whorls separated by a moderately deep suture. Aperture oval to pyriform, surrounded by a well-marked continuous lip (Fig. 2). Shell yellowish and translucent. Soft parts unpigmented (Fig. 2), eyes absent. Female reproductive organs (Fig. 9), unknown until now, include a short and broad pallial accessory gland complex, a large bursa copulatrix with a moderately long duct, a broad loop of oviduct and one receptaculum seminis, small and cylindrical in shape, in the position of rs_1 (after RADOMAN 1973, 1983, SZAROWSKA 2006). Penis (Figs 10–12) simple, bent at half its length, and gradually narrowing, with a glandular outgrowth on its left side, at approximately half penis length.

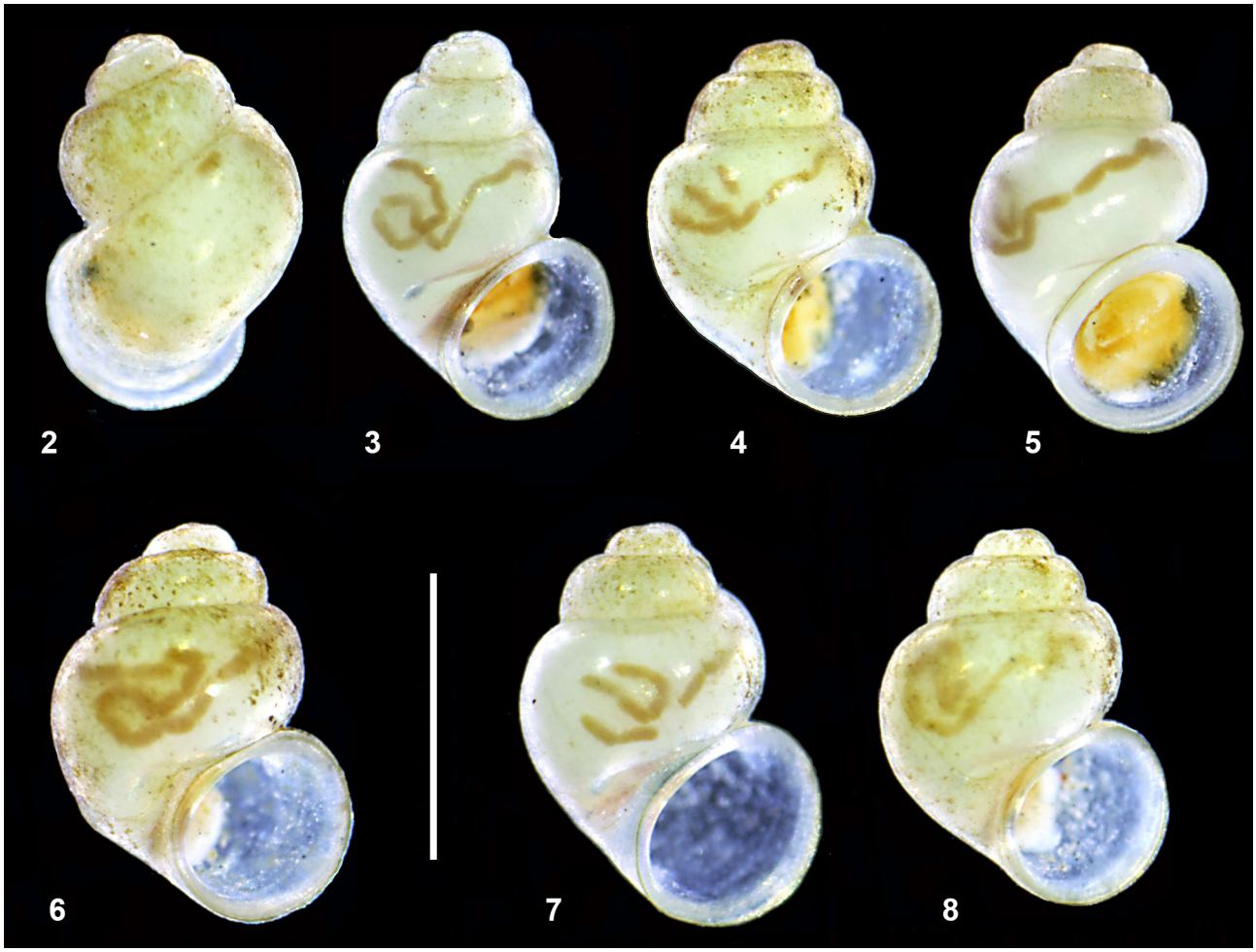
Balkanospeum Georgiev, 2012

Balkanospeum schniebsae (Georgiev, 2011)

Material: Zdravkovets village, Machanov Trap cave, Stara Planina Mts (locality 2).
Shell (Figs 13–15) elongate-conical, with a rather high spire formed by 4.5–5 regularly growing whorls, moderately convex and separated by a moderately deep suture. Aperture oval, surrounded by a well-marked continuous lip (although less developed than in *Balkanica*). Shell whitish, shiny and translucent. Soft parts unpigmented (Figs 13–15), eyes absent (Fig. 16). Female reproductive organs similar to those of *Balkanica*. Penis (Figs 16–17) simple, bent, long and narrow, with a characteristic outgrowth on its left side, approximately at half penis length.

Devetakia Georgiev et Glöer, 2011

GEORGIEV & GLÖER (2011) described a new genus *Devetakia*, with two species: *D. krushunica* Georgiev et Glöer, 2011 from Urushka Maara cave (type species) (locality 4) and *D. pandurskii* Georgiev



Figs 2–8. Shells of *Balkanica yankovi*, bar equals 1 mm

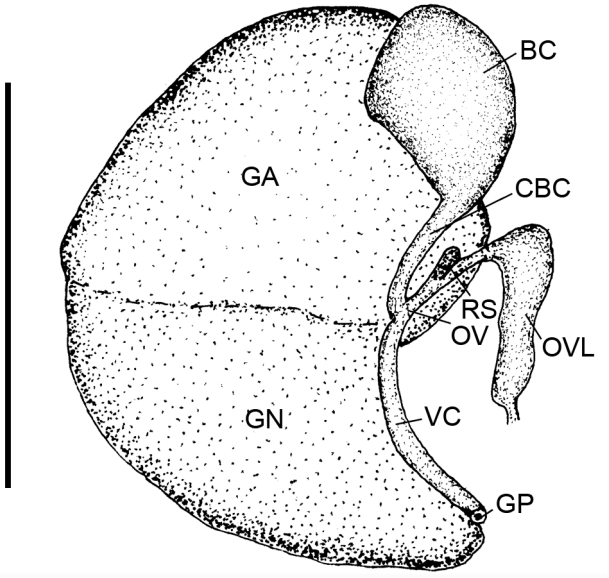
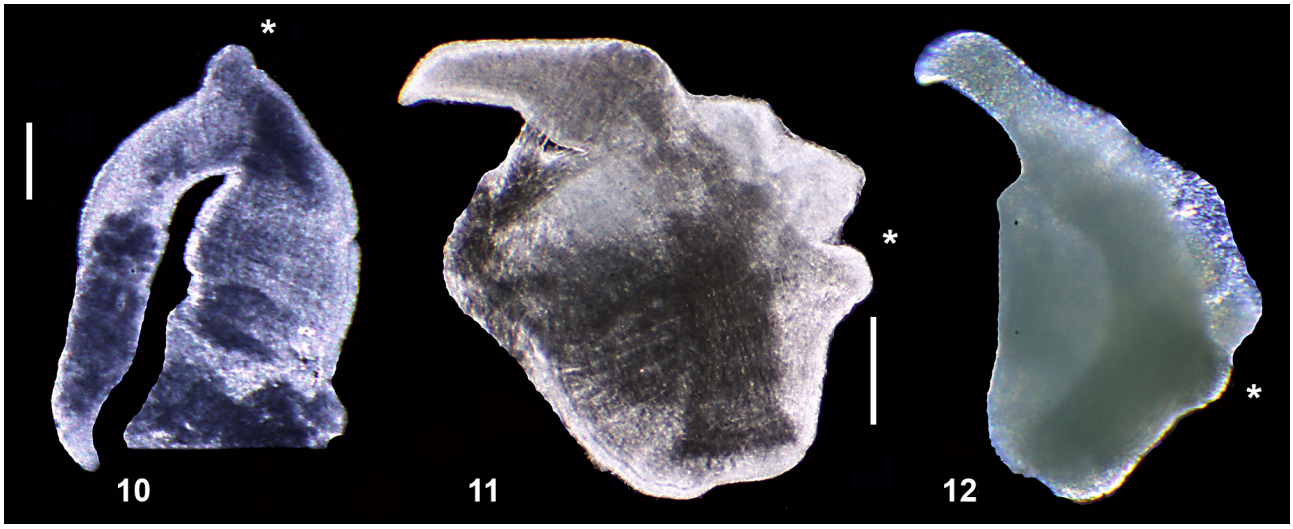


Fig. 9. Female reproductive organs of *Balkanica yankovi*, bar equals 0.5 mm (BC – bursa copulatrix, CBC – duct of bursa copulatrix, GA – albuminoid gland, GN – nidamental gland, GP – gonoporus, OV – oviduct, OVL – loop of (renal) oviduct, RS – receptaculum seminis, VC – ventral channel)

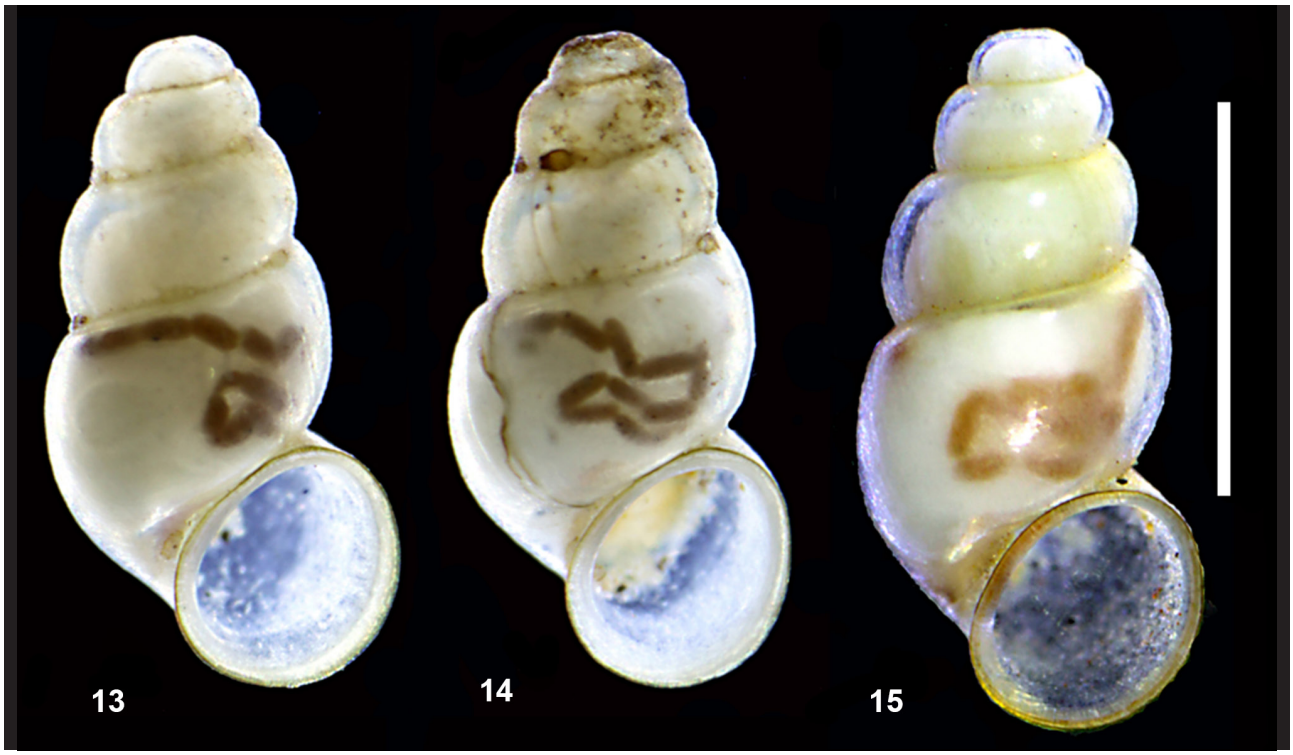
et Glöer, 2011 from Devetashka cave (locality 6). It should be noted that GEORGIEV (2011c) described, also from Devetashka cave, *Belgrandiella pandurskii* Georgiev, 2011, which actually represents the genus *Pontobelgrandiella* and should not be confused with *Devetakia pandurskii*. The shells of the two nominal species of *Devetakia* were practically identical. All the descriptions were based on the shells alone, thus the species distinctness of these taxa resulted mainly from a belief that cave fauna must be isolated and inevitably speciates without any stasis. Later, GEORGIEV (2012) described a third nominal species of *Devetakia*, *D. mandrica* Georgiev, 2012, from Mandrata cave. The shell of *D. mandrica* was different from those of its two congeners, as the latter had much deeper sutures and more convex whorls. In this paper, all the morphological data concern *D. mandrica*, since the material of the remaining species was not sufficient.

Devetakia mandrica Georgiev, 2012

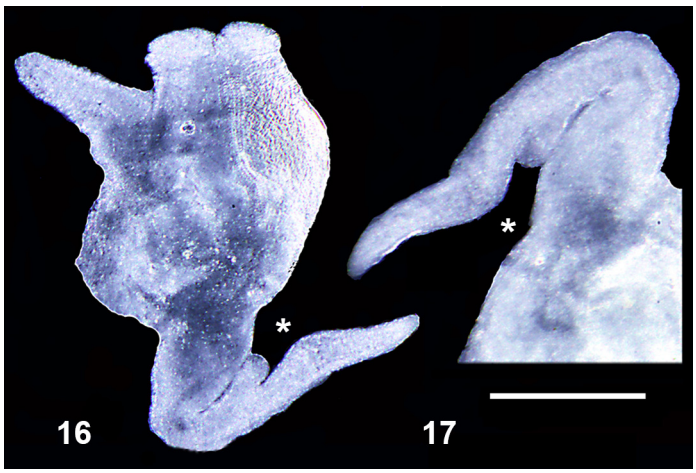
Material: Chavdartsi village, Mandrata cave, N foothills of Stara Planina Mts (locality 3).
Shell (Figs 18–24) cylindrical or nearly cylindrical, with 4–4.5 flat whorls separated by a shallow suture.



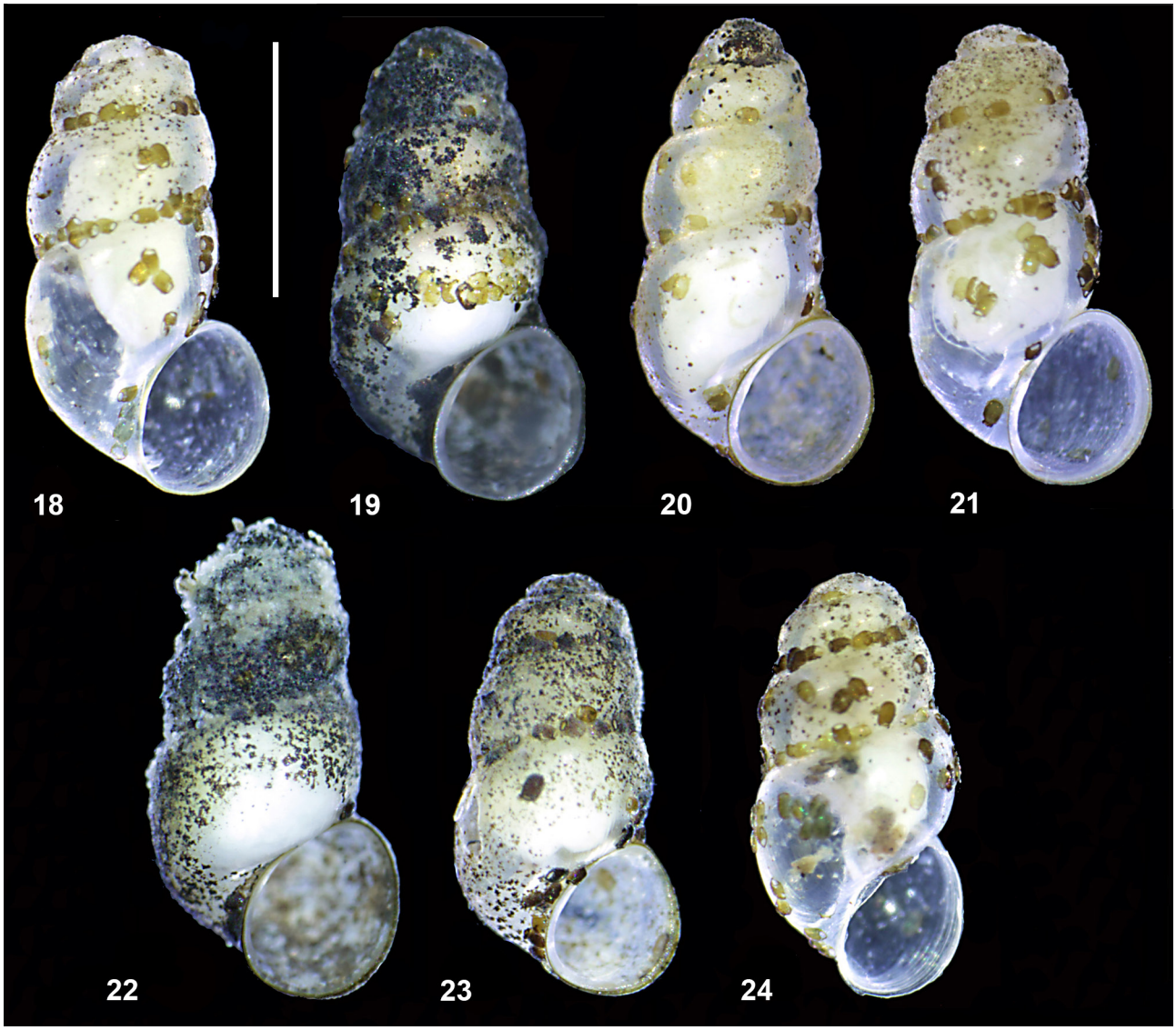
Figs 10–12. Penes of *Balkanica yankovi*, asterisks indicate the glandular outgrowth, bars equal 0.1 mm



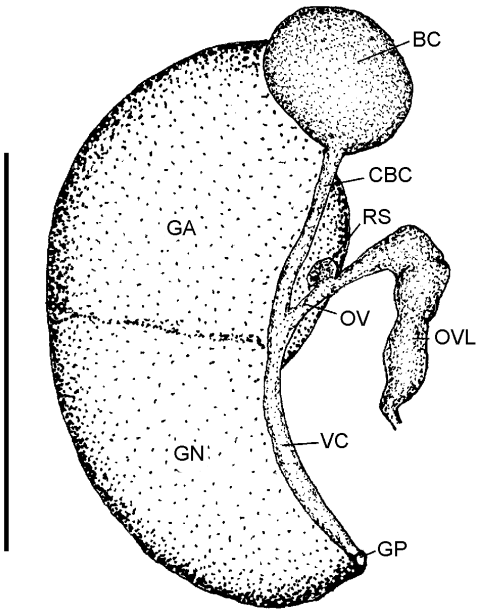
Figs 13–15. Shells of *Balkanospeum schniebsae*, bar equals 1 mm



Figs 16–17. Soft parts of male *Balkanospeum schniebsae*, bar equals 0.2 mm: 16 – eyeless head with penis, visible left cephalic tentacle and radula; 17 – penis; asterisks indicate the outgrowth on the left side

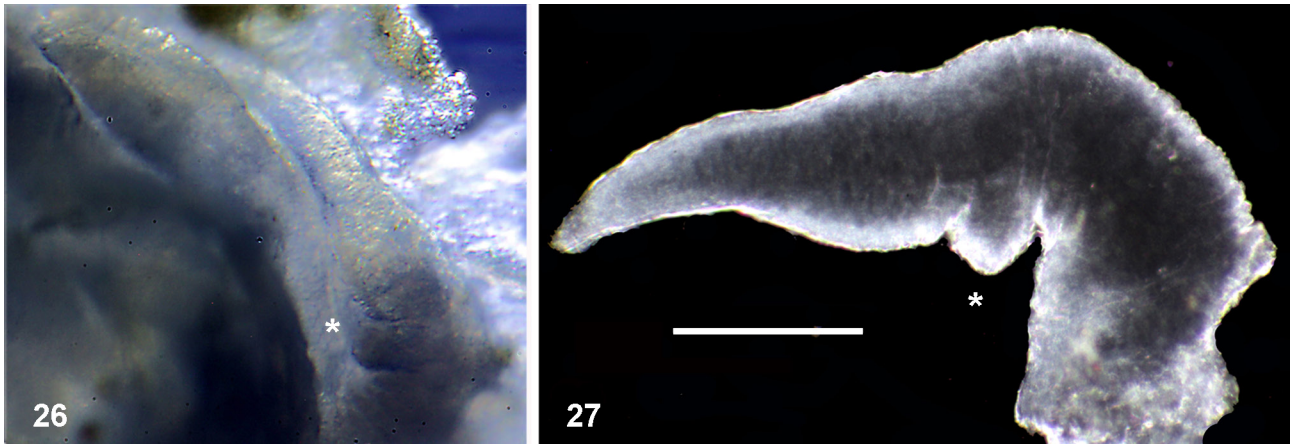


Figs 18–24. Shells of *Devetakia mandrica*, bar equals 1 mm



Spire high, apex flat. Aperture oval, peristome continuous, umbilicus slit-like, outer lip slightly developed; shell translucent, fragile and shiny. Soft parts unpigmented (Figs 18–24), eyes absent. Female reproductive organs (Fig. 25), unknown so far, with a moderately broad pallial accessory gland complex, a small and spherical bursa copulatrix with a long duct, broad loop of oviduct and one receptaculum seminis, small and spherical in shape, in the position of rs_1. Penis (Figs 26–27) bent, long and narrow, gradually and regularly narrowing to the tip, with an outgrowth close to the base on its left side; this outgrowth was overlooked by GEORGIEV (2012).

Fig. 25. Female reproductive organs of *Devetakia mandrica*, bar equals 0.5 mm (BC – bursa copulatrix, CBC – duct of bursa copulatrix, GA – albuminoid gland, GN – nidamental gland, GP – gonoporus, OV – oviduct, OVL – loop of (renal) oviduct, RS – receptaculum seminis (in position of RS_1, close to duct of bursa joining oviduct), VC – ventral channel)



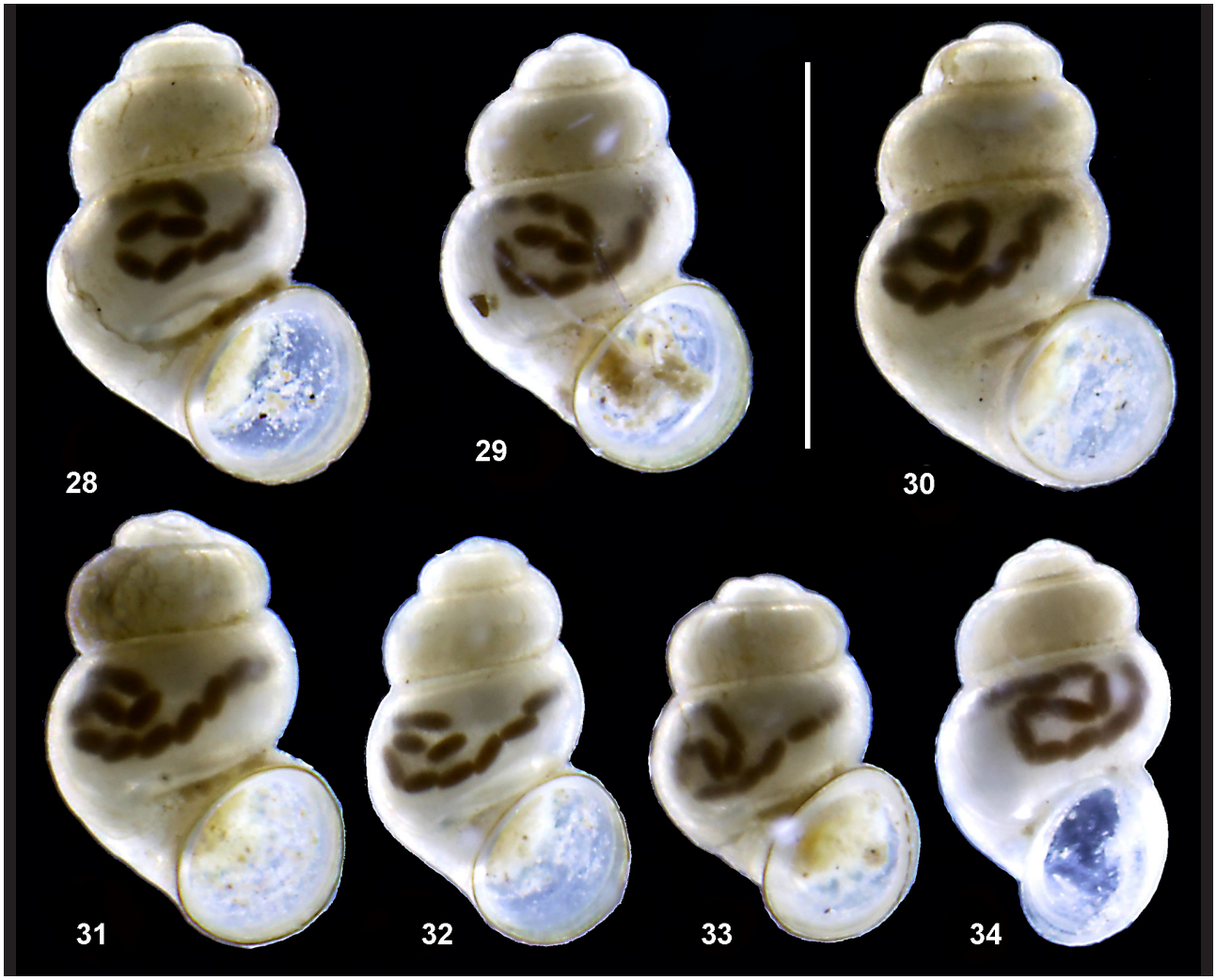
Figs 26–27. Penis of *Devetakia mandrica*: 26 – in situ, with right cephalic tentacle; 27 – under cover slip; bar equals 0.1 mm; asterisks indicate the outgrowth on the left side

Cavernisa Radoman, 1978

Cavernisa zaschevi (Angelov, 1959)

Material: Tserovo village, Vodnata cave (locality 12) and Tserovo village, Yamata cave (locality 13).

At locality 12, a few specimens were found (Figs 28–34) with very small (slightly above 1 mm) shells resembling the ones from Yamata cave (locality 13). Operculum thin and colourless, except for the orange nucleus. Soft parts unpigmented, eyes absent.



Figs 28–34. Shells of *Cavernisa zaschevi*, locality 12, bar equals 1 mm

Fig. 35. Penis of *Cavernisa zaschevi*, bar equals 0.1 mm

Penis (Fig. 35) simple and bent, without any outgrowths. RADOMAN (1983) drew a blunt and narrow outgrowth on the left side of the penis. Female reproductive organs include a large bursa copulatrix and one receptaculum seminis, in the position of rs_1.

At locality 13, six specimens were found with barrel-shaped shells (Figs 36–39) with 4.5 convex whorls separated by a rather deep suture. Aperture oval, peristome continuous, with a slightly marked outer lip, umbilicus in the form of broad slit. Shell white, thin-walled and translucent. Soft parts unpigmented (Figs 36–39), eyes absent. Female reproductive organs resemble the ones from locality 12 and consist of one receptaculum (in the position of rs_1) and a large bursa copulatrix with a short duct. Penis simple, with a small outgrowth on its left side.

Devetakiola Georgiev n. gen.

Type species: *Bythiospeum devetakium* Georgiev et Glöer, 2013; monotypic genus

The diagnosis and description are identical with those presented for *Bythiospeum devetakium* by GEORGIEV & GLÖER (2013). The shells resemble those of *Pontobelgrandiella*, the soft part morphology and anatomy are unknown. *Devetakiola* is recognised on molecular basis (OSIKOWSKI et al. in press), as it forms a molecularly distinct clade (Fig. 40), evidently no less distinct than the other taxa representing the genera included in this study. Thus, as long as we do not consider all those taxa as congeneric, *Devetakiola* deserves a genus rank.

Devetakiola devetakium (Georgiev et Glöer, 2013)

Material: Alexandrovo, Brashlyanskata cave, N foothills of Stara Planina Mts, (locality 10) and Prevala

Figs 36–39. Shells of *Cavernisa zaschevi*, locality 13, bar equals 1 mm

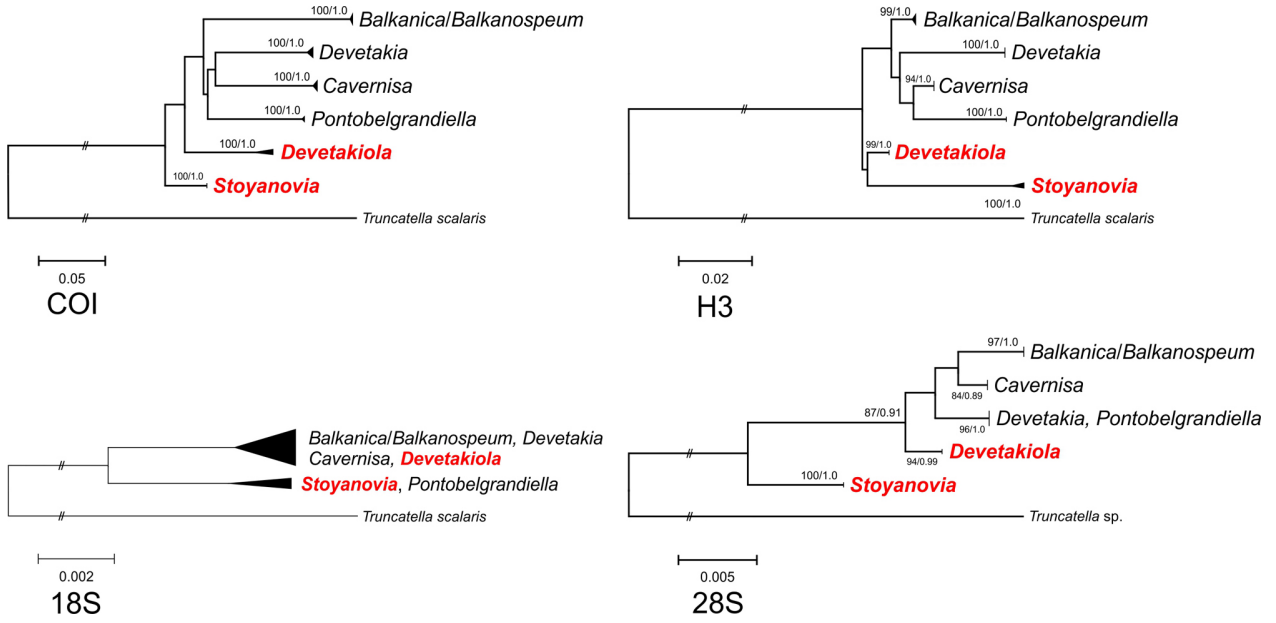


Fig. 40. Maximum likelihood trees of the Bulgarian stygobiont Truncatelloidea, based on molecular data: mitochondrial cytochrome oxidase subunit I (COI) and three nuclear loci: histone 3 (H3), ribosomal DNA (18S and 28S); note that *Devetakiola* and *Stoyanovia* lineages (bold and red) are no less distinct than the other genera within the clade, and that phylogenetic relationships within this stygobiont clade remain unclear. Based on OSIKOWSKI et al. in press

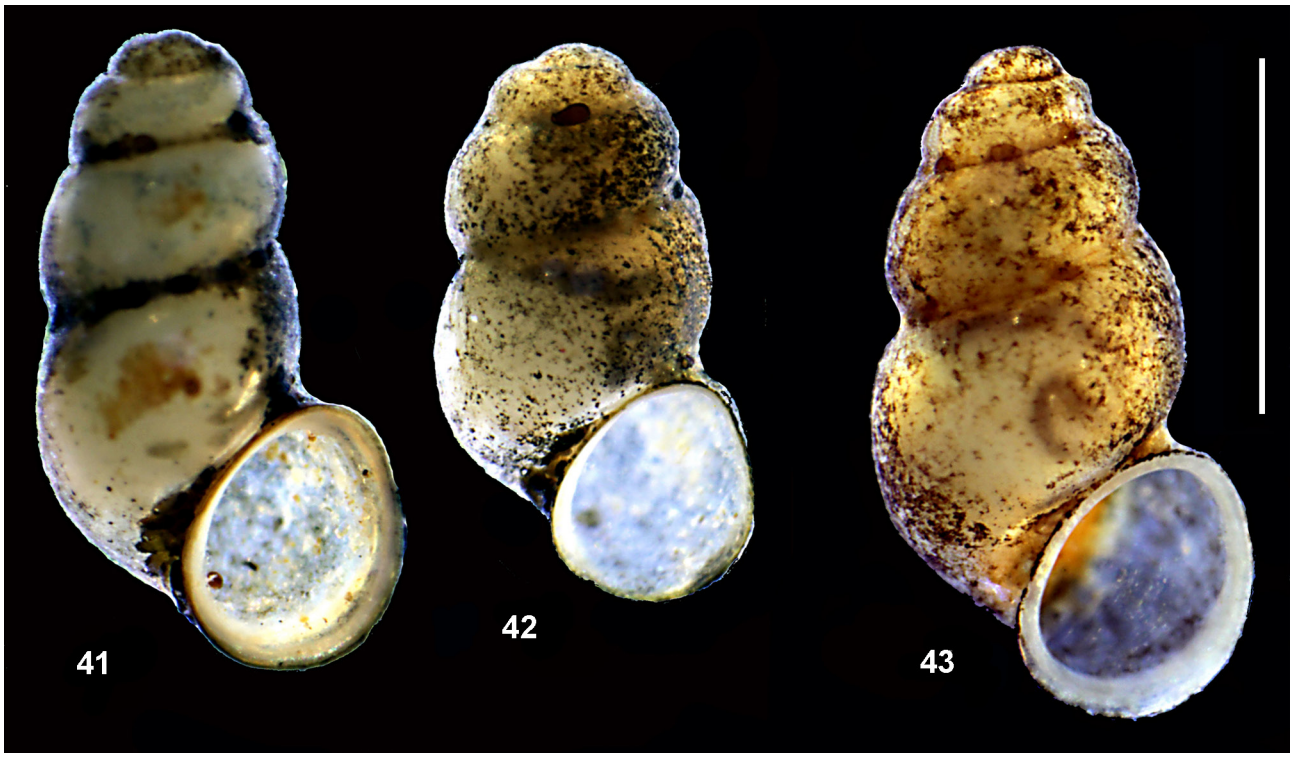
village, spring near Vreloto cave, N foothills of Stara Planina Mts (locality 11).

At localities 10 (Figs 41–42) and 11 (Fig. 43), three snails were collected, illustrated in the corresponding figures. Their soft part morphology and anatomy remain unknown; more material is needed for morphological studies.

Stoyanovia Georgiev n. gen.

Type species: *Bythiospeum stoyanovi* Georgiev, 2013; monotypic genus

The diagnosis and description are identical with those presented for *Bythiospeum stoyanovi* by GEORGIEV (2013). Shell conical, with the narrowest and most



Figs 41–43. Shells of *Devetakiola devetakium*: 41–42 – from locality 10, 43 – from locality 11; bar equals 1 mm

Figs 44–48. Shells of *Stoyanovia stoyanovi*, bar equals 1 mm

slender spire among the taxa discussed in this paper. *Stoyanovia* forms a molecularly distinct clade (Fig. 40), evidently no less distinct than the other genera included in this study (Osikowski et al. in press). Thus, as long as we do not consider all those taxa as congeneric, *Stoyanovia* deserves a genus rank.

***Stoyanovia stoyanovi* (Georgiev, 2013)**

Material: Bezhanovo village, Parnitsite cave, N foothills of Stara Planina Mts. (locality 9).
Shell (Figs 44–48) ovate-conical with a high spire formed by 4–4.5 rather flat and rapidly growing whorls separated by a rather shallow suture. Aperture small and oval, peristome continuous, with a slightly marked outer lip, umbilicus absent. Shell thin-walled, whitish and slightly translucent. Soft parts unpigmented (Figs 44–48), eyes absent. Female reproductive organs unknown (no well-fixed female specimens were available), penis simple, conical, tapered distally, with a rounded tip.

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