BULGARIA, A HOT SPOT OF BIODIVERSITY (GASTROPODA: RISSOOIDEA)?

PETER GLÖER[1] & DILIAN GEORGIEV[2]

[1]Schulstrasse 3, D-25491 Hetlingen, Germany
[2]Department of Ecology and Environmental Conservation, University of Plovdiv, Tzar Assen Str. 24, BG-4000 Plovdiv, Bulgaria

Abstract *It is demonstrated that the diversity of* Bythinella *species in Bulgaria is higher than was formerly realised. The Balkan Peninsula is probably a major centre of endemicity for the genus* Bythinella*, and more species are expected, as some of the localities pointed out in the old literature were not investigated (Vitosha Mts, Petrokhan Pass in Stara planina Mts). We found eleven new* Bythinella *spp., 7 species in springs and 4 species in small streams. The vertical spread of* Bythinella *spp. in Bulgaria is not wide, with 14 species living below 1000 m a.s.l., 13 of them below 500 m, and only 3 species reaching springs higher than 1000 m. Multivariate cluster analysis reveals the distinctness of the newly described species and an identification key to the 17* Bythinella *spp. known so far from Bulgaria is provided.*

Key words Bythinella*, non-adaptive radiation, Bulgaria, Rissooidea*

INTRODUCTION

The small freshwater spring snails from the genus *Bythinella* Moquin-Tandon 1856 (Gastropoda: Risooidea: Bythinellidae) are probably the most diverse of all hydrobioids in Europe (Radoman, 1976; Ponder *et al.*, 2008). These minute snails are distributed from N-Africa (Boeters, 1998) to south-eastern Turkey (Yıldırım *et al.*, 2006), and they are considered to have at least one centre of species richness, in France, which has a total of 42 known species (Bichain *et al.*, 2007). For a long time it appeared that the *Bythinella* species number decreased to the east because only a few species were described from the Balkans and Asia Minor. For example in the region of Serbia there were only three species known for the genus *Bythinella*: *B. serborientalis* Radoman 1978; *B. austriaca* (Frauenfeld 1857); and *B. opaca* (Gallenstein 1848) (Radoman, 1983). Four were known from Greece: *B. charpentieri charpentieri* (Roth 1855); B. *charpentieri cabirius* P. Reischütz 1988; *B. cretensis* Schütt 1980; and *B. kosensis* Schütt 1980 (Bank 2006). Six are recorded from Romania: *B. molcsany* (J. Wagner 1941); *B. dacica* Grossu 1946; *B. grossui* Falniowski, Szarowska & Sîrbu 2009; *B. radomanii* Falniowski, Szarowska & Sîrbu 2009; *B. calimanica* Falniowski, Szarowska & Sîrbu 2009; and *B. viseuiana* Falniowski, Szarowska & Sîrbu 2009 (Glöer & Sîrbu, 2006; Falniowski *et al.*, 2009). There are two in Turkey: *B. turca* Radoman, 1976, *B. occasiuncula* Boeters & Falkner, 2001 (Yıldırım *et al.*, 2006). In later publications it was shown that the diversity of the genus *Bythinella* is higher in the Balkans (and possibly in Asia Minor) than it was previously believed (Glöer & Pešić, 2006; Glöer, 2008; Falniowski *et al.*, 2009; Glöer & Georgiev, 2009; Georgiev, 2009; and unpublished materials of the authors (from Greece)). Angelov (2000: vii) supposed that a probable second centre of radiation of this group was present in Bulgaria.

The first data on the occurrence of *Bythinella* in Bulgaria was given by Angelov (1960). This author reported four localities for the species *Bythinella austriaca*: 1. Borovets resort complex in Rila Mountain, in a small stream on silicate terrain and spruce forest, 1400 m alt. (A. Angelov Leg., 12.09.1948); 2. Sinia Vir karstic spring near Peshtera town, northern slope of the West Rhodopes Mts (A. Valkanov Leg., 18.08.1955); 3. Koprivshtitsa town, Sredna Gora Mts (A. Valkanov Leg., 14.07.1910); 4. Chuchura karstic spring near Smolyan town, West Rhodopes (A. Angelov leg., 04.08.1953). The same author comments on the believed general distribution of this species as: "Austria, Hungary, Czech Republic, Poland, Romania, North Thyrol and West Ukraine". For a long time *Bythinella austriaca* was believed to be the only representative of its genus in Bulgaria.

Angelov (2000) added a few more localites for "*B. austriaca*" in the Vitosha and Stara Planina Mts: "Mainly in karst springs in West

Contact author : gloeer@malaco.de

and South-West Bulgaria. Very frequent and abundant in the karst area in Smolyan Region. Sometimes on silicate grownds: Vitosha Mts, Rila Mts, Petrokhan Pass." Based on all the literature sources, it was believed that this species occurred in the mountains of Bulgaria from 500 up to 1720 metres a.s.l. (Hubenov, 2007).

Materials and Methods

In 2009 D. Georgiev collected freshwater molluscs in Bulgaria. The snails were collected by hand sieving. Samples were stored in 75% ethanol. Dissection and measurements of the genital organs and shells were performed using a stereo microscope (Zeiss); photographs were taken with a (digital) Leica R8.

To differentiate and describe the *Bythinella* spp. we used the following characteristics: (i) the shell – shape, height, ratio of height of the shell to the width of the last whorl, umbilicus, aperture, and (ii) the male copulatory organ – ratio of the length of the penis to the penial appendix, flagellum length, width of the proximal and distal parts of the flagellum, colour of the penis. Usually the flagellum is not considered by many authors but we believe that it is an important feature in distinguishing *Bythinella* spp. We did not measure the ratio of shell height to width because using this value, slim shells with a broad aperture cannot be separated from broad shells with a slim aperture. The male copulatory organ was studied on at least three specimens from each sample.

We did not examine the female reproductive tract, because the differences do not, in our opinion, provide useful features to distinguish between the *Bythinella* species under discussion.

Multivariate cluster analysis was made using WinStat software program.

Species concepts based on reproductive isolation as a criterion are not applicable because all of the species live allopatrically. Our approach uses a combination of morphology and anatomy. We accept that two populations are distinct (species)

Table 1 The distinguishing features of *Bythinella* spp. of Bulgaria.

Taxon	Shell shape	H	H:W	A:H	Umbilicus	Aperture	Penis/ appendix	Flagellum prox.	Flagellum dist	Flagellum length	Penis
B. gloeeri	1	0	2, 1	0,4 4	0	0	0	0	0	0	0
B. markovi	2	0	1, 8	0,4 8	0	1	1	3	1	0	0
B. valkanovi n.sp.	1	0	2, 0	0,5 8	1	0				0	0
B. slaveyae n.sp.	1	0	1, 7	0,4 8	0	0	1	0	2	1	0
B. kleptuzica n. sp.	1	1	2, 4	0,4 3	0	1	2	2	2	0	0
B. elenar n. sp.	0	1	2, 1	0,5 9	0	0	2	0	1	0	1
B. hansboetersi	0	1	2, 0	0,4 1	1	1	3	2	4	0	0
B. margritae n.sp.	0	1	1, 7	0,4 8	0	1	2	2	0	0	1
B. dierckingi n.sp.	1	1	2, 0	0,4 5	0	2	1	0	3	1	0
B. smolyanika n.sp.	1	2	2, 2	0,4 3	0	3	3	0	2	0	0
B. dedovi. n.sp.	2	2	2, 0	0,5 2	0	0	3	0	1	2	1
B. izvorica n.sp.	0	1	2, 0	0,4 7	0	2	3	0	1	1	1
B. angelovi n.sp.	1	2	2, 2	0,4 2	0	2	1	2	5	0	0
B. rhodopensis n.sp.	1	2	2, 1	0,4 8	0	0	4	1	5	1	0
B. srednogorica	0	2	2, 1	0,4 3	0	1	1	2	0	0	0
B. ravnogorica	1	2	2, 3	0,4 0	1	0	0	1	3	1	0
B. walkeri	0	2	2, 1	0,4 3	0	0	1	2	0	2	0

Shape of the shell: normal = 0; slim = 1; broad body whorl = 2. Height of the shell: <2.5 mm = 0; >2.5<3.0 = 1; >3 = 2. Umbilicus: closed = 0; slit-like = 1. Ratio of penis to penial appendix: 0.5:1 = 0 0.25:1 = 1; 1:1 = 2; 0.75:1 = 3; 1.25:1 = 4. Shape of the aperture: oval = 0; circular = 1; oval, angled = 2; oblique oval = 3. Flagellum, proximal: slim = 0; very slim = 1; thin = 2; broad = 3. Flagellum distal: regularly slim = 0; slightly thickened = 1; club-shaped = 2; thickened = 3; slim = 4; regularly broad = 5. Length of the flagellum: normal = 0; short = 1; long = 2. Penis: not pigmented = 0; pigmented by a dark spot = 1.

if shell and anatomical characters indicate that differences are fixed and speciation or at least differentiation has taken place. The main problem with this method is finding those constant features by which the species can be separated. It goes without saying that this can only be done successfully with large samples.

RESULTS

Determination of taxa that belong to the genus *Bythinella* is not easy because they are not equipped with a wealth of distinguishing features. Nevertheless it was possible to distinguish seventeen species by making use of the following species concept: each species has at least one constant feature that allows separation from other species; a single constant distinguishing feature shows that speciation has taken place. Thus we only used differentiating characters, which are different in some species (Table 1).

Using the features given in Table 1 we were able to analyse using multivariate cluster analysis as shown in Fig. 1. This illustrates distinctions between the *Bythinella* ssp., and enables the construction of an identification key.

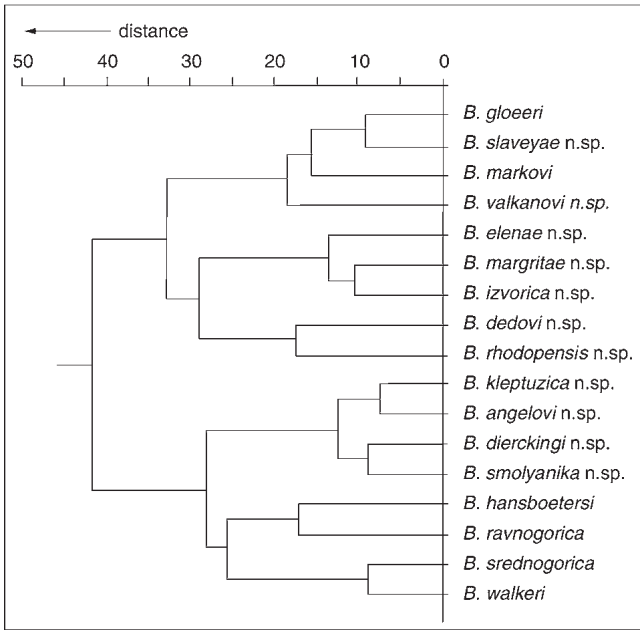

Figure 1 Multivariate cluster analysis of the *Bythinella* spp. of Bulgaria.

IDENTIFICATION KEY FOR THE *BYTHINELLA* SPP. OF BULGARIA

1. Shell height < 2.6 mm 2
1′ Shell height > 2.6 mm 5

2. Body whorl broad, H : W = 1.5, Flagellum short and regularly thick ..... *B. markovi*
2′ Shell slim, whorls regularly increasing 3

3. Aperture relative large (A:H = 0.57–0.60), umbilicus slit-like ..... *B. valkanovi*
3′ Umbilicus closed, A:H = 0.43–0.50 4

4. Aperture slim oval, A:H = 0.43–0.46, flagellum (distal) not thickened ..... *B. gloeeri*
4′ Aperture broad oval, A:H=0.47–0.50, flagellum (distal) thickened ..... *B. slavayae*

5. Shell height 2.4–2.9 mm, aperture circular, slim (H:W=1.6–1.7) ..... *B. kleptuzica*
5′ Aperture not circular 6

6. Penis dorsal with a dark spot 7
6′ Penis without a dark spot 10

7. Flagellum (distal) thickened ..... *B. elenae*
7′ Flagellum (distal) not thickened 8

8. Penis shorter than penial appendix *B. dedovi*
8′ Penis and penial appendix of the same length 9

9. Aperture oval, angled at the top ... *B. izvorica*
9′ Aperture nearly circular ..... *B. margritae*

10. Flagellum (distal) thickened 11
10′ Flagellum (distal) not thickened, proximal thin to very thin 13

11. Shell large, up to 3.3 mm, slim with a slim and oblique aperture, flagellum (distal) club-shaped ..... *B. smolyanica*
11′ Shell smaller, up to 3.1 mm, flagellum (distal) regularly thick 12

12. Penis very short, penis:penial appendix = 0.25, aperture broad oval, slightly angled at the top ..... *B. dierckingi*
12′ Penis:penial appendix >0.5, aperture regular ..... *B. ravnogorica*

13. Penis longer that the penial appendix, penis:penial appendix = 1.25, flagellum regularly thick, proximate flagellum very thin, shell height up to 3.3 mm, aperture oval ..... *B. rhodopensis*
13′ Penis : penial appendix <1, Flagellum proximal thin 14

Figure 2 The shells of the *Bythinella* spp. of Bulgaria. 1–6: Shells of previously described *Bythinella* spp. 7–28: Paratypes of the new *Bythinella* spp.: 1 *Bythinella hansboetersi*; 2 *B. gloeeri*; 3 *B. walkeri*; 4 *B. markovi;* 5 *B. ravnogorica*; 6 *B. srednogorica;* 7–8 *B. angelovi;* 9–10 *B. dedovi;* 11–12 *B. dierckingi;* 13–14 *B. elenae;* 15–16 *B. izvorica;* 17–18 *B. kleptuzica;* 19–20 *B. rhodopensis;* 21–22 *B. slaveyae;* 23–24 *B. smolyanica;* 25–26 *B. valkanovi;* 27–28 *B. margritae*.

14. Penis: penial appendix = 0.75, shell H:W = 1.6–1.7 ..... *B. hansboetersi*
14′ Penis: penial appendix = 0.25 15

15. Shell slim, H:W = 2.2 ..... *B. angelovi*
15′ Shell broader H:W = 2.1 16

16. Aperture circular ..... *B. srednogorica*
16′. Aperture not circular ..... *B. walkeri*

Bythinella valkanovi n. sp.

Material examined 11 empty shells from type locality, 05.09.2009, Dilian Georgiev, Slaveya Stoycheva leg.

Holotype Height = 2.5 mm, width = 1.5 mm. ZMH 79050.

Paratypes 3 ex., empty shells. ZMH 79051.

Locus typicus Sinia Vir spring at the southern part of Peshtera town close to the northern slope of the Rhodopes Mts, Upper Thracian Lowland, N 42° 02′ E 24° 19′, between 400–425 m alt.

Etymology Named after Prof. Valkanov, who was the first to collect specimens of this species on 18.08.1955, which were reported as *Bythinella austriaca* by Angelov (1960).

Description Shell horn-coloured, cylindrical and slim, with 4–4.5 regularly convex whorls with deep sutures. Surface silky and finely striate. Apex obtuse, umbilicus closed to slit-like. Aperture oval with sharp periostome. Shell height 2.2–2.4 mm, width 1.1–1.4 mm, apertural height:shell height = 0.57–0.60.

Distribution Known only from the type locality, which is the only *Bythinella* habitat currently known in the Upper Thracian Lowland.

Notes on the ecology and conservation Angelov (1960) reported a water temperature in the spring of 18°C. The species was collected from beneath stones, dead wood and nylon and metal rubbish. The karstic spring Sinia Vir is situated in the suburbs of Peshtera the waters of which flow through periodically mowed wet meadows with some shrubs and trees, but mainly *Salix* sp. There is a large concrete building for the catchment of a large part of the spring waters, which possibly negatively affects the population of *B. valkanovi*. It lives with *Radix labiata*.

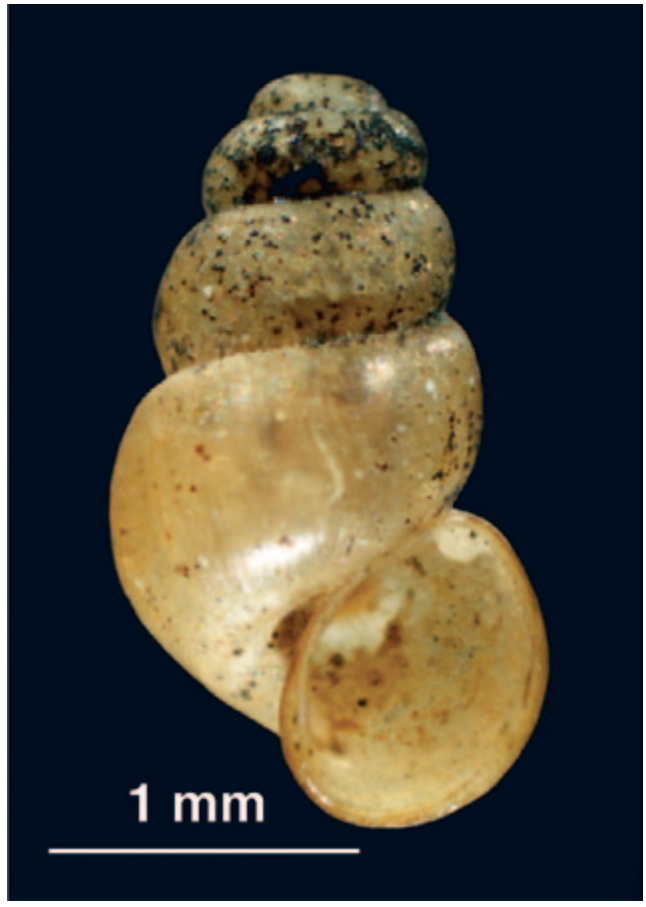

Figure 3 Shell of *Bythinella valkanovi* n. sp. (holotype).

Bythinella smolyanica n. sp.

Material examined 44 ex. from the type locality, 13.09.2009, Dilian Georgiev, Slaveya Stoycheva leg.

Holotype Height = 3.2 mm, width = 1.8 mm. ZMH 79052.

Paratypes 5 ex. in ethanol. ZMH 79053.

Locus typicus A small stream flowing into one of the Smolyanski Ezera lakes, West Rhodopes Mts, N 41° 36′ 20.7″ E 24° 40′ 25.9″, 1298 m alt. This locality was first reported by Angelov (2000: 8) under *Bythinella austriaca*.

Etymology Named after the town in the vicinity of which the species was found.

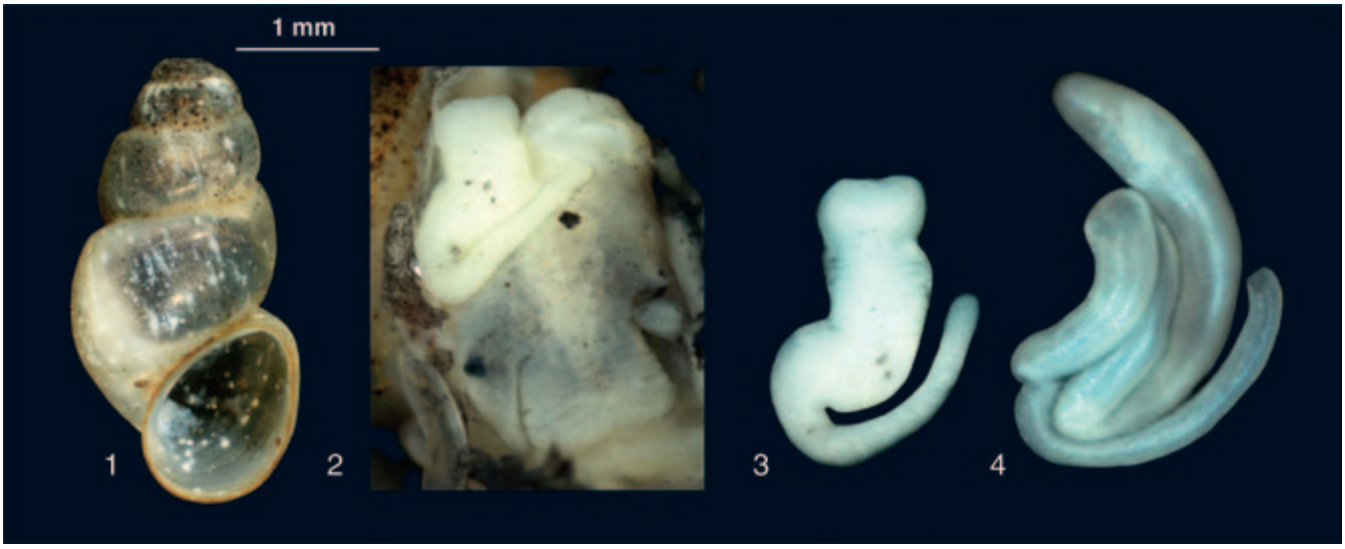

Figure 4 *Bythinella smolyanica n*. sp.: 1 holotype; 2 penis in situ; 3 penis with penial appendix; 4 flagellum.

Description Shell whitish, cylindrical with 4.0–4.5 slightly rounded whorls with a deep suture. Surface silky and finely striate. Apex obtuse, umbilicus closed. Aperture oval with a sharp periostome. Shell height 2.8–3.3 mm, width 1.8–1.9 mm, aperture height:shell height = 0.42–0.45.

Animal Mantle white with light grey snout and tentacles.

Anatomy Penial appendix as long as penis, flagellum slightly thickened at the distal end, not very thin at the proximal end.

Distribution Known only from the type locality, but could possibly be expected in any the streams flowing into the Smolyanski Ezera lakes.

Notes on the ecology and conservation Living in a small stream beneath stones and dead wood. Banks of the stream overgrown with high grass vegetation but dominated by *Urtica* sp. Water temperature measured on the day of collection, 11.4° C. Stream, situated close to a road, polluted by obvious dumping.

***Bythinella elenae* n. sp.**

Material examined 201 ex. from type locality, 27.09.2009, Elena Stoeva leg.

Holotype Height = 2.7 mm, width = 1.7 mm. ZMH 79054.

Paratypes 5 ex. in ethanol from type locality. ZMH 79055.

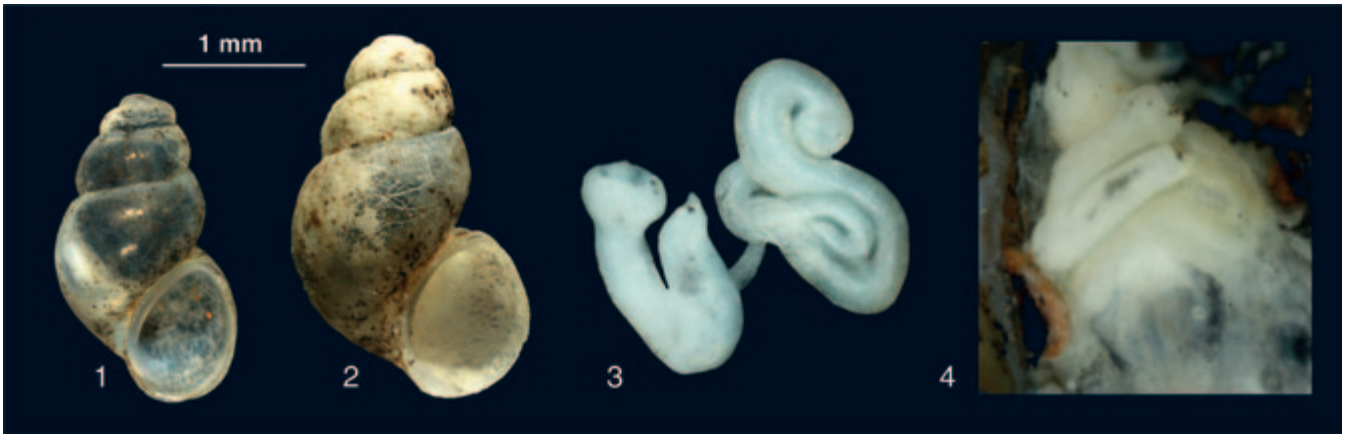

Figure 5 *Bythinella elenae* n. sp.: 1 Holotype; 2 paratype; 3 penis showing penial appendix and flagellum; 4 penis in situ.

Locus typicus Karstic spring flowing from a cave west of Malko Tarnovo town, Strandza Mt., N 42° 00′ 33.5″ E 27° 27′ 25.7″, between 400–425 m alt.

Etymology Named after Elena Stoeva (NGO "Green Balkans") who collected the species.

Description Shell whitish, cylindrical of 4.0–4.5 sluightly convex whorls, with deep sutures. Shell surface silky and smooth. Apex obtuse, umbilicus closed. Aperture oval with sharp periostome. Shell height 2.5–2.8 mm, width 1.5–1.7 mm, aperture height:shell height = 0.56–0.63.

Animal Mantle black with broad, light grey, border.

Anatomy Penis broad and as long as penial appendix. Flagellum thickening regularly distally, thinner proximally. Penis with dark-pigmented dorsum.

Distribution Known only from the type locality.

Notes on the ecology and conservation Collected from stones on the bottom of a karstic spring, therefore possibly a calcareous species.

***Bythinella dedovi* n. sp.**

Material examined 33 ex. from type locality, 26.09.2009, Dilian Georgiev leg.

Holotype Height = 3.1 mm, width = 2.0 mm. ZMH 79056.

Paratypes 5 ex. in ethanol from type locality. ZMH 79057.

Locus typicus Karstic spring flowing directly into the river passing Mladejko village and in the river 10–15 metres away from the spring, west of Mladejko village, Strandza Mt., N 42° 09′ 03.7″ E 27° 21′ 35.5″, 216 m alt.

Etymology Named after Dr Ivailo Dedov (Central Laboratory of General Ecology, BAS) a Bulgarian malacologist specialised on terrestrial gastropods who contributed so much to studies of the Bulgarian and Macedonian faunas.

Description Shell whitish to horn-coloured, cylindrical with 4.0–4.5 regularly rounded whorls, with deep sutures. Surface silky and finely striate. Apex obtuse, umbilicus closed. Aperture oval with a sharp periostome, slightly thickened at the columella. Shell height 2.8–3.3 mm, width 2.0–2.1 mm, aperture height:shell height = 0.50–0.54.

Animal Mantle black with a broad, light grey, border.

Anatomy Penis broad and shorter than penial appendix. Flagellum not thickened distally and thin proximally. Penis with dark spot dorsally.

Distribution Known only from the type locality.

Notes on the ecology and conservation Collected from stones by a spring in a karstic river. The only Bulgarian *Bythinella* species found to live

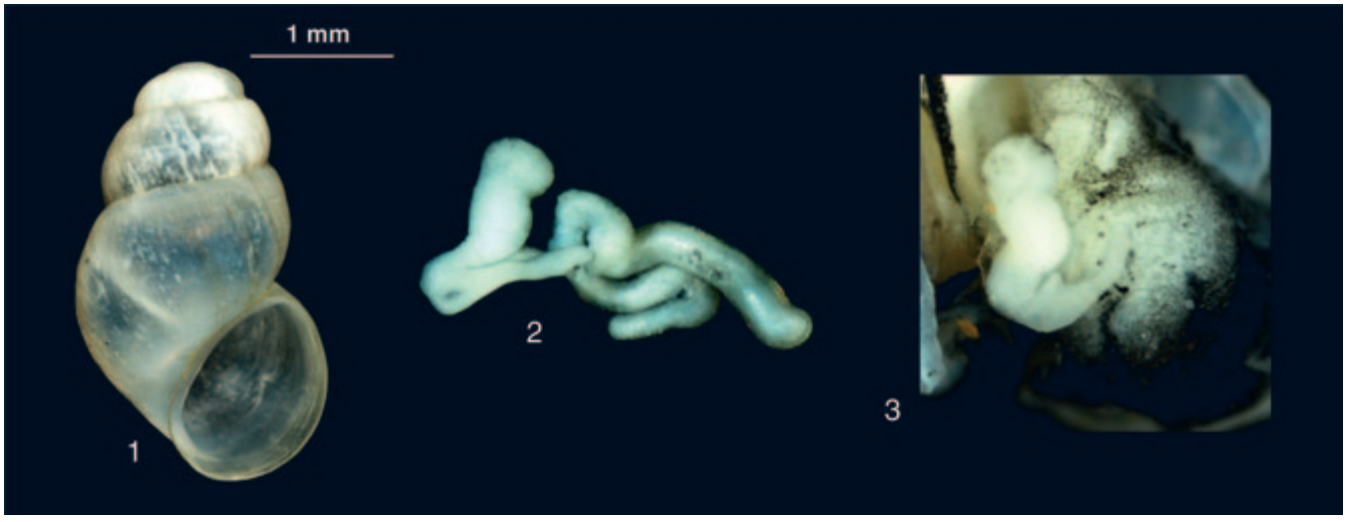

Figure 6 *Bythinella dedovi* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

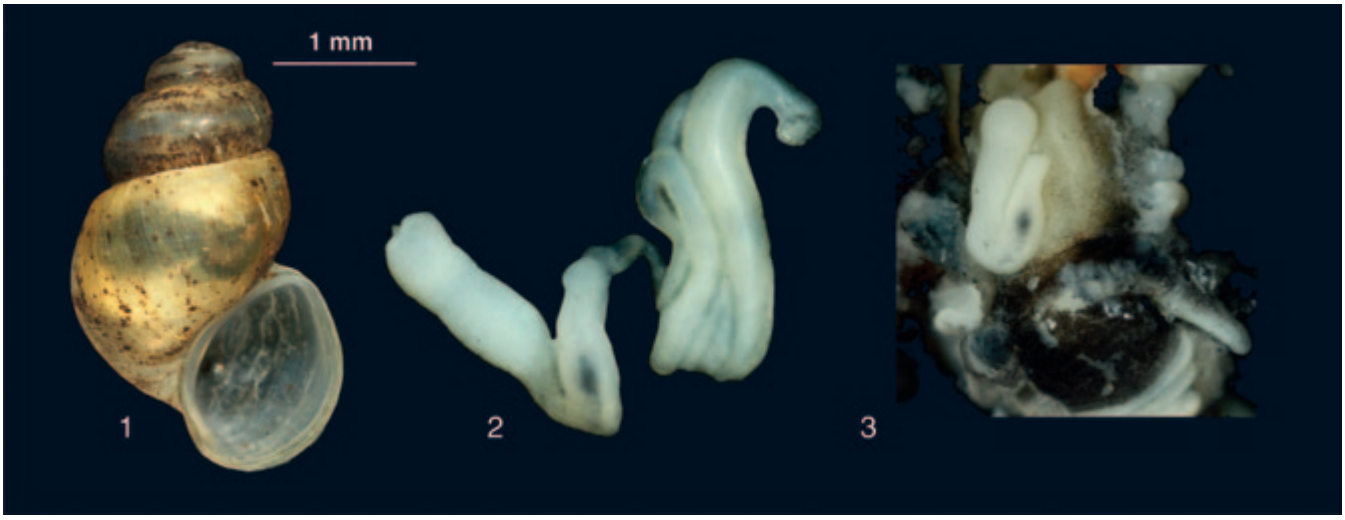

Figure 7 *Bythinella izvorica* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

some distance (10–15 m) from a spring, and in an open river.

Bythinella izvorica n. sp.

Material examined 15 ex. from type locality, 26.09.2009, Dilian Georgiev leg.

Holotype height 3.9 mm, width 1.6 mm. ZMH 79058.

Paratypes 5 ex. in ethanol from type locality. ZMH 79059.

Locus typicus In the spring emerging from the Izvora cave, west of Mladejko village, Strandza Mt., N 42° 09′ 03.7″ E 27° 21′ 27.3″, 215 m alt.

Etymology Named after the Izvora cave at the entrance of which the species was found.

Description Shell whitish, cylindrical of 4–4.5 whorls, these regularly rounded with deep sutures. Surface silky and finely striated. Apex obtuse, umbilicus closed. Aperture oval, slightly angled above, with sharp periostome. Shell height 2.8–3.0 mm, width 1.6–1.8 mm, aperture height:shell height = 0.45–0.50.

Animal Mantle black with border broad, light grey.

Anatomy Penis shorter than penial appendix, flagellum relatively short and not thickened distally.

Distribution Known only from the type locality.

Notes on the ecology and conservation Collected from stones only in the spring area at the Izvora cave entrance, and possibly a calcareous species. Endangered by pollution and crushing by tourists who often visit this area.

Bythinella margritae n. sp.

Material examined 22 ex. from type locality, 26.09.2009, Dilian Georgiev leg.

Holotype Shell height 2.7 mm, width 1.9 mm. ZMH 79060.

Paratypes 4 ex. in ethanol from type locality. ZMH 79061.

Locus typicus Small stream below a water source west of Mladejko village, Strandza Mt., N 42° 09′ 04.6″ E 27° 21′ 49.3″, 129 m alt.

Etymology Named after Margrit Falkner. Together with her the senior author collected his first *Bythinella* sp. in Bavaria.

Description Shell whitish, cylindrical consisting of 4.0–4.5 regularly convex whorls, which have deep sutures. Surface silky. Apex obtuse, umbilicus closed. Aperture a rounded oval with sharp periostome, slightly thickened at the columella. Shell height 2.7–2.9 mm, width 1.9–2.0 mm, aperture height:shell height = 0.47–0.50.

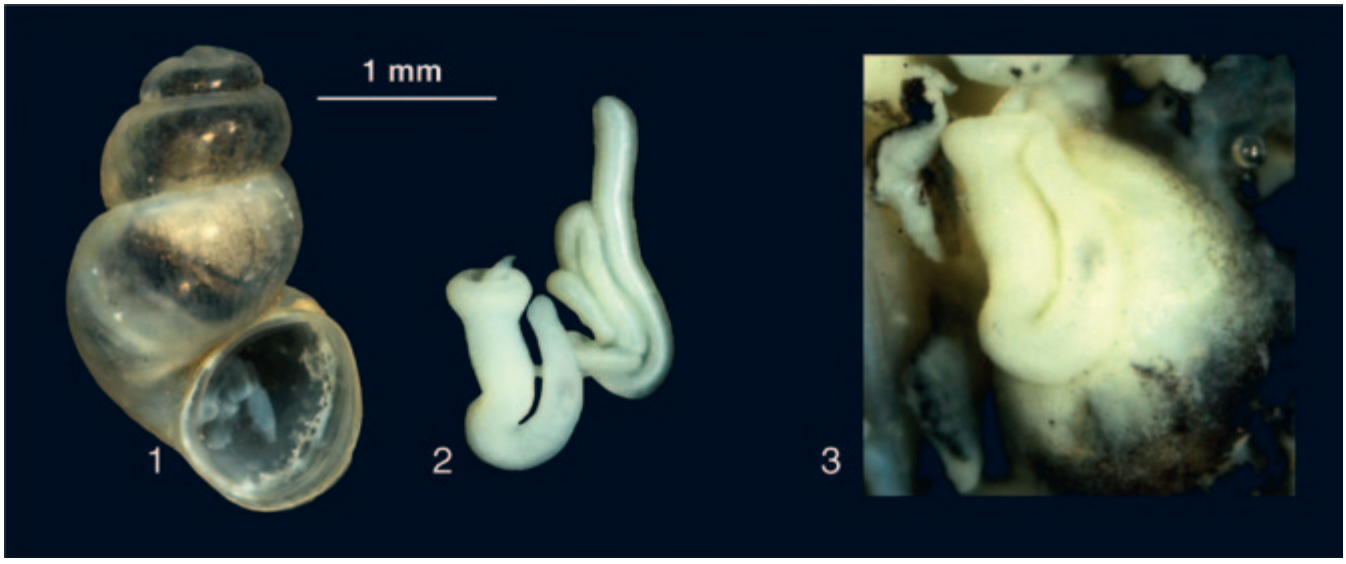

Figure 8 *Bythinella margritae* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Animal Mantle black with a broad light grey border.

Anatomy Penis as long as the penial appendix, flagellum slim and not thickened at the distal end, tapered at the proximal end.

Distribution Known only from the type locality.

Notes on the ecology and conservation Collected on stones in a stream within a karstic area. We consider the species is vulnerable from any disturbance of the habitat where it lives.

***Bythinella kleptuzica* n. sp.**

Material examined 26 ex. from type locality, 05.09.2009, Slaveya Stoycheva, Dilian Georgiev leg.

Holotype Shell height 2.9 mm, width 1.7 mm. ZMH 79062.

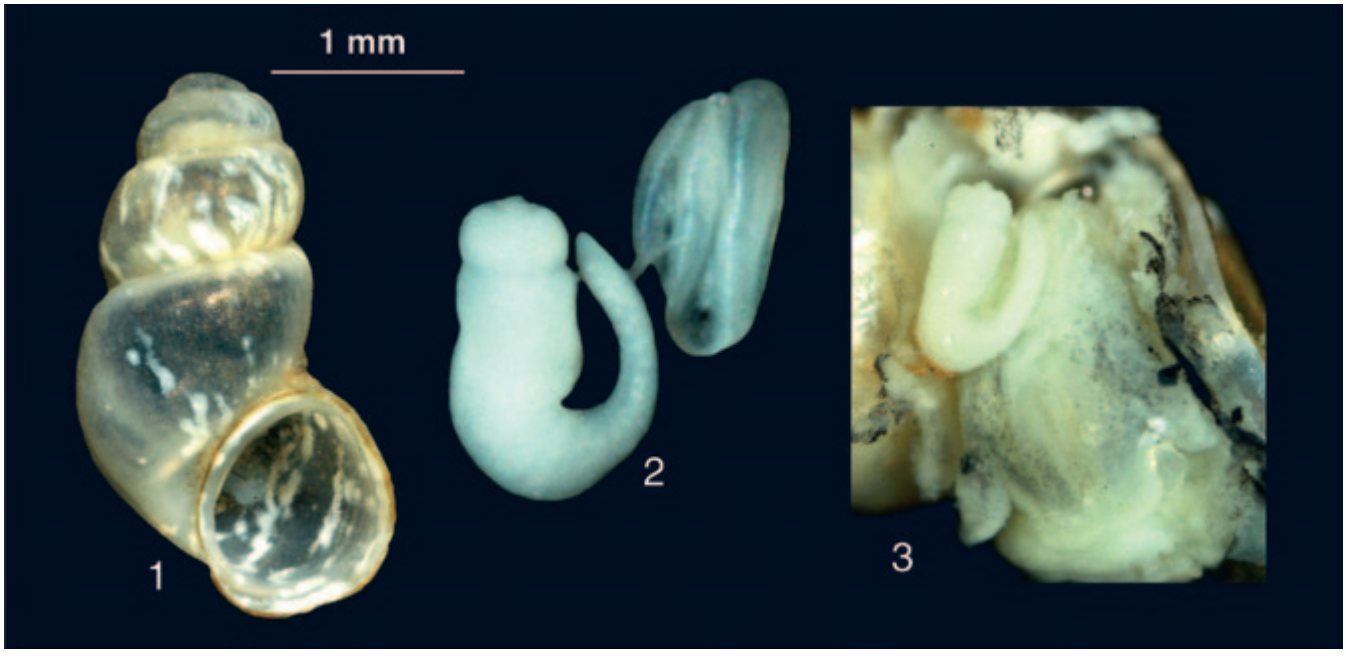

Figure 9 *Bythinella kleptuzica* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Paratypes 5 ex. in ethanol from type locality. ZMH 79063.

Locus typicus Kleptuza spring, Velingrad town, West Rhodopes Mts.

Etymology Named after the Kleptuza spring, where the species lives.

Description Shell whitish, cylindrical, slim and consisting of 4–4.5 whorls, these regularly convex with deep sutures. Surface silky. Apex obtuse, umbilicus closed. Aperture oval, rounded with sharp periostome, this slightly thickened at the columella. Shell height 2.4–2.9 mm, width 1.5–1.7 mm, aperture height:shell height = 0.41–0.45.

Animal Mantle black with a broad light grey border.

Anatomy Penial appendix as long as penis. Flagellum very thin proximally, and not thickened distally.

Distribution Known only from the type locality.

Notes on the ecology and conservation Lives in a large, cold (4°C), karstic spring among moss. Co-existing with *Ancylus* sp. The spring is situated in an urbanised area near a hotel and restaurant and is very vulnerable to any kind of disturbance.

***Bythinella rhodopensis* n. sp.**

Material examined 16 ex. from type locality, 14.06.2009, Dilian Georgiev leg.

Holotype Shell height 2.9 mm, width 1.8 mm. ZMH 79064

Paratypes 5 ex. in ethanol from type locality. ZMH 79065

Locus typicus A stream south of village of Lilkovo, West Rhodopes Mts, near Modar Peak, N 41° 52′ 38.7″ E 24° 33′ 21.4″, 1516 m alt.

Etymology Named after the Rhodopes Mts where the species lives.

Description The yellowish to horn-coloured shell is cylindrical and consists of 4.0–4.5 whorls, which are regularly rounded with a deep suture. The surface is silky. The apex is obtuse, the umbilicus closed. The aperture is oval with a sharp periostome. Shell height 2.9–3.3 mm, width 1.8–2.0 mm, aperture height to shell height 0.47–0.50.

Animal Mantle black with a broad light grey border.

Anatomy Penial appendix as long as the penis. Flagellum very thick, and tapered at the proximal end.

Figure 10 *Bythinella rhodopensis* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Figure 11 *Bythinella dierckingi* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Distribution Known only from the type locality.

Notes on the ecology and conservation Lives in small streams in *Picea abies* forest, which pass over limestone and granite rocks, and found under small pieces of dead wood and stones, also on sand. Water temperature of the spring (measured on 14.06.2009) 11.8° C.

***Bythinella dierckingi* n. sp.**

Material examined 26 ex. from type locality, 22.11.2009, Dilian Georgiev leg.

Holotype Shell height 3.0 mm, width 1.7 mm. ZMH 79066

Paratypes 5 ex. in ethanol from type locality. ZMH 79067

Locus typicus West of Ravnogor village, West Rhodopes Mts, a spring situated on the left bank of Batashka Ryaka river.

Etymology Named after Reinhard Diercking, for his engagement in conservation of natural environments of the freshwater molluscs in Hamburg.

Description Shell whitish or horn-coloured, cylindrical and consisting of 4–4.5 regularly rounded whorls, with a shallow to deep suture. Surface silky. Apex obtuse, umbilicus closed. Aperture oval, angled at the top with sharp periostome, this slightly thickened at the columella. Shell height 2.7–3.0 mm, width 1.6–1.7 mm, aperture height:shell height = 0.45–0.46.

Animal Mantle black with broad, light grey, border.

Anatomy Penial appendix much longer than the penis. Flagellum very thickened distally, but slimmer at the proximal end.

Distribution Known only from the type locality.

Notes on the ecology and conservation Lives in a small stream in *Picea abies* forest, in an area of granite rocks, under small pieces of dead wood and stones. The population of this species is endangered by the interest of local people in extracting spring waters for drinking. Active deforestation of the area was also observed during the collection of material.

***Bythinella slaveyae* n. sp.**

Material examined 34 ex. from type locality, 27.06.2009, Dilian Georgiev leg.

Holotype Shell height 2.2 mm, width: 1.5 mm. ZMH 79068.

Paratypes 5 ex. in ethanol from type locality. ZMH 79069.

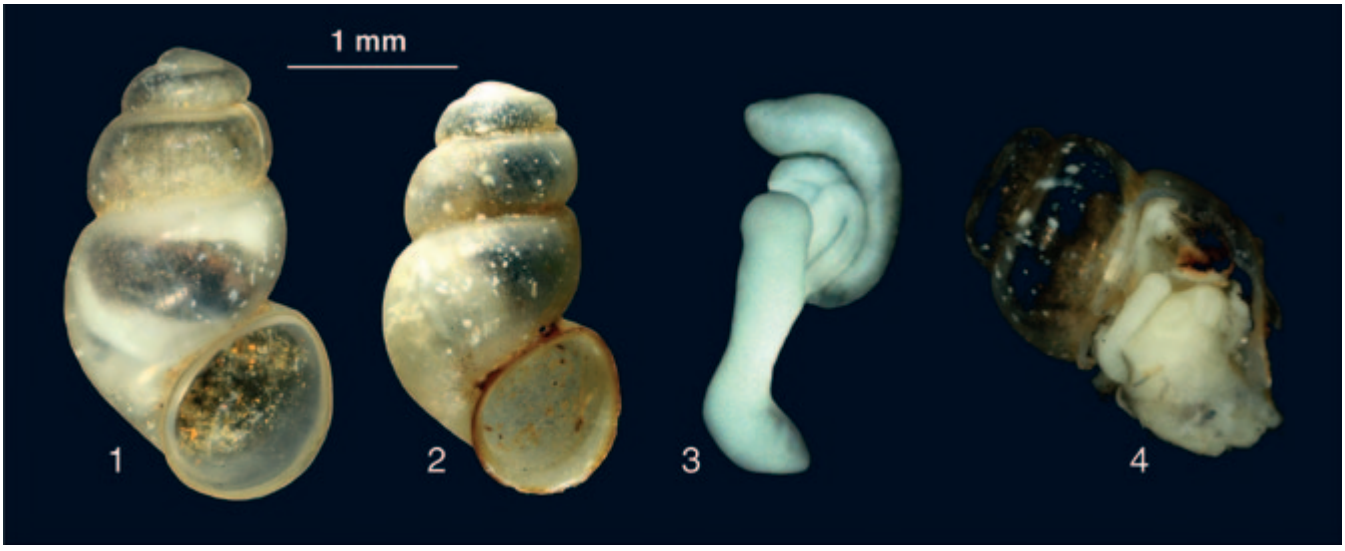

Figure 12 *Bythinella slaveyae* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Locus typicus Belasitsa Mt., south of Belasitsa village, N 41° 21′ 06.7″ E 23° 09′ 18.6″, 687 m alt.

Etymology Named after Slaveya Stoycheva, who found the type locality of this species and helped so much in the field studies of Dilian Georgiev on the Bulgarian, Greek and Turkish molluscs.

Description **S**hell whitish, cylindrical, comprising 4.0–4.5 regularly convex whorls, with deep sutures. Surface silky. Apex obtuse, umbilicus closed. Aperture oval with sharp periostome. Shell height 2.2–2.3 mm, width 1.4–1.5 mm, aperture height:shell height = 0.47–0.50.

Animal Mantle black with broa,d light grey, border.

Anatomy Penial appendix much longer than penis. Flagellum thick and claviform distally, slender proximally.

Distribution Known only from the type locality. Belasitsa Mt. is situated in three countries: Bulgaria, Greece and Macedonia, so there is a possibility that this species occurs outside the borders of Bulgaria.

Notes on the ecology and conservation Lives in a non limestone area, in a small spring falling from granite rocks in *Castanea sativa* forest.

Bythinella angelovi n. sp.

Material examined 25 ex. from type locality, 10.01.2010, Dilian Georgiev, Slaveya Stoycheva leg.

Holotype Shell height 3.2 mm, width 1.8 mm. ZMH 79070.

Paratypes 5 ex. in ethanol from type locality. ZMH 79071.

Locus typicus A stream near the monument of the Bulgarian revolutionary Georgi Benkovski in Koprivshtitsa town, Sredna Gora Mts, N 42° 38′ 14.8″ E 24° 21′ 57.4″, 1134 m alt.

Etymology Named in memory of Professor Angel Angelov, who reported the Koprivshtitsa town area as a locality of a *Bythinella* species, and contributed a lot to study of the Bulgarian freshwater molluscs.

Description Shell whitish, cylindrical, slender, with 4.0–4.5 regularly rounded whorls, with deep sutures. Surface silky and finely striate. The apex is obtuse, the umbilicus closed. The aperture is oval with a sharp periostome. Shell height 2.9–3.2 mm, width 1.7–1.8 mm, aperture height:shell height = 0.41–0.44.

Animal Mantle black with a broad light grey border.

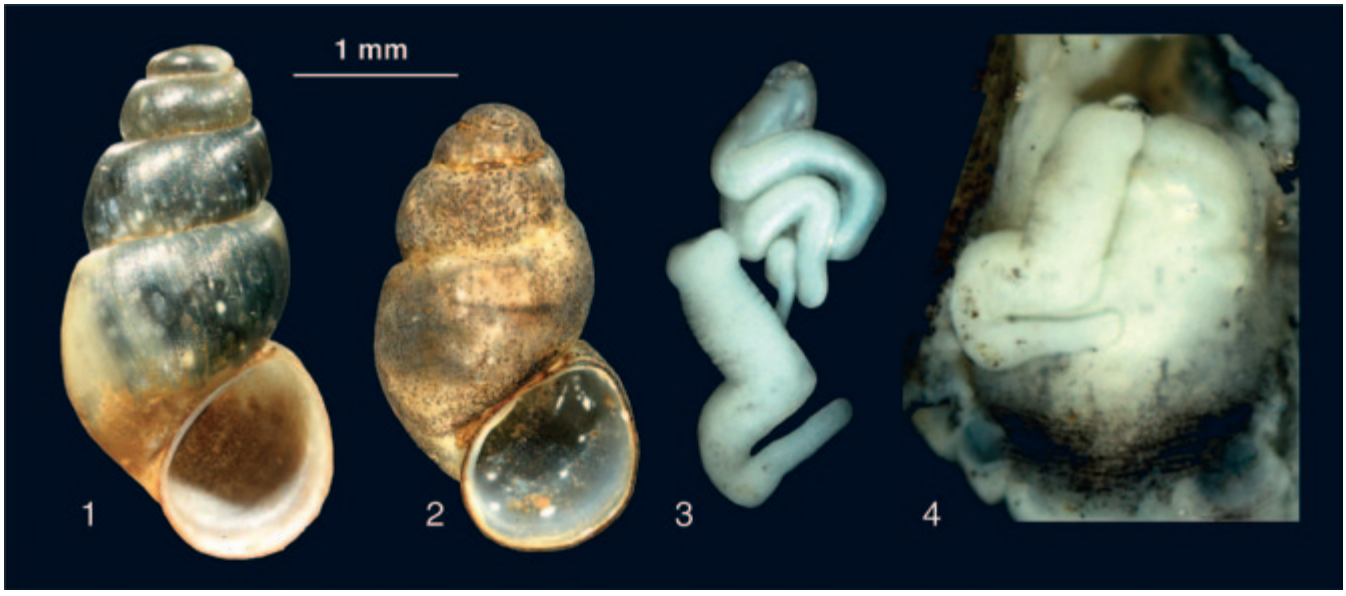

Figure 13 *Bythinella angelovi* n. sp.: 1 holotype; 2 penis with penial appendix and flagellum; 3 penis in situ.

Anatomy Penial appendix longer than penis. Flagellum very thin proximally, and thickened distally.

Distribution Known only from the type locality.

Notes on the ecology and conservation Lives in a stream flowing through *Pinus* and *Picea abies* forests on granite rocks, and found under stones and dead wood. Water temperature was 6.4°C on 10.01.2010.

DISCUSSION

Haase *et al.* (2007) stated that the identification of *Bythinella* spp. should be based on the integration of morphological, anatomical, and genetic investigations. On the other hand Bichain *et al.* (2007) stated that analyses of ITS1 and COI in some clades of *Bythinella* are in conflict with taxonomic delineations, probably because of different genetic evolutionary histories. Bichain *et al.* (2007) concluded from DNA sequencing, that *Bythinella bicarinata* and *Bythinella dunkeri*, in our opinion two well defined species, are conspecific. That distinct species can be genetically similar has been shown by Haase *et al.* (2007) for *B. robiciana*/*B. opaca* from Slovenia. That means that species identification may be done without genetic investigations if the morphological and anatomical data reveal constant features, as Haase (2008: 101) wrote: "… fixed morphological differences rather than genetic distances are a criterion for distinguishing species."

Considering Wilke *et al.* (2010), there occurred a non-adaptive radiation of *Bythinella* ssp. in Pliocene/Pleistocene in Central and Southern Europe. They summarised the criteria of non-adaptive radiation from the available literature: "(1) There is no clear niche differentiation, (2) species usually have a low degree of phenotypical variation, and (3) species usually evolve in allopatry (or peripatry)". They found eight radiations and some species which could not be assigned to radiations. The material studied by Wilke *et al.* (2010) was collected in Germany, France, Italy, Romania, and the Balkans, excluding Bulgaria. The seventeen *Bythinella* spp. known from Bulgaria represent possible endemic species which fulfill the criteria of non-adaptive radiation mentioned above. This is a result of the geological history of Bulgaria.

After the Oligocene the whole of Bulgaria was dry land, but at the beginning of the Chokrak Period, some eastern parts of the country became lowered and were filled with the Crimean-Caucasian Sea. Large inland water basins existed in Bulgaria till the end of the Pliocene. During the Miocene, Bulgaria was largely a dry steppe area, and the only inland basin was the Sarmatian Sea, slightly penetrating what are today the country's borders with the Danube Valley (Yordanov, 1962; Hughes & Woodword, 2008). Therefore the

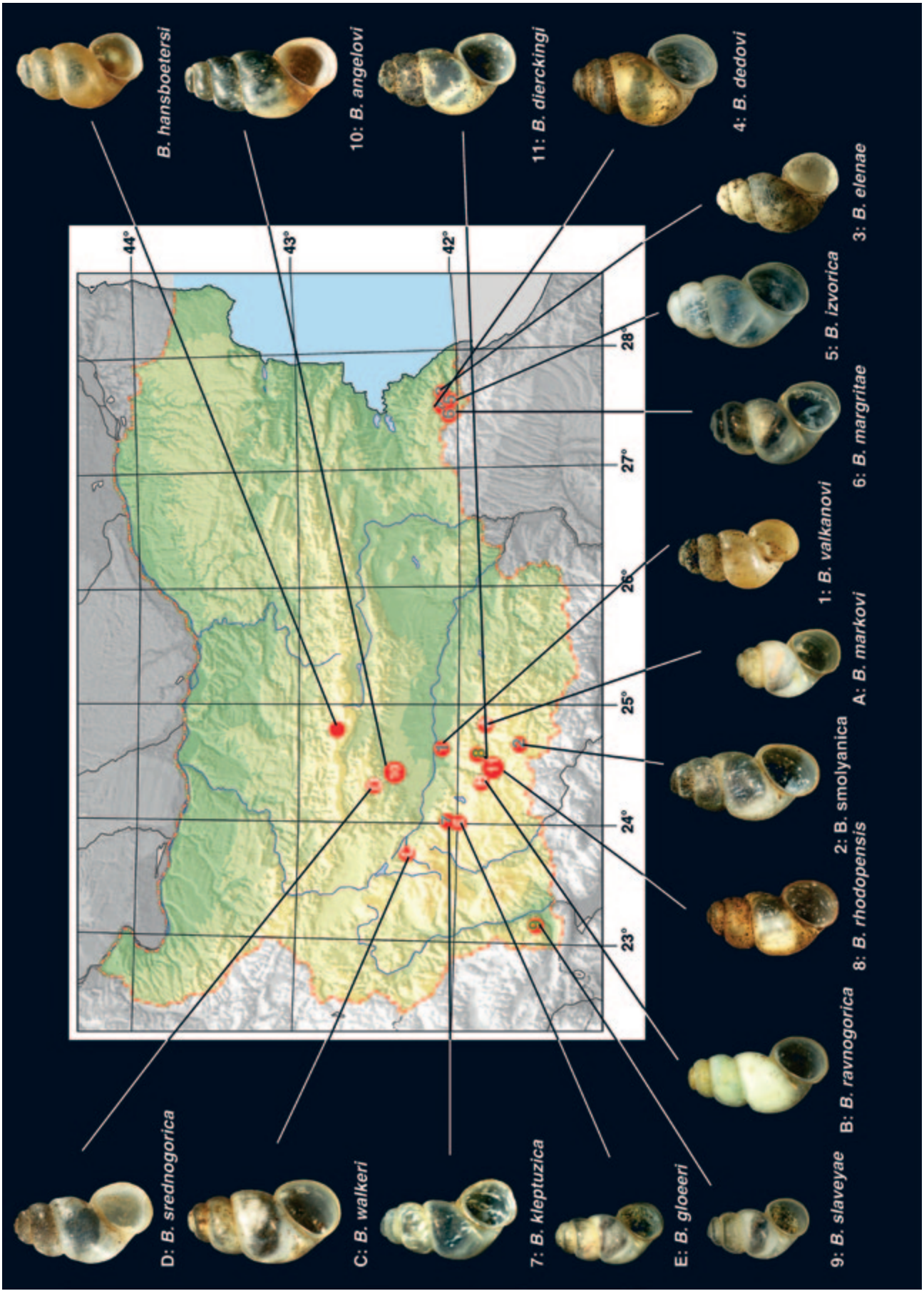

Figure 14 The *Bythinella* spp. from Bulgaria.

radiation of *Bythinella* spp. in Bulgaria has a younger history. On the other hand, this allopatric speciation could take place over the whole Pleistocene time frame because no glaciation, except some local glaciations in the Pirin Mts, happened in Bulgaria, and the *Bythinella* spp. were able to survive.

The question arises, how could this radiation take its course. The distribution pattern that we found (Fig. 14) implies an allopatric speciation. Thus we consider special aspects of the dispersal, including a species richness gradient, as important reasons for the allopatric speciation in Bulgaria.

Passive dispersal is the main process that distributes freshwater molluscs into new lentic habitats outside drainage systems. Migrating birds are important for long-distance dispersal, something shown by Wesselingh *et al*. (1999) for neogene *Planorbarius* spp. distributed along the routes of migration. Dispersal by waterbirds is a major advantage for molluscs because they tend to carry them to other aquatic habitats (Green & Figuerola, 2005). In addition, Boeters (1993) reported that small spring snails lay their egg capsules on hibernating beetles, which will transport the eggs to other springs in neighbouring regions. Falniowski (1992: 137) found that adult specimens of *Bythinella* are rather resistant to desiccation (up to six weeks), and dispersal by birds or on leaves blown with the wind is possible.

The vertical range of *Bythinella* spp. in Bulgaria is limited, with 14 species living below 1000 m asl., 13 of them lower than 500 m, and only 3 species reaching springs higher than 1000 m. In some springs *Bythinella* spp. are very abundant but passive dispersal may only take a few species to other suitable habitats or sites. Thus genetic drift, bottleneck effect, and founder effect will promote speciation.

The extremely retarded immigration rate in the mountainous region of Bulgaria gives isolated species a chance for separate development, and even after another immigration occurs, reproduction may be prevented by gene disharmonies. On the other hand, spatially and temporally varying environments forces species to evolve adaptively.

The species richness conforms with the latitudinal variation of diversity in European freshwater animals, discussed by Hof *et al.* (2008), who found that β-diversity increases along a gradient from N- to S-Europe with about 60% – 80% in Greece, dependent on habitats, and Strong *et al.* (2008) mentioned that neighbouring Greece is one of the worldwide hot spots of freshwater gastropod diversity. Willig *et al*. (2003: 284) thoroughly discussed latitudinal gradients of biodiversity (Rapoport's rule), which increase from higher to lower latitudes, and are usually explained with the species-energy hypothesis. But the authors could show that this effect is less pronounced over areas that extend over less than 20° in latitude. Roy *et al.* (1998) analysed a strong relationship between the mean sea surface temperature and diversity patterns, especially in extratropical latitudes along coastal regions. In Europe, however, we have to consider the fact that the fauna in parts of Europe has been wiped out over large regions during Pleistocene glacial-interglacial climate swings, and re-colonisation after the last Ice Age has not yet come to its end. On the other hand, the mountains in southern Europe provide isolated water bodies, like springs, which are suitable for isolating species over long periods. The latter fact seems to be the main argument to explain the increasing species richness gradient from north to south in Europe.

In summary, we can say that the distribution pattern of the *Bythinella* spp. in Bulgaria is stochastical. On the other hand most of the springs are isolated, and water systems are not connected. Thus habitat jumping is retarded. At least it is impossible to estimate how radiation in fact proceeds in this region, and future studies of genetic analyses may reveal new insights.

In addition there are a lot of potential localities where species new to science may be found in Bulgaria, as for example the isolated mountains of Sakar and Slavyanka. The long mountain ridge of Stara Planina is not well investigated either, and even the Rhodopes, from which very few new *Bythinella* spp. have been described in recent papers, could still hold some undetected species.

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