Review of the Genus *Grossuana* Radoman, 1973 (Gastropoda: Truncatelloidea) from Bulgaria, with a Description of a New Species

Dilian Georgiev¹, Peter Glöer², Ivailo Dedov³, Atanas Irikov¹

¹ Department of Ecology and Nature Conservation, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", 24 Tsar Assen Street., 4000 Plovdiv, Bulgaria; E-mail: diliangeorgiev@abv.bg; irikov@abv.bg
 ²Biodiversity Research Laboratory, 3 Schulstraße Str., D-25491 Hetlingen, Germany; E-mail: gloeer@malaco.de
 ³Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street., 1113 Sofia, Bulgaria; E-mail: idedov@gmail.com

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Abstract: A synopsis of the published information about the genus *Grossuana* Radoman, 1973 from Bulgaria is presented. Some original data on the distribution and ecology of species is provided. *Grossuana falniowskii* sp. n. is described from a spring in a park forest at the city of Stara Zagora (Southern Bulgaria).

Keywords: spring, description, snail, taxonomy, distribution, ecology, Balkan Peninsula.

Introduction

The genus Grossuana Radoman, 1973 consists of species inhabiting springs and streams in the Eastern Balkan Peninsula (RADOMAN 1983, SZAROWSKA et al. 2007). Till now, seven species of this genus have been reported from Bulgaria. The history of studies on the genus Grossuana in Bulgaria started with an article by WAGNER (1927). Based on the shell morphology, he described the subspecies Pseudamnicola consociella euxina Wagner, 1927 from a spring near Devnya River and Aladzha Monastery (Northeastern Bulgaria). URBAŃSKI (1960) re-collected P. consociella euxina from its type locality and reported a new locality of this species, *i.e.* springs at Zlatni Pyasatsi Resort (Varna Region). GROSSU (1946) described Pseudamnicola codreanui Grossu, 1946 from Bulgaria, with the type locality in the spring Ak Bunar at the Botanical Garden of the town of Balchik (Northern Black Sea coast).

RADOMAN (1973) erected the new genus *Grossuana*, which is characterised by conical penis with a hardly visible outgrowth on its left side. The Bulgarian species described as *Pseudamnicola* were assigned to this genus. RADOMAN (1983) consid-

ered all the representatives of *Grossuana* in Serbia, Bulgaria and Romania as subspecies of *G. serbica* Radoman, 1973. He also reported two further localities of *Grossuana* in Bulgaria, where he identified *G. serbica codreanui* (Grossu, 1946), *i.e.* the spring Manastir by the south bank of Beloslavsko Lake and a spring in the village of Buchin Prohod, Kostinbrod Municipality (Western Stara Planina Mountains). ANGELOV (2000) and HUBENOV (2007) summarised the information on the Bulgarian freshwater molluscs using the name *Pseudamnicola* for the two known species in the country.

Based on molecular studies using CO1 gene, SZAROWSKA *et al.* (2007) found that *G. codreanui* from Bulgaria (village of Yasenovo, newly reported locality in this paper) was not related with *G. codreanui* from Techirghiol Lake, Romania. However, specimens from the type locality of the latter species were not included in that study.

ZETTLER (2008) made a synopsis of the studies on *Grossuana codreanui* and reported a new locality of this species from Bulgaria, *i.e.* a water source at the village of Avren, Varna Region. He also stated that Pseudamnicola consociella euxina seemed to be a species of the genus Grossuana, probably conspecific with G. codreanui. GLÖER, GEORGIEV (2009) described two new species of Grossuana: G. angeltsekovi Glöer, Georgiev, 2009 (from springs in Western Rhodopes Mountains and the lower slopes of Pirin Mountains from Mesta River valley) and G. thracica Glöer, Georgiev, 2009 (from the Chirpan Bunar spring, Upper Thracian Lowland, Southern Bulgaria). GEORGIEV (2011) reported a new locality of G. thracica, i.e. a spring at Tri Voditsi village (Upper Thracian Lowland, Southern Bulgaria). He believed that Sadleriana virescens bulgarica (Wagner, 1927) from the same area, identified using shell morphology only (ANGELOV 2000) could, in fact, be G. thracica. GEORGIEV (2012) described G. avtosensis from a water source near the town of Avtos (Eastern Stara Planina Mountains) and G. radostinae from a stream near Madara Town (Northeastern Bulgaria). GEORGIEV, GLÖER (2013) described two further species, G. slavyanica (Slavyanka Mountain, Southwestern Bulgaria) and G. derventica (Dervent Heights, Southeastern Bulgaria). GEORGIEV (2013) studied a collection of the Hungarian Natural History Museum and found G. angeltsekovi in samples from the Greek part of the Rhodopes (Papikio Oros, near Vronti).

The aims of this paper are: (i) to describe a new species of the genus *Grossuana*, (ii) to summarise the available information about the genus *Grossuana* from Bulgaria and (iii) to propose an identification key to the species of this genus from Bulgaria based on original and already published data.

Material and Methods

Live specimens were collected by hand and preserved in 75% ethanol. The empty shells were collected by sieving through 1 x 1 and 2 x 2 mm mesh size sieves. The dissections and measurements of the shells were carried out using stereomicroscope and an eyepiece micrometer considering the criteria of RADOMAN (1983) and HERSHLER, PONDER (1984). The photographs were taken with a digital camera system.

The opinion of RADOMAN (1983) that most of the species of Rissooidea (see Truncatelloidea, CRISCIONE, PONDER 2013) differ in their penis morphology was accepted for this paper. The female genitalia was not investigated.

Abbreviations used: H – shell height, W – shell width, AH – aperture height, AW – aperture width, ZMH – Zoological Museum of Hamburg, MTD – Zoological Museum of Dresden. The type material was deposited in the Zoological Museum of Hamburg (ZMH, Germany).

Results

Genus Grossuana Radoman, 1973

Grossuana falniowskii sp. n.

Material examined: Five males were dissected, 15 shells were measured.

Holotype: H = 1.7 mm, W = 1.35 mm, ZMH 79681.

Paratypes: six ex. + one penis in ethanol, eight specimens, coll. Glöer, from the type locality, ZMH 79682.

Additional material (not paratypes): 11 empty shells in the deposits of Bedechka River, Krayrechen Park, city of Stara Zagora, Bulgaria, 15.02.2004; 45 live specimens collected from three springs along the same stretch of Bedechka River: spring 1 (N42 26 24.9 E25 38 24.0, the type locality), 15 specimens in ethanol, 19.03.2009; spring 2 (N42 26 21.8 E25 38 25.3), 10 specimens in ethanol, 18.03.2013; spring 3 (N42 26 51.7 E25 38 03.3), 20 specimens in ethanol, 23.03.2014.

Type locality: Spring directly flowing into Bedechka River, Krayrechen Park, city of Stara Zagora, Bulgaria, situated right on the river bank, close to the main flow, N42 26 24.9 E25 38 24.0, 219 m a.s.l. It is the type locality of *Belgrandiella zagoraensis* Glöer & Georgiev, 2009 as well.

Measurements: H = 1.39-1.65 mm, W = 1.09-1.42 mm, AH = 0.79-0.99, AW = 0.66-0.86, W/H = 0.72-0.86, AH/H = 0.53-0.61.

Description: Shell small, ovoid-conical, white, consisting of 4-4.5 regularly growing whorls. Whorls slightly rounded, with relatively deep suture. Shell surface glossy, with fine growth lines. Umbilicus

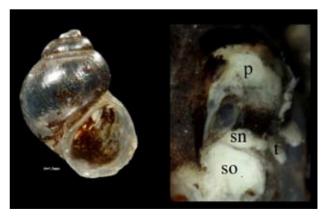


Fig. 1. Grossuana falniowskii sp. n., the shell of the holotype (left) and a sp. soft body with penis (right) photographed in ethanol: p - penis, sn - snout, t - tentacle, so - sole



Fig. 2. Live specimens of G. falniowskii sp. n. from Locality 3, Krayrechen Park, Stara Zagora

slit-like. Aperture ovoid and slightly rounded at the top, with sharp peristome thickened at the columella (Fig. 1). Operculum red. Mantle black, with white or light grey border. Head and tentacle base black to grey; snout, sole and distal tentacle parts white or light grey (Figs. 1 & 2). Penis conical and pointed at its apical part, with broad base, bearing small single lobe on its left side. Patches of black pigmentation found all over the penis, as well as a dark spot (generic characteristic).

Differentiating diagnosis: With its gradually tapering, broad-based penis, *G. falniowskii* sp. n. is most similar to *G. thracica*. It differs from the latter species in the deeper shell suture, the shape of the aperture, which is rounded at its top, and the smaller shell height. In addition, the penis of *G. thracica* is white with one black spot while the penis of the new species has patches of black pigments. No other species of *Grossuana* from Bulgaria are known to have such penis pigmentation (Radoman, 1983).

Etymology: Named after our colleague Professor Andrzej Falniowski (Poland), who contributed greately to the studies of the Truncatelloidea and helped Dilian Georgiev through providing literature sources and some good dissecting instruments for laboratory work. **Ecological data:** Habitat (Fig. 3). All three springs were rich in calcium, with limestone substrate within a broadleaf river bank forest. In more details:

Locality 1_(type locality, N42 26 24.9 E25 38 24.0, 219 m a.s.l.). Spring directly flowing into Bedechka River situated right on its bank, close to the main flow. Water temperature measured on 18.03.2013 was 7.4°C while the air temperature was 4.2°C. Oxygen levels measured on 14.03.2009 were 9.4 mg/l (at water temperature 8.2°C). The dominant plant species at this locality were Alnus glutinosa, Populus sp., Amorpha fruticosa and various species of mosses and grasses. The sandy bottom of the spring was filled completely with dead leaves and detritus. The maximum depth of the spring water was 3 cm. Grossuana specimens were found on dead leaves, sand, woody and plastic trashes. Associated molluscs: Belgrandiella zagoraensis [in GLÖER, GEORGIEV (2009), individuals of G. falniowskii sp. n. were erroneously reported as Radomaniola bulgarica Glöer & Georgiev, 2009].

Locality 2 (N42 26 21.8 E25 38 25.3, 185 m a.s.l.). Spring and stream flowing into Bedechka River. The spring is situated 30 m away from the river bank in a forest with thick bushy understory. The



Fig. 3. The spring localities of *G. falniowskii* sp. n. along Bedechka River, Stara Zagora: 1. The type locality; 2. Locality 2; 3 and 4. Locality 3

dominant plant species were Juglans regia, Salix sp., Ulmus sp., Corylus sp., Robinia pseudoacacia, Rubus sp., Sambucus sp., Humulus lupulus, Clemathis sp., Hedera helix, Rannunculus sp., and various species of mosses. The water was very rich in calcium (since a relatively thick layer of CaCO₂ was recorded on the surfaces of the submerged substrates). Water temperature measured on 18.03.2013 was 10°C while the air temperature was 4.2°C. The spring and stream area was about 1 cm deep (the maximum water depth was 2 cm). Some parts of this water body were filled with leaf detritus from the surrounding broadleaf plants and fine sand and small stones formed its bottom. Grossuana specimens were found on various substrates: stones, sand, dead leaves and branches and even on plastic and glass trashes. Associated molluscs: Belgrandiella zagoraensis.

Locality 3 (N42 26 51.7 E25 38 03.3, 191 m a.s.l.). Spring and stream about 15 m away from the river bank. Water temperature measured on 18.03.2013 was 12°C. The dominant plant species at this locality were *Juglans regia*, *Acer negundo*, *Alnus glutinosa*, *Populus* sp., *Rubus* sp., *Sambucus* sp., *Hedera helix*, and *Rannunculus* sp. Sand and small stones formed the bottom. Associated molluscs: *Belgrandiella zagoraensis*.

Population density: At Locality 2 (on 18.03.2013) in 11 randomly taken 2 x 2 cm samples from submerged dead tree branches and glass bottles, we found 0-11 specimens (av. three specimens per 2 x 2 cm). For comparison, the co-existing *Belgrandiella zagoraensis* had a similar density of 2 specimens per 2 x 2 cm in the same samples.

At Locality 3 (on 18.03.2013), in three randomly taken 2 x 2 cm samples, the population density was higher: 9-15 specimens; av. 11 specimens per 2 x 2 cm.

Seasonal activity: Active specimens were observed during the whole year.

Diet: Green algae were found in the faecal pellets of the collected specimens.

Distribution: *G. falniowskii* sp. n. was recorded living at three spring areas along Bedechka River, Stara Zagora city, Krayrechen Park, Upper Thracian Lowland at the foothills of Sarnena Gora Mountains: two areas are located below Zagorka pond and one is located above the pond. Empty shells of *G. falniowskii* sp. n. were collected also from the river deposits found both upstream out of the city and at the south slope of Sarnena Gora Mountains. The species is likely a local or regional endemic.

Discussion

Morphology and anatomy

Shell: Most of the Bulgarian Grossuana species have ovate-conical shells. Only the shell of G. slavyanica is elegant, elongate-conical. Some shells of G. aytosensis tend to be cylindrical-conical, similar to shells of *Bythinella* spp. The number of the whorls of all the known species ranges from 3.5 to 5.5 and usually is 4-4.5. The suture is weak in most of the species, and relatively deep in G. falniowskii sp. n.. Shell height varies between 1.4 and 2.2 mm. The smallest species are G. derventica and G. falniowskii sp. n., having shell heights below 1.8 mm. The largest species are G. radostinae, G. thracica and G. angeltsekovi (height up to 2.2 mm). WAGNER (1927) described Pseudamnicola consociella euxina using shell morphology of specimens from the spring of Devnya River and Aladza Manastir (Varna Region) and ZETTLER (2008) speculated it could be a species of Grossuana. These specimens had quite large shells: height 2.7 mm, and width 1.5 mm, and could be the largest species known of this genus. The shell surface is mostly shining with fine growth lines with the exception of G. aytosensis where it is rougher. The aperture of the Bulgarian species is ovoid, and could be rounded or angled at the top. In G. slavy*anica*, the aperture shape is oval. The operculum in most cases is red or reddish-orange and only in *G. radostinae* is brown.

Soft body: All the known species in Bulgaria have black-coloured mantle with a white or grey border. The base of tentacles, snout and sole is always black or dark grey, and the rest of the soft body parts are white or light grey.

Penis: There are three different penial forms in the Bulgarian Grossuana spp. known until now (Fig. 5). The first penis form is regularly broad along its length and tapers smoothly towards the top (G.angeltsekovi, G. avtosensis, G. radostinae, G. codreanui and G. slavyanica). The second form is characterised by an expanded base and it tapers sharply towards the top (G. thracica and G. falniowskii sp. n.). The third form has an expanded base followed by thin middle part and sharply tapering distal part (G. derventica). In all the cases, the penis is white with one black spot, with the exception of G. radostinae and G. falniowskii sp. n., which have several irregular dark pigmented patches all over the penis. The penis outgrowth could be small, single and hardly visible (G. thracica and G. falniowskii sp. n.) or well visible, and divided in two lobes (G. radostinae, G. aytosensis and others).



Fig. 4. *Grossuana* species known from Bulgaria: 1. *G. falniowskii* sp. n., shell collected in river deposits of Bedechka River, Stara Zagora; 2. *G. thracica*, holotype, ZMH 51458; 3. *G. thracica*, paratype, ZMH 51459; 4. *G. derventica*, holotype, ZMH 79337; 5. *G. aytosensis*, paratype, coll. Glöer; 6. *G. angeltsekovi*, holotype, ZMH 51460; 7. *G. angeltsekovi*, holotype, ZMH 51461; 8. *G. slavyanica*, paratype, ZMH 79336; 9. *G. codreanui*, Balchik (type locality); 10. *G. radostinae*, paratype, MTD SNSD Moll S4385

Distribution

Grossuana angeltsekovi was considered the most widespread species in Bulgaria by GLÖER, GEORGIEV (2009). After this publication, the species was registered also at the Greek part of the Rhodopes Mountains (GEORGIEV 2013). We found some further localities of *Grossuana* populations having shell and penis characters similar to those of *G. angeltsekovi*: spring at the village of Smolitchane (Osogovo Mountains, Western Bulgaria); Popov Izvor spring near the village of Bosnek (Vitosha Mountains, Western Bulgaria) and several other localities in the Western Rhodopes and Pirin Mountains (unpublished data). Further molecular studies could provide information if they belong to one or more sibling species. Until now, they are assigned to *Grossuana* cf. *angeltsekovi*.

All the other species from the country are known only from one or few closely situated locali-

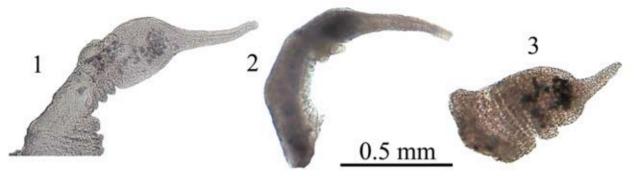


Fig. 5. The three types of penis morphology of the Bulgarian *Grossuana* species (light microscope pictures, 40x): 1. broad-based, thin at its middle part (penis of *G. derventica*); 2. regularly broad (penis of *G. angeltsekovi*); 3. broad-based, conical (penis of *G. falniowskii* sp. n.)

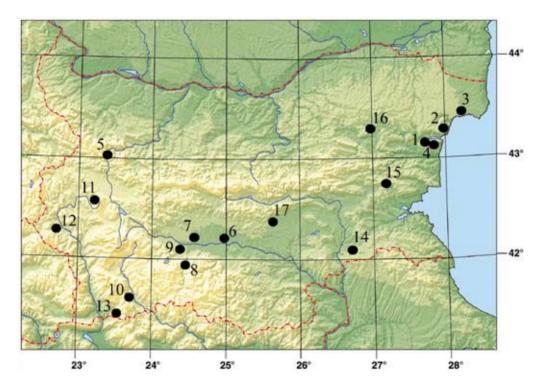


Fig. 6. Known distribution of the genus *Grossuana* in Bulgaria: 1 - "Pseudamnicola consociella euxina" (Devnya River and Aladzha Monastery); <math>2 - "Pseudamnicola consociella euxina" (Zlatni Pyasatsi Resort); 3 - G. codreanui (Baltchik town); 4 - G. codreanui (Avren village); 5 - "G. serbica" (Petrohan Pass); 6 - G. thracica (Chirpan Bunar cave); 7 - G. thracica (Tri Voditsi village); 8 - G. angeltsekovi (Batchkovo village); 9 - G. angeltsekovi – complex (Belashtitsa village); 10 - G. angeltsekovi – complex (Musomishta village); 11 - G. angeltsekovi – complex (Bosnek village); 12 - G. angeltsekovi – complex (Smolichane village); 13 - G. slavyanica (Goleshovo village); 14 - G. derventica (Melnitsa village); 15 - G. aytosensis (Aytos town); 16 - G. radostinae (Madara monument); 17 - G. falniowskii sp. n. (Stara Zagora city) (For more details, see the introduction)



Fig. 7. A spring locality of *G*. cf. *angeltsekovi* used by wild boars (*Sus scrofa*) as a bathing site (Western Rhodopes Mountains, Vatcha Gorge, south of the town of Krichim)

ties. Based on the present knowledge on their distribution, they could be considered local endemics (Fig. 6).

The available data on the altitudinal distribution show that the Bulgarian *Grossuana* spp. are distributed at lowland and hilly areas between 100 (*G. thracica*) and 762 m a.s.l. (*G. slavyanica*).

Some possible ways of dispersion by migrating birds was suggested by GEORGIEV (2012). A finding in the Rhodopes Mountains adds some new data: one of the localities of *Grossuana* cf. *angeltsekovi* (spring at Vacha River Gorge, south of the town of Krichim) was found to be used by wild boars (*Sus scrofa*) as a bathing site (Fig. 7). It seems possible that *Grossuana* individuals could attach onto the boar fur and to the dry mud remaining inside the fur and, therefore, can be transported to other bathing sites. However, it is not known how long *Grossuana* species can survive outside the water.

Ecology

The ecology of the *Grossuana* spp. is poorly studied. They are known to inhabit clean waters with oxygen levels between 8.4 and 9.4 mg/l. All species registered in Bulgaria inhabit springs; some populations can also live along streams. Only *G. radostinae* was found in a rivulet.

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From the Bulgarian species, only *G. derventica* was found in a stream inside a cave and can be considered as troglophilous. In contrast, *G. thracica*, another species described from a cave spring, was not found inside the cave and was restricted at distance of a few meters in the spring area out of the cave entrance.

The *Grossuana* spp. are mostly benthic, and can be found on the bottom crawling on sand, stones, submerged dead wood and leaves, on aquatic plants and artificial materials. All species known tend to be calciphilous, living in karstic springs.

The diet of *Grossuana* spp. is known to consist of unidentified green algae.

Identification key to the species of the genus *Grossuana* recorded from Bulgaria

1. Shell ovoid-conical
2. Penis regularly broad
3. Operculum red
4. Penis thin at its middle part, whorls 3.5-4
- Penis regularly tapering upwards, whorls > 46.
5. Shell surface shinning
 6. Suture weak, aperture angled at the top, shell height 2.1-2.2 mmG. thracica Suture deeper, aperture rounded at the top, shell height < 2.0 mmG. falniowskii sp. n.
7. Whorls 4-4.5G. codreanui - Whorls 5-5.5G.angeltsekovi

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