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RESEARCH ARTICLE

Revealing the stygobiont and crenobiont Mollusca biodiversity hotspot in Caucasus: Part II. *Sitnikovia* gen. nov., a new genus of stygobiont microsnails (Gastropoda: Hydrobiidae) from Georgia

Выявление центров биоразнообразия стигобионтных и кренобионтных моллюсков на территории Кавказа: Часть II. *Sitnikovia* gen. nov. – новый род стигобионтных микрогастропод (Gastropoda: Hydrobiidae) из Грузии

E.M. Chertoprud*, D.M. Palatov & M.V. Vinarski

Е.М. Чертопруд*, Д.М. Палатов, М.В. Винарский

Elizaveta M. Chertoprud, Lomonosov Moscow State University, 1 Leninskie gory, Moscow 119991, Russia. E-mail: skytuna@yandex.ru

Dmitry M. Palatov, Lomonosov Moscow State University, 1, Leninskie gory, Moscow 119991, Russia; A.N. Severtzov Institute of Ecology and Evolution, 33, Leninsky prospect, Moscow 117071, Russia. E-mail: triops@yandex.ru

Maxim V. Vinarski, St. Petersburg State University, 7/9 Universitetskaya Emb., Saint Petersburg 199034, Russia. E-mail: radix.vinarski@gmail.com

Abstract. A new genus of stygobiont hydrobiid snails, endemic to Georgia, is described. *Sitnikovia* **gen. nov.** includes two species: *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.**, known only from their type localities (Garakha and Sakishore caves). The data of shell characters, penial morphology, and radula of the new genus are provided.

Резюме. Описан новый род эндемичных для Грузии стигобионтных моллюсков-гидробиид. *Sitnikovia* **gen. nov.** объединяет два вида, *S. megruli* **sp. nov.** и *S. ratschuli* **sp. nov.**, – известных только из типовых местообитаний (пещеры Гараха и Сакишора). В работе приведены отличительные признаки раковин, радулы и анатомии мужской половой системы.

Key words: aquatic malacofauna, stygobiont gastropods, taxonomy, Transcaucasia, Littorinimorpha, Hydrobiidae, new genus, new species

Ключевые слова: водная малакофауна, стигобионтные гастроподы, таксономия, Закавказье, Littorinimorpha, Hydrobiidae, новый род, новые виды

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Introduction

Traditionally, the endemic species of the Caucasian hydrobiid snails were placed into genera described from other territories, e.g. the Balkan Peninsula (Lindholm, 1913; Shadin, 1932; Starobogatov, 1962; Schütt & Şeşen, 1993; Grego et al., 2017). The recent works, including those based on a molecular approach, have shown that most probably all species of this family inhabiting Caucasus and Transcaucasia must be classified within the genera endemic to this territory (Vinarski et al., 2014; Glöer et al., 2015; Anistratenko et al., 2017; Sitnikova et al., 2018; Vinarski & Palatov, 2019;

*Corresponding author

Grego et al., 2020). It seems that no Balkan hydrobiid genera occur in the waters of Caucasus. To date, five endemic genera of the Caucasian hydrobiids have been described: *Shadinia* Akramowski, 1976, *Motsametia* Vinarski, Palatov et Glöer, 2014, *Pontohoratia* Vinarski, Palatov et Glöer, 2014, *Nicolaia* Glöer, Bößneck, Walther et Neiber, 2016, and *Tschernomorica* Vinarski et Palatov, 2019 (Vinarski & Kantor, 2016; Vinarski & Palatov, 2019).

In this paper, a new genus and two new species of stygobiont hydrobiids endemic to Georgia are described. The first part of this series was published by Grego et al. (2020).

Material and methods

The material for the present study was collected in two caves of Western Georgia (Sakishore and Garakha) and examined by us in 2017 and 2019. These caves belong to two geologically and hydrologically isolated karstic areas, namely the Odishi Plain (Samegrelo-Zemo Svaneti) and Shaori Lake hollow (Racha) (Fig. 1). Snails were collected from subterranean waterbodies situated inside the caves. Living molluscs were hand-picked from submerged stones and pebbles as well as from the surface of rimstone pools. Empty shells were sampled by means of a sieve with 0.2 mm mesh from the sand. Sampled specimens were fixed with 90% ethanol.

Digital images of 22 and 21 shells from the Sakishore Cave and the Garakha Cave, respectively, and the type specimens of the two new species were made by a camera attached to Stereoscopic Microscope Micromed MC-5 ZOOM LED. ImageJ 1.48v software (https://imagej.nih.gov/ij) was used for morphometric measurements. Six standard measurements were taken from each specimen: shell height (SH) and width (SW), aperture height (AH) and width (AW), body whorl height (BWH) and width (BWW). The measurements were analysed by the principal component analysis (PCA) using the PAST 2.0 statistical software.

The scanning electron microscopy (SEM) images of the shells were made in the Yu.A. Orlov at

Fig. 1. *Sitnikovia* **gen. nov.** Type localities of *S. megruli* **sp. nov.** (1) and *S. ratschuli* **sp. nov.** (2). **a** – karst massif of Central Mengrelia approximately corresponding to the Odish Plateau, **b** – karst massif of Racha (after Tintilozov, 1976).

the Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow, by means of Cam Scan S2 and Vega3 Tescan microscopes. The protoconchs and radulae were examined using a Cam Scan S2 SEM in the Electronic Microscopy laboratory of the Biological Faculty of the Moscow State University, Moscow. For counting of protoconch whorls and for measurements we followed techniques described by Anistratenko (2005). Among others, the diameter of the whole protoconch and the width of the initial non-spiral part of the embryonic shell (WE) were measured (Fig. 2).

The holotypes and a part of the paratypes of the new taxa were deposited in the collection of the Zoological Institute of the Russian Academy of Sciences (ZIN), Saint Petersburg, Russia. Other paratypes are kept in the Zoological Museum of the Moscow State University (ZMMU), Moscow, Russia, and in the collections of the authors.

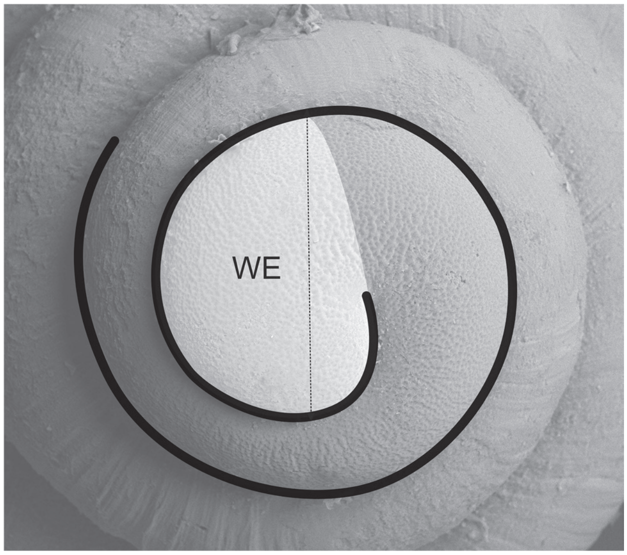

Fig. 2. *Sitnikovia megruli* **sp. nov.** Schematic illustration of the initial non-spiral embryonic shell part width measurement (WE).

Taxonomic part

Order **Littorinimorpha** Golikov & Starobogatov, 1975

Superfamily **Truncatelloidea** Gray, 1840

Family **Hydrobiidae** Stimpson, 1865

Genus ***Sitnikovia*** **gen. nov.**

Type species *Sitnikovia megruli* **sp. nov.**

Diagnosis. Shell high-conical, relatively slender (SW/SH ratio 0.40–0.60), with four to six slowly increasing whorls. Spire high, body whorl moderately inflated. Operculum ovate, corneous, thin and flat, without protrusions on the inner side, paucispiral with submarginal nucleus, usually orange or yellowish. Animal blind. Inner callus not developed. Radula of taenioglossate type, with formula 5-1-5-1-1; central (or rachidian) tooth with single pair of basal cusps. Lateral tooth resembles rachis in its shape and position of cusps, and bears eight or nine cusps (three or four at inner side, one largest, four at outer side); inner marginal tooth with 21–24 cusps, outer marginal tooth with 16 cusps.

Penis with a single dorso-lateral lobe, whose appearance varies from a well-developed conical offshoot to massive bulging with small knobby tip. The distal part of the penis is narrow and pointed; in its central part the penis is swollen; its width slowly decreases towards the basal part.

From all genera of the Caucasian hydrobiid snails, established to the date, *Sitnikovia* **gen. nov.** differs by a unique combination of general shell shape and the penis structure.

Etymology. Named after Dr. Tatiana Ya. Sitnikova, a renowned Russian expert in systematics of the freshwater Caenogastopoda.

Species composition. Sitnikovia **gen. nov.** includes two species described herein, both are endemic to Georgia.

Sitnikovia megruli **sp. nov.**
(Figs 3a, 3c, 4a, 4c, 4e, 4g, 4i)

"*Paladilhiopsis*" sp.: Chertoprud et al., 2020: 275–289.

Holotype. Adult mollusc, **Georgia**, *Samegrelo-Zemo Svaneti*, Chkhorotsqu district, Odishi plain, left side of the Bulebe River, subterranean spring inside Garakha Cave, 42°31′7.91″N 42°10′6.54″E, 207 m a.s.l., 03.II.2017, leg. D. Palatov, No. 514/1 (ZIN).

Paratypes. 21 adult molluscs, same collecting data as for the holotype, five under No. 514/2 (ZIN), three under No. Lc-40753 (ZMMU) and the rest in collections of the authors.

Shell dimensions of the holotype (mm). WN 4.75; SH 2.1; SW 1.15; BWH 1.15; BWW 1.0; AH 0.75; AW 0.65. For morphometric characteristics of the entire type series see Table 1.

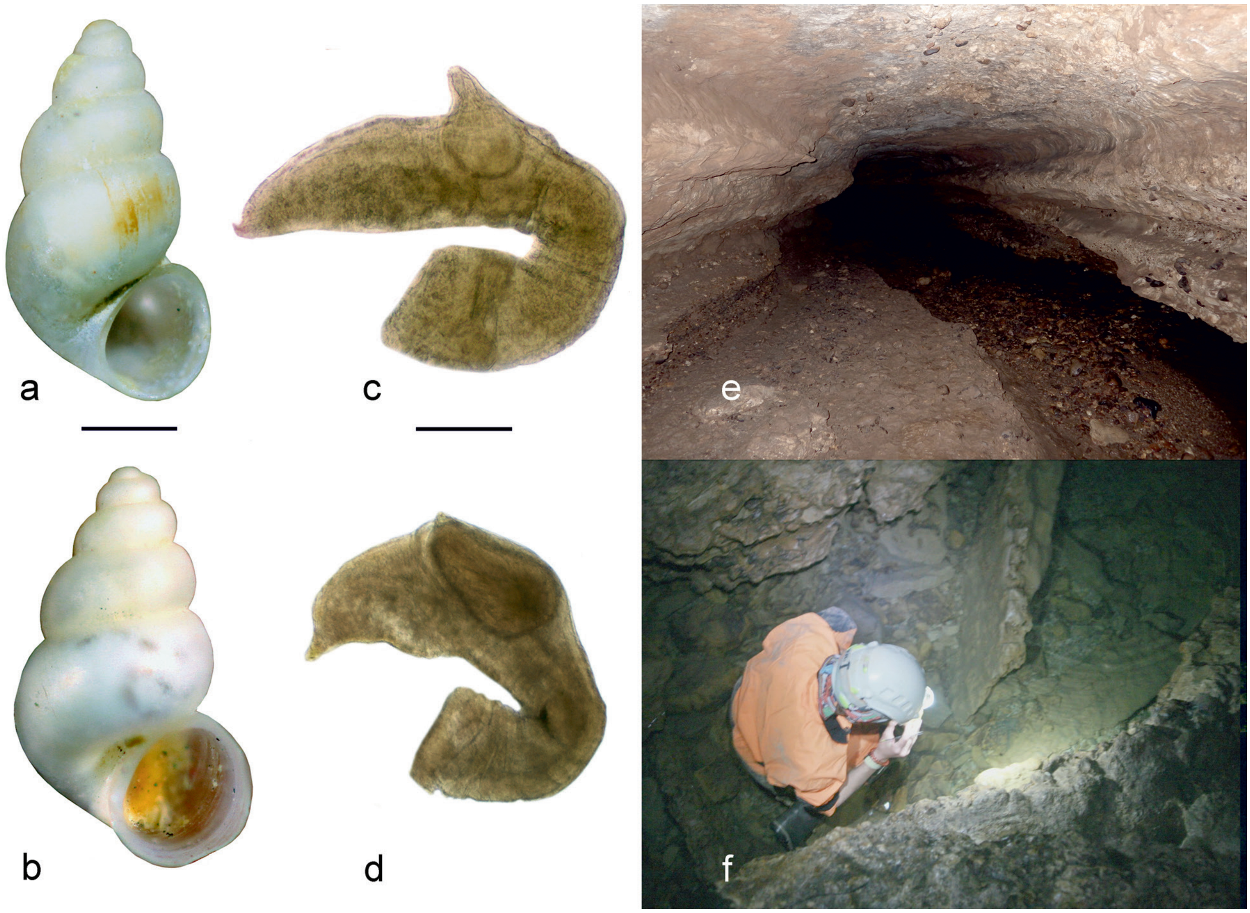

Fig. 3. *Sitnikovia megruli* **sp. nov.** (a, c, e) and *S. ratschuli* **sp. nov.** (b, d, f). **a**, **b** – shell, holotype; **c**, **d** – penial morphology; **e**, **f** – type locality (e – Garakha Cave, f – Sakishore Cave). Scale bars: 0.5 mm (a, b); 0.2 mm (c, d).

Table 1. *Sitnikovia megruli* **sp. nov.** and *S. ratschuli* **sp. nov.**, conchometric characteristics of the type specimens.

Character / index	Species	
	S. megruli (n = 21)	*S. ratschuli* (n = 22)
Whorl number	4.3–5.0 (4.7 ± 0.2)	4.0–5.1 (4.4 ± 0.3)
Shell height, mm (SH)	1.7–2.2 (2.0 ± 0.1)	1.5–2.6 (1.9 ± 0.3)
Shell width, mm (SW)	0.9–1.2 (1.0 ± 0.1)	0.9–1.4 (1.2 ± 0.3)
Body whorl height, mm (BWH)	1.2–1.3 (1.2 ± 0.1)	1.0–1.7 (1.3 ± 0.1)
Body whorl width, mm (BWW)	0.8–1.0 (0.9 ± 0.1)	0.9–1.2 (1.1 ± 0.2)
Aperture height, mm (AH)	0.6–0.8 (0.7 ± 0.05)	0.6–1.0 (0.7 ± 0.1)
Aperture width, mm (AW)	0.5–0.7 (0.6 ± 0.05)	0.5–0.9 (0.7 ± 0.1)
SW/SH	0.45–0.57 (0.51 ± 0.03)	0.53–0.68 (0.60 ± 0.01)
BWH/SH	0.59–0.69 (0.73 ± 0.02)	0.63–0.71 (0.67 ± 0.02)
BWW/BWH	0.65–0.79 (0.73 ± 0.04)	0.71–0.94 (0.83 ± 0.01)
AH/SH	0.30–0.38 (0.34 ± 0.02)	0.34–0.43 (0.38 ± 0.01)
AW/AH	0.79–1.01 (0.90 ± 0.05)	0.82–0.98 (0.91 ± 0.04)

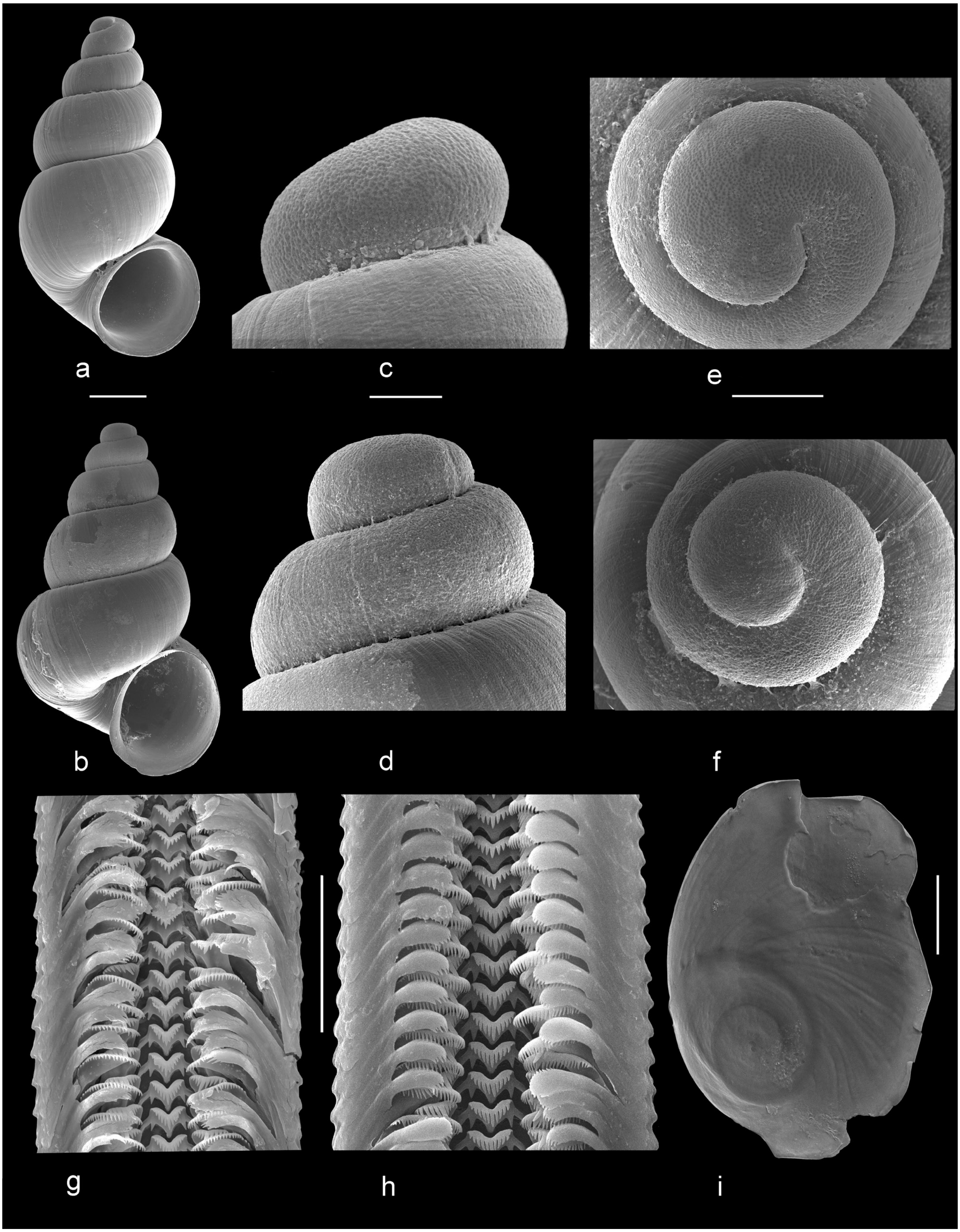

Fig. 4. *Sitnikovia megruli* **sp. nov.** (a, c, e, g, i) and *S. ratschuli* **sp. nov.** (b, d, f, h). **a**, **b** – shell, paratype; **c**, **d** – protoconch, lateral view; **e**, **f** – protoconch, apical view; **g**, **h** – radulae; **i** – operculum. Scale bars: 300 μm (a, b); 100 μm (c–i).

Description. Shell small (SH < 2.25 mm), high conical, relatively narrow and slender (SW/SH ratio 0.45–0.60), whitish. Whorl number up to 5.00. Whorls rounded and visibly convex, separated by deep oblique suture. Spire high, its height 0.60 SH or more. Body whorl high and moderately inflated, its width slightly exceeds width of penultimate one. Tangent line slightly convex. Aperture ovate, with obtuse angle in its upper part, more or less detached from body whorl wall forming a rather wide umbilicus. Shell surface almost smooth, with rare growth lines. Protoconch broad, low domed, consists of 1.5 whorls (330 µm in diameter, WE 220 µm). Surface of protoconch covered by mesh-like microsculpture with cellular pits of variable density. Pits of irregular shape, evenly immersed in protoconch surface. Protoconch separated from teleoconch by distinct and thin axial line. Operculum (Fig. 4i) ovate, corneous, thin, flat, without protrusions on inner side, paucispiral with submarginal nucleus, generally orange or yellowish.

Radula (Fig. 4g). Two basal cusps on each side of rachis and five cusps on each side of median cusp of rachis. Median cusp only slightly longer than adjacent ones, all cusps relatively long and narrow. On lateral tooth, there are four-five cusps on each side of largest cusp (formula: (5)4-1-4), all cusps long and narrow like those of rachis. Inner marginal tooth with 21–24 cusps, outer marginal tooth with 16 cusps.

Penis (Fig. 3c) with well-developed dorso-lateral lobe looking as short, conical, obtusely terminated process. Distal pointed part of penis very short and wide.

Animal blind.

Differential diagnosis. This species is similar to *S. ratschuli* **sp. nov.**, except for being smaller (SH does not exceed 2.2 mm) and having a relatively narrower shell (SW/SH ratio 0.45–0.57). Body whorl is smaller and moderately inflated. Shell proportions are noticeably slenderer. Aperture is relatively small with similar proportions. The protoconch of *S. megruli* **sp. nov.** is noticeably bigger than that of *S. ratschuli* **sp. nov.** (Figs 4e, 4f). Surface of protoconch has a similar mesh-like sculpture, although its pattern is slightly different: pits are irregularly shaped and equally impressed. The penial lobe in *S. megruli* **sp. nov.** is elongated and narrow, whereas in *S. ratschuli* **sp. nov.** it is rather shorter and swollen. There are no noticeable differences in radular morphology between *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.**

Etymology. Named after Samegrelo (Georgian სამეგრელო), a historic province in the western part of Georgia.

Distribution. Endemic to Western Georgia. Known only from the type locality.

Habitats and ecology. Inhabits a subterranean spring in Garakha Cave (Fig. 3e), prevailing on solid substrates. The densest aggregations of snails were found in the small left tributary of the main cave stream. Molluscs were collected from the submerged stones and from the surface of rimstone pools. Water indicators: T 11 °C, pH 8.5, total dissolved solids (TDS) 130 ppm.

Sitnikovia ratschuli **sp. nov.**

(Figs 3b, 3d, 4b, 4d, 4f, 4h)

Holotype. Adult mollusc, **Georgia**, *Racha-Lechkhumi*, vicinity of Kveda Tlughi Village, Shaori Lake basin, subterranean river inside Sakishore Cave, 42°26′31.1″N 43°09′30.9″E, 1188 m a.s.l., 13.VIII.2019, E. Chertoprud leg., No. 513/1 (ZIN).

Paratypes. 22 adult molluscs, same collecting data as for the holotype, three under No. 513/2 (ZIN), three under No. Lc-40754 (ZMMU) and the rest in collections of the authors.

Shell dimensions of the holotype (mm). WN 4.9; SH 2.15; SW 1.2; BWH 1.35; BWW 1.05; AH 0.8; AW 0.75. For morphometric characteristics of the entire type series see Table 1.

Description. Shell small (SH < 2.6 mm), high conical, relatively slender (SW/SH ratio 0.56–0.68), whitish. Whorl number up to 5.10. Spire high, its height 0.46 SH or more, and can reach 0.9 mm. Body whorl high and rather convex, its width extends beyond width of penultimate one. Tangent-line slightly concave. Surface of teleoconch with rare growth lines. Whorls rounded and visibly convex, separated by deep oblique suture. Aperture ovate, with obtuse upper angle. Umbilicus well developed. Protoconch consists of 1.5 whorls (270 µm in diameter, WE 158 µm). Surface of protoconch with mesh-like microsculpture. Protoconch separated from teleoconch by distinct axial line. Operculum ovate, corneous, thin and flat.

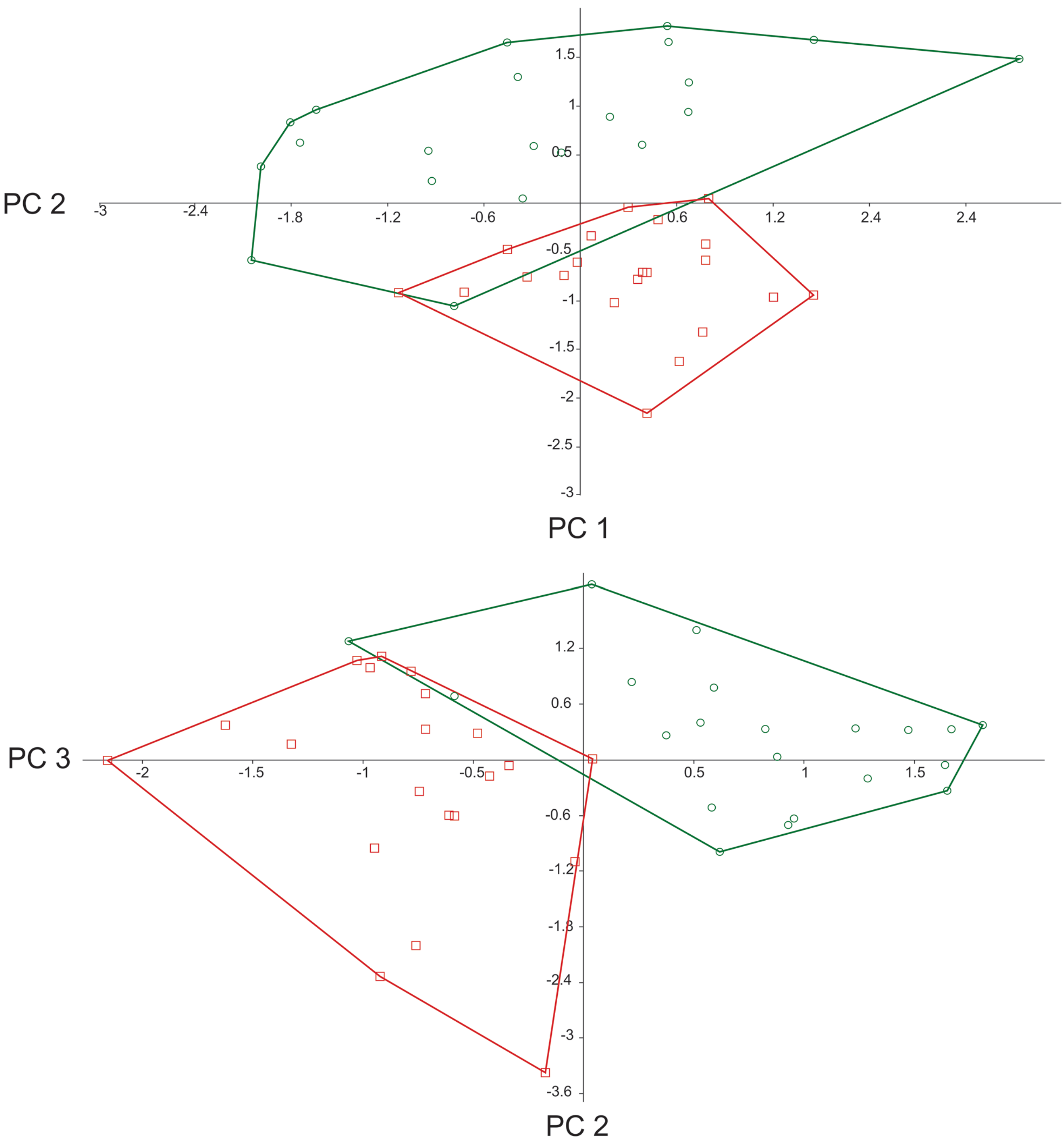

Fig. 5. *Sitnikovia* **gen. nov.** Principal component analysis of whorl numbers and shell measurements (see Table 1 for data). Green circles – *S. ratschuli* **sp. nov.**, red squares – *S. megruli* **sp. nov.** Three first principal components (PC) combined account for 97.3% of variance (PC1 70.4%, PC2 24.2%, PC3 2.7%).

Radula (Fig. 4h). Central (rachidian) tooth has a pair of basal cusps, large median cusp and five lateral cusp on each side of it. Lateral tooth asymmetric, with eight cusps, three at inner side, one largest, four at outer side (formula: 4-1-3). Median cusp elongated, almost twice as long as adjacent ones, and overlaps adjacent inner marginal tooth. Inner marginal tooth with 24 cusps, outer marginal tooth with 16 cusps.

Penis (Fig. 3d) massive and swollen in central part, with bulging dorso-lateral lobe slightly spaced from penis, and having miniature pointed tip. Distal part of penis abruptly tapers and forms short, hook-shaped tip, curved downwards.

Animal blind.

Differential diagnosis. This species is similar to *S. megruli* **sp. nov.**, but is larger (SH up to 2.6 mm) and having a relatively wider shell (SW/SH ratio 0.56–0.64). Body whorl is higher and more inflated, noticeably wider than the preceding one. Aperture of similar proportions but larger. The protoconch of *S. ratschuli* **sp. nov.** is noticeably smaller than that of *S. megruli* **sp. nov.** (Figs 4e, 4f). Surface of protoconch has a similar mesh-like sculpture although the pattern is slightly different with irregular axial elevations forming feebly defined ridges surrounding the depressions. The penial lobe in *S. ratschuli* **sp. nov.** is low and wide, whereas in *S. megruli* **sp. nov.** it is rather slender and pointed. There are no noticeable differences in radular morphology between *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.**

Etymology. The name is derived from Racha (Georgian რაჭა), a historical highland region of Western Georgia, located in the upper Rioni River and at the foothills of the Greater Caucasus Range.

Distribution. Endemic to Western Georgia. Known only from the type locality.

Habitats and ecology. Inhabits subterranean river flowing in the Sakishore Cave (Fig. 3f). Molluscs live on the massive stones immersed in water. *S. ratschuli* **sp. nov.** may be characterized as a rheophilic species that prefers submerged hard substrates. Molluscs prevail in a truly underground part of a watercourse, whereas in the part of the cave where the sunlight penetrates only empty shells were found.

Discussion

The two new species, united here under the generic name *Sitnikovia* **gen. nov.**, are characterized by the similar features of penial anatomy, specifically by the presence of a dorso-lateral lobe. The main anatomical difference between the two species is the shape and proportions of this lobe. Significant differences were found in the dimensional characteristics of the protoconch. Compared to *S. ratschuli* **sp. nov.**, *S. megruli* **sp. nov.** has a noticeably larger embryonic shell which can be explained by the lecitotrophic type of embryonic development, when the nutrients for developing embryos come from the large and yolk-rich oocyte. The differences between the teleoconchs are not so apparent, but the PCA analysis has shown that *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.** are more or less distinct: the “clouds” of points, corresponding to the two taxa, overlap only slightly (Fig. 5). However, we are unable to report any shell measurement or ratio allowing one to distinguish the shells of the two species. The values of all morphological indices of *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.** overlap (see Table 1).

However, the anatomical differences between the two studied forms alongside with a spatial isolation allow us to affirm their species distinctness. The habitats of *S. megruli* **sp. nov.** and *S. ratschuli* **sp. nov.** are located in two different speleoregions, which are not interconnected either spatially or geologically. Samegrelo Odishi cave region is a cluster karst massif with a predominance of the Neogene age conglomerates, while the Racha cave region is located on the highlands and is composed of Upper Cretaceous limestone (Tintilozov, 1976). Thus, two anatomically similar species are distributed in currently isolated karst areas. The disjunction of their ranges was probably formed relatively recently, as a consequence of a marine transgression caused the flooding of the Colchis Lowland. A molecular phylogenetic study is required to test this hypothesis.

Moreover, the Racha region, specifically the Shaori karst plateau, is elevated by more than 1000 m a.s.l., while most of the previously investigated caves are located at lower altitudes (e.g. Garakha Cave lies at 207 m a.s.l.). Thus *S. ratschuli* **sp. nov.**, a mountain species, has the most elevated habitat among other stygobiontic gastropods that have already been described. Due to the isolated location of the Shaori karst region, it is rather poorly studied in terms of the subterranean biology.

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