

## New diagnostic characters of *Kolhymorbis angarensis* (Dybowski & Grochmalicki, 1925) (Gastropoda: Pulmonata: Planorbidae)

E.V. SOLDATENKO

E.V. Soldatenko, Zoological Institute, Russian Academy of Sciences, Universitetskaya Emb. 1, St. Petersburg 199034, Russia. E-mail: sold.zoo@mail.ru

The radula morphology and the anatomy of the copulatory apparatus in *Kolhymorbis angarensis* were examined using light microscopy, scanning electron microscopy (SEM) and histological methods. *Kolhymorbis angarensis* was shown to have the stylet and the penial sac with a glandular appendage (flagellum), the characteristics, previously unknown for any species of this genus. The significance of these findings for the taxonomy of the genus is discussed.

**Key words:** molluscs, morphology, radula, stylet, Gastropoda, Pulmonata, Planorbidae, *Kolhymorbis*

### INTRODUCTION

*Kolhymorbis angarensis* (Dybowski & Grochmalicki, 1925) (Fig. 1a–c) was first described as a member of the genus *Segmentina* (*Segmentina nitida* forma *angarensis*) on the basis of a conchological similarity. Later, a new species, *Kolhymorbis maacki* Starobogatov & Streletzkaia, 1967, was described and placed into its own genus, *Kolhymorbis* Starobogatov & Streletzkaia, 1967, based on shell morphology and the anatomy of the copulatory apparatus. *Kolhymorbis maacki* was later recognized by Moskvicheva (1974, 1977) as a synonym of *K. angarensis*, a conclusion that was accepted by Starobogatov et al. (2004).

The diagnostic characters of the genus *Kolhymorbis* (and, hence, those of *K. angarensis*) are the penial sac lacking glandular appendages and the penis with a soft papilla. These characters, however, have not been confirmed by the re-examination of alcohol-preserved specimens of this species.

### MATERIAL AND METHODS

Examined material consists of eight samples of *K. angarensis* in the collections of the Zoological Institute of Russian Academy of Sciences, St. Petersburg (ZIN).

ZIN 1; Galutuy L. near Baikal L., September 1938; Baikal expedition of the Acad-

emy of Science. ZIN 7; *Primorsky Terr.*, Yakovlevsky Distr., Varfolomeevka, temporary ponds, 25 Sept. 1956; leg. Ya.I. Starobogatov. ZIN 9; *Primorskiy Terr.*, Vladivostoksky Distr., a pool in the flood-plain of Bol'shaya Klenochnaya R., 30 Sept. 1956; leg. Ya.I. Starobogatov. ZIN 11; *Primorsky Terr.*, Vladivostoksky Distr., Razdol'noye, spring brook, 30 Sept. 1956; leg. Ya.I. Starobogatov. ZIN 27; *Amur Prov.*, a lake in the flood-plain of Zeya R., 28 May 1973; leg. V.A. Dvorjadkin. ZIN 35; *Khabarovsk Terr.*, upper reach of Manoma R., puddle, 19 June 1980; leg. M.N. Zatravkin. ZIN 44; *Khabarovsk Terr.*, forest 12 km from Sulukh, 12 July 1982; leg. I.M. Moskvicheva.

A total of 52 specimens were examined, of which 13 were dissected (ZIN 7, 9, 11, 27, 35, 44). Pharynxes and reproductive organs were removed under a Leica MZ 9<sub>5</sub> dissecting microscope; the structures extracted from the animals and their fragments were studied using various techniques as follows.

Six specimens were prepared as unstained whole mounts. These whole-mount preparations were examined and photographed on a Leica DMLS-2 microscope equipped with a CCD camera; the line drawings were copied from photographs according to their original proportions (Fig. 2).

Four specimens were prepared for scanning electron microscopy. Each extracted penis was dehydrated in ethanol and then

air dried for 20 min in hexamethyldisilazane (Bock, 1987). The radulae were washed from the accompanying tissues with a weak aqueous solution of sodium hypochlorite, rinsed in distilled water and 96% alcohol (Röepstorff & Riedel, 2004), coated with platinum in a HITACHI IB-5 ion sputter and viewed on a HITACHI S-570 scanning electron microscope.

Three specimens were taken for histological observations. The dissected copulatory apparatus was fixed in 70% alcohol and after dehydration in xylene was embedded in paraffin using standard histological techniques (Lillie, 1969). Serial sections 6  $\mu\text{m}$  thick were stained with hematoxylin and eosin. Histological sections were viewed and photographed on a Leica DM LS-2 microscope.

The radular teeth were measured according to Röepstorff & Riedel (2004).

## RESULTS

The radula of *K. angarensis* is 0.60–0.65 mm long and 0.11–0.12 mm wide. The number of transverse tooth rows ranges from 162 to 170; the rows are almost straight and each row is bilaterally symmetrical relative to the central tooth. Radula formula: 1 + (20–21). The teeth are slightly spaced apart, especially along the periphery of the radula (Fig. 1d). The central tooth is small, with a wide base and short (less than  $\frac{1}{2}$  of base height) dental plate (Fig. 1e). The dental plates are pectinate; the central plate has 5, occasionally 7, cusps of various lengths, of which 2 are significantly larger than the rest. All marginal teeth have 7 cusps, of which 3 and 2 cusps are the most pronounced in the primary and secondary marginals, respectively. The central tooth is 5.0–6.0  $\mu\text{m}$  high and 3.5–4.0  $\mu\text{m}$  wide. The primary marginals are 5.0–5.5  $\mu\text{m}$  high and 3.5–3.7  $\mu\text{m}$  wide. The secondary marginals are 4.8–6.4  $\mu\text{m}$  high and 4.0–5.5  $\mu\text{m}$  wide.

A detailed light-microscopic study reveals a small flat pale-yellow stylet in the copulatory apparatus. The structure is partially or completely covered with lateral cells that

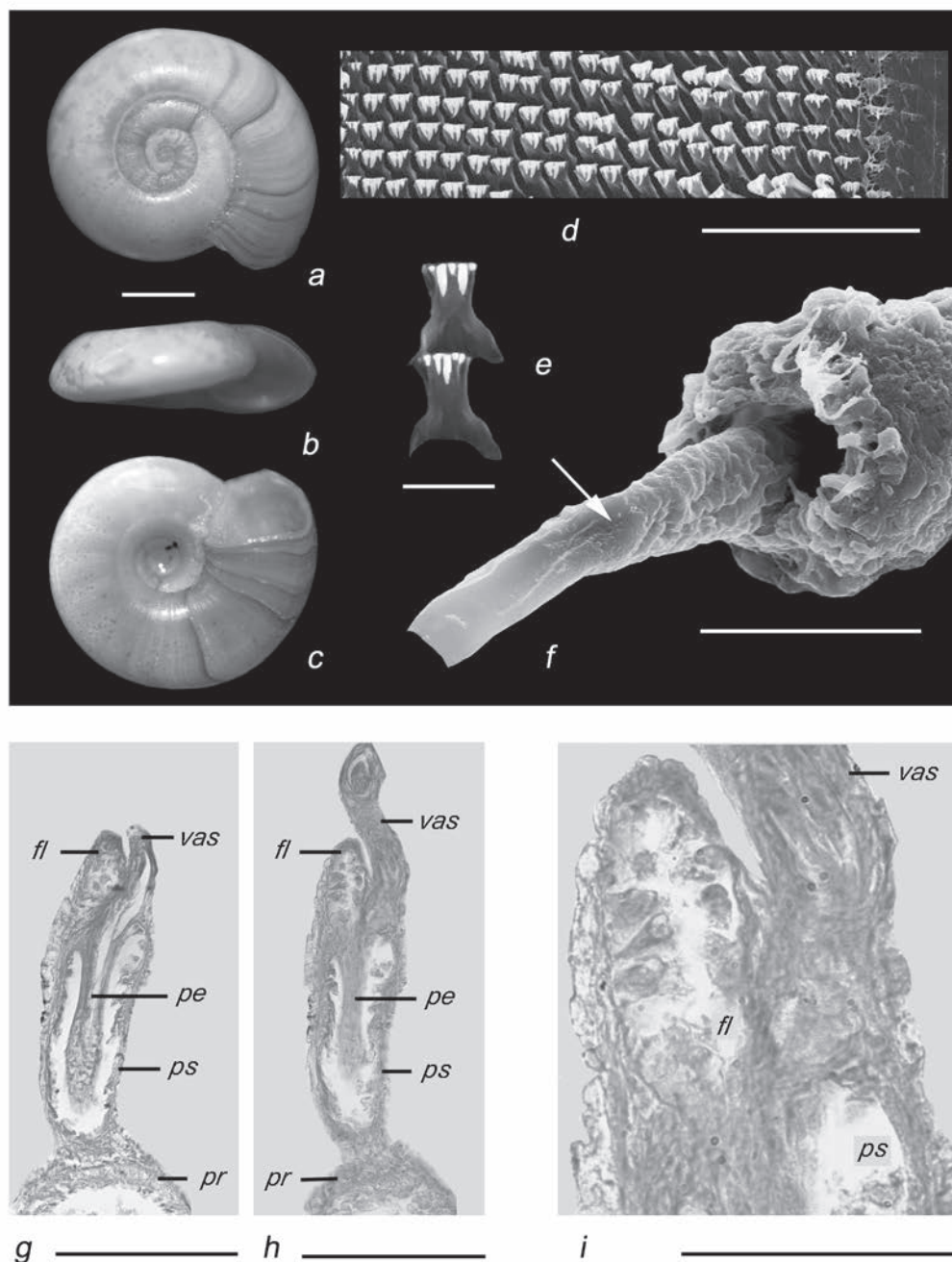
participate in stylet formation (Soldatenko, 2009). The opening of vas deferens is terminal and lies at the base of the stylet. In the proximal portion of the sac is a small enlargement with irregular walls (Fig. 2, *f*).

The SEM study of the penis with partially excised penial sac confirms the presence of a stylet on the penis tip (Fig. 1f). The stylet is a plate-like structure, with the edges curved toward vas deferens in its proximal portion, and completely flat in its distal portion. The stylet is 20–25  $\mu\text{m}$  long and 10–12  $\mu\text{m}$  wide.

A series of histological sections through the copulatory apparatus reveals a gland (flagellum, according to Baker, 1945); this gland lies closely adjacent to the sac wall, except in the proximal portion, where it is slightly separated from the wall (Fig. 1g–i). The gland duct opens into vas deferens. The length of the gland is more or less equal to that of the entire sac (100–110  $\mu\text{m}$ ).

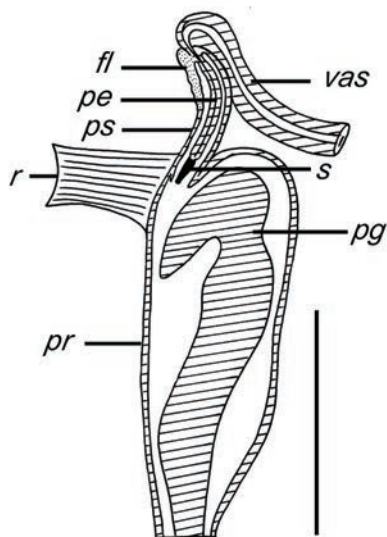
## DISCUSSION

SEM allows the detailed examination of the radula morphology and reveals the structures in the copulatory apparatus of *K. angarensis* that had previously been overlooked because of their small size. The copulatory apparatus of *K. angarensis* was shown to have a plate-like stylet on the penis tip and the gland, slightly projecting from the sac. The radula structure is similar to that of *Helicorbis* Benson, 1855, *Polypylis* Pilsbry, 1906, and *Segmentina* Fleming, 1818, but the overall size of the radula and the sizes of individual teeth in *K. angarensis* are considerably smaller. The presence of a gland in the penial sac of *K. angarensis* is not unexpected, because the shell is lens-shaped and copulation is likely to be impossible without some additional fixation. The plate-like shape of the stylet, however, is unusual, because this stylet shape has never been described before for any species of the Planorbidae Rafinesque, 1815, although such possibility has been suggested on theoretical grounds (Meier-Brook, 1983). These structures and other characters of the copulatory apparatus (small flagellum,



**Fig. 1.** *Kolhymorbis angarensis*: shell (**a** – apical view; **b** – frontal view; **c** – umbilical view); radula (**d** – right side of radula showing several tooth rows, with central tooth on left side; **e** – central tooth); penis with plate-like stylet projecting from penial sac (**f**) (arrow indicates opening of vas deferens); microphotographs of histological sections through copulatory apparatus (**g–i**). Abbreviations: *fl*, flagellum; *pe*, penis; *pr*, preputium; *ps*, penial sac; *vas*, vas deferens. Scale bars: 1 mm (a–c); 30  $\mu$ m (d, f); 5  $\mu$ m (e); 100  $\mu$ m (g, h); 25  $\mu$ m (i).





**Fig. 2.** Copulatory apparatus of *Kolhymorbis angarensis* (diagram of longitudinal section). Abbreviations: *fl*, flagellum; *pe*, penis; *pg*, preputial gland; *pr*, preputium; *ps*, penial sac; *r*, retractor; *s*, stylet; *vas*, vas deferens. Scale bars: 0.5 mm.

terminal position of the opening of vas deferens, weakly developed preputium) are an indication of an early phylogenetic divergence of the genus *Kolhymorbis* within the tribe Segmentinini Baker, 1945, where it was placed by Starobogatov, 1967.

It would be premature to make any changes in the diagnosis of *Kolhymorbis* until all relevant anatomical characteristics of every member of this genus, and, especially, of the type species, *K. shadini* Starobogatov & Streletzkaia, 1967, are sufficiently studied. It should, however, be noted that the presence of the new structures does not contradict the placement of *Kolhymorbis* within the tribe Segmentinini of the subfamily Planorbinae Rafinesque, 1815.

#### ACKNOWLEDGMENTS

The ZIN collections used for this study are supported by Rosnauka for UFC no. 2-2.20. The author is grateful to L.L. Yarokhnovich, curator of the ZIN collection of fresh-water molluscs, for her assistance during this study, and T.K. Tsogoyev, engineer of the Electron Microscopy Department, ZIN, for his help with SEM.

#### REFERENCES

- Baker, F. C.** 1945. *Molluscan family Planorbidae*. Urbana: University of Illinois Press. 530 p.
- Bock, C.** 1987. A quick and simple method for preparing soft insect tissues for scanning electron microscopy using Carnoy and hexamethyldisilazane. *Beiträge Elektronenmikroskopische Direktabbildung und Analyse von Oberflächen*, **20**: 209–214.
- Dybowski, B. & Grochmalicki, J.** 1925. Przyczynki doo znajomoci mięczaków jeziora Bajkalskiego. (Contributions à la connaissance des Mollusques du lac Baikal). *Wladislavii-dae nom. fam. Kosmos (Lwow)*, **50**: 819–881.
- Lillie, R.D.** 1969. *Histopathologic Technic and Practical Histochemistry*. New York: 3rd edition, McGraw-Hill Book Co. 645 p.
- Moskvicheva I.M.** 1974. Freshwater molluscs of the lower reaches of the Amur River basin: Thesis Synopsis. ZIN, AN SSSR. Leningrad. 20 p. (In Russian).
- Moskvicheva I.M.** 1977. Ecological groupings of the molluscs in the Amur River basin. *In: Biogeografiya Priamurya* [Biogeography of the Amur River Region], **17**: 83–88. (In Russian).
- Röepstorf, P. & Riedel, F.** 2004. Deepwater gastropods endemic to Lake Baikal and SEM study on protoconchs and radulae. *Journal of Conchology*, **38**: 253–282.
- Soldatenko, E.V.** 2009. Postembryonic development of the copulative apparatus in representatives of three genera of the family Planorbidae (Gastropoda: Pulmonata). *Trudy Zoologicheskogo Instituta RAN*, **313**: 168–182. (In Russian).
- Starobogatov, Ya.I.** 1967. On the systematization of freshwater Pulmonate molluscs. *Trudy Zoologicheskogo Instituta AN SSSR*, **42**: 280–304. (In Russian).
- Starobogatov, Ya.I. & Streletzkaia, E.A.** 1967. Composition and zoogeographical characteristics of freshwater malacofauna of the East Siberia and northern part of the Far East. *Trudy Zoologicheskogo Instituta AN SSSR*, **42**: 221–268. (In Russian).
- Starobogatov, Ya.I., Prozorova, L.A., Bogatov, V.V. & Sayenko, E.M.** 2004. Molluscs. *In: Tsalolikhin, S.Ya.* (Ed.) *Opredelitel' presnovodnykh bespozvonochnykh Rossii i sopredel'nykh territoriy* [Key to freshwater invertebrates of Russia and adjacent lands], 6. Nauka, St. Petersburg. 492 p. (In Russian).

Received 19 September 2009 / Accepted 30 November 2009