

Two tiny streams in Thimphu, with two new gastropods (Caenogastropoda, Rissooidea: Pomatiopsidae and Amnicolidae)

CHOKI GYELTSHEN 

National Biodiversity Centre, Serbithang, Ministry of Agriculture and Livestock, Thimphu, Bhutan

ADRIAAN GITTENBERGER 

GiMaRIS, Rijksstraatweg 75, 2171 AK Sassenheim, The Netherlands

EDMUND GITTENBERGER 

National Biodiversity Center Naturalis, P.O. Box 9517, 2300 RA Leiden, The Netherlands;
GiMaRIS, Rijksstraatweg 75, 2171 AK Sassenheim, The Netherlands; egittenberger@yahoo.com



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MATERIAL AND METHODS

The material was collected visually by the first author in cooperation with Phuntsho Namgyel. The specimens, including the holotypes, are kept in the National Biodiversity Centre, Serbithang, Thimphu, Bhutan. Some duplicates are in the National Biodiversity Center Naturalis, Leiden, The Netherlands. Photographs of the holotype of *Tricula mahadevensis* Neemann, Shah & Tachamo, 2007, were made by Ms Sara Schnedl and put at our disposal by Ms Anita Eschner (both Naturhistorisches Museum, Vienna, Austria).

The number of whorls was counted in conformity with Kerney & Cameron (1979: 13). The superfamilia and the familia are indicated in accordance with Bouchet et al. (2017). Locality numbers are between square brackets. Photographs were made by E. G. with a Canon EOS 7D, using a Canon Macro MP-E 65mm lens with a Macro Ring Lite MR-14EXII.

For the DNA-extractions crushed shells were used and a 3-day CTAB based method as described by van Haaren et al. (2021). In the PCR the marker COI was targeted with the primers mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC; universal COI forward primer developed by Leray et al., 2013) and COI_Gastr_R (ACTTCDGGRTGDCCAAAAAYCA; a “new” GiMaRIS primer developed to be Gastropoda-specific) within the following program: hold 95°C 4', 55 cycles of 60' at 95°C, 60' at 48°C and 60' at 72°C, and final a hold at 72°C for 8'. The PCR was done in a Qiagen RotorGene Q apparatus with a HRM analysis at the end to check whether the PCR product formed melted at the temperature expected for a COI product. The PCR products were cleaned with a ExoCleanUp FAST PCR reagent, following the manufacturer's (vwr) instructions. Sequencing was done in both directions by Macrogen Europe. The resulting sequences were submitted to GenBank.

In a spring area near Thimphu two species of micro-gastropods were discovered, which are described as new to science here. The endemic *Tricula tashiae* spec. nov. is next to the more widespread *T. montana* Benson, 1843, and is the second *Tricula* species known now from Bhutan. *Erhaia benjii* spec. nov. is described as the fifth endemic *Erhaia* species for the country.

Key words: Gastropoda, *Tricula*, *Erhaia*, Bhutan, Thimphu.

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INTRODUCTION

Aquatic molluscs may be found in a broad variety of habitats with water present, at least temporary. These habitats got special attention within the scope of the Bhutan Evertebrata Inventory Project of the National Biodiversity Centre (Serbithang, Thimphu, Bhutan), the Ugyen Wangchuck Institute for Conservation and Environmental Research (Bumthang, Bhutan) and Naturalis Biodiversity Center (Leiden, the Netherlands). This resulted in the discovery and description of several new species of micro-gastropods. Here we present the results of an exploration of some tiny streams in the neighbourhood of Serbithang and Ngabiphu in south Thimphu.

Abbreviations: B = shell breadth; H = shell height; n = number of specimens; NBCB = National Biodiversity Centre, Serbithang, Thimphu, Bhutan; NHMW = Naturhistorisches Museum, Wien, Austria; RMNH = National Biodiversity Center Naturalis, Leiden, the Netherlands.

SYSTEMATICS

Subclass Caenogastropoda Cox, 1960
Superfamily Rissooidea Gray, 1847
Family Pomatiopsidae Stimpson, 1865

Genus *Tricula* Benson, 1843

Type species (by monotypy): *Tricula montana* Benson, 1843.

Remark: the name *Tricula* is introduced by Benson (1843: 465-466) as “allied to *Melania*” and “Sub-genus”. In the text the species is introduced as *T. montana*. Therefore, the basionym is *Tricula montana*, not *Melania (Tricula) montana* as given by Gittenberger et al. (2020).

***Tricula tashiae* spec. nov.**

Figs 2-5

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Type series. — Holotype (NBCB1280), paratypes (NBCB1281/7 + 5 juveniles in ethanol 70%; RMNH.MOL.181986/3), Bhu-

tan, Thimphu Dzongkhag: [S121] temperate conifer forest between Royal Thimphu College and Serbithang, 27°24'41"N 89°39'35"E, 2583 m a.s.l., Choki Gyeltshen & Phuntsho Namgyel leg. 14.v.2022 (Fig. 1).

Description (n = 11). — Shell elongated conical, with ca. 5 narrowly shouldered, convex whorls, separated by a deep suture. Protoconch heavily encrusted or missing. Teleoconch with coarse growth lines and barely discernible, dense, spiral lirae. Aperture obliquely piriform, with a slightly curved inner lip and a regularly curved outer lip. Peristome continuous and prominently thickened, slightly protruding at the parietal side, next to a long umbilical chink.

Measurements. — H ca. 3.6-4.3 mm; B 1.7-2.3 mm.

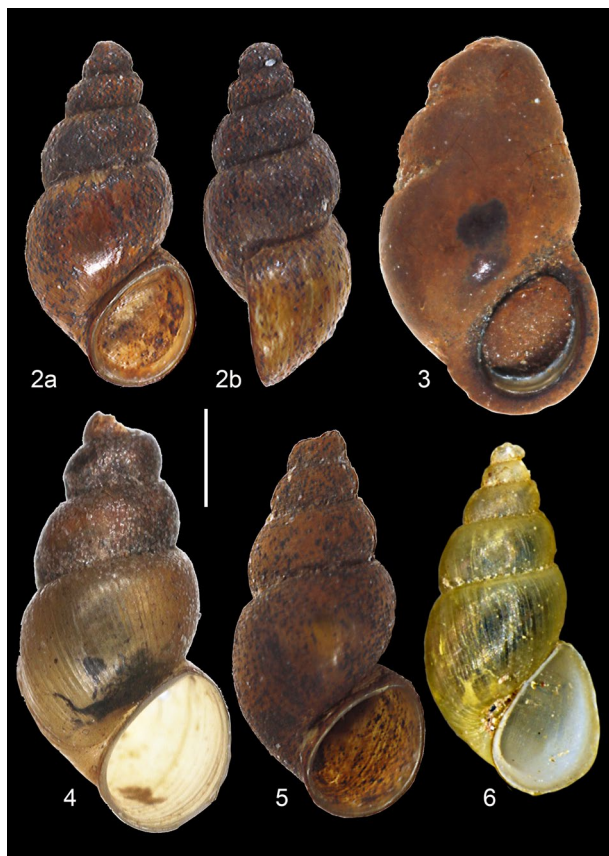
Differentiation. — Shells identified as *Tricula montana* Benson, 1843 from central and eastern Bhutan (Gittenberger et al., 2020) are smaller, the aperture is more triangular, with a nearly vertical palatal side and a broadly rounded basal part. The parietal part of the inner lip is nearly completely attached to the penultimate whorl so that there is hardly an umbilical chink.

Tricula mahadevensis Neesemann, Shah & Tachamo, 2007, described from Nepal, Bhaktapur district, over 400 km west of *T. tashiae* spec. nov. (see Neesemann et al., 2007: 63) also differs by a triangular shape of the aperture (Fig. 6). Like *T. tashiae* spec. nov. it has a thickened, continuous peristome, but the shell is less slender, its whorls are less convex and the adapical part of the inner lip is nearly entirely connected to the shell wall.

We have not thoroughly compared the *Tricula* species



Fig. 1. Type locality of *T. tashiae* spec. nov. and *E. benjii* spec. nov. Bhutan, Thimphu Dzongkhag, near Royal Thimphu College in Ngabiphu near Serbithang. Photograph by C. Gyeltshen.



Figs 2-6. *Tricula* spec. **Figs 2-5** *T. tashiae* spec. nov. **Fig. 2** holotype NBCB1280 (H 3.6 mm) & **Figs 4-5** paratypes NBCB1281 (H 4.4 + 4.1 mm), Bhutan, Thimphu Dzongkhag, near Royal Thimphu College in Ngabiphu near Serbithang; **Fig. 3.** NBCB1419 (H 4.0 mm), Bhutan, Thimphu Dzongkhag, temperate conifer forest between Royal Thimphu College and Serbithang. **Fig. 6.** *Tricula mahadevensis* Neesemann, Shah & Tachamo, 2007, holotype NHMW104160 (H 3.7 mm), Nepal, Bhaktapur. Photographs 2-5 by E. Gittenberger and 6 by Ms Sara Schnedl (©NHMW, Molluskensammlung). Scale bar = 1 mm.

from China, Yunnan, occurring ca. 1000 km east of Bhutan and dealt with by Davis et al. (1986), considering it unlikely that one of these species occurs also in Bhutan.

DNA data. — The DNA of one crushed specimen was sequenced, i.e. GiMaRIS isolate AG_6498, resulting in a 292 bp long partial sequence of COI (GenBank accession number OQ221738). The closest matches found through a Blast search on GenBank, i.e. both with 92.12 %, concern GenBank accession numbers KC832691.1, *Tricula hudiequanensis* Davis & Y.-H. Guo, 1986 and KC832688, *Hubendickia schuetti* (Brandt, 1968), both published in Liu et al. (2014).

Habitat. — The animals were collected in a temperate forest from damp mud along two tiny streams, 15 meters apart. The tiny spring freshwater is of pH ca. 6.5. The temperate forests consist of blue pine, few oak species, rhododendron species, populus and some bamboo species.

Notes. — Nearly all *Tricula* shells are heavily encrusted by a thick brown layer that cannot easily be removed without damaging the shell (Fig. 3). The slightly protruding parietal side cannot be a consequence, since this character state is also seen in a shell without this encrustation (Fig. 4).

Etymology. — The epithet *tashiae* refers to Dr. Tashi Yangzome Dorji, former Program Director of NBCB and current Director of Department of Livestock, for her support in spearheading the National Invertebrates Inventory project in Bhutan and her role in enhancing biodiversity conservation and sustainable utilisation in the country.

Family Amnicolidae Tryon, 1863

Genus *Erhaia* Davis & Y.-H. Kuo, 1985

Type species (by original designation): *Erhaia daliensis* Davis & Y.-H. Kuo, 1985.

Erhaia benjii spec. nov.

Figs 7-10

urn:lsid:zoobank.org:act:F8D9C531-CD8C-4869-A486-69517FAA99F1

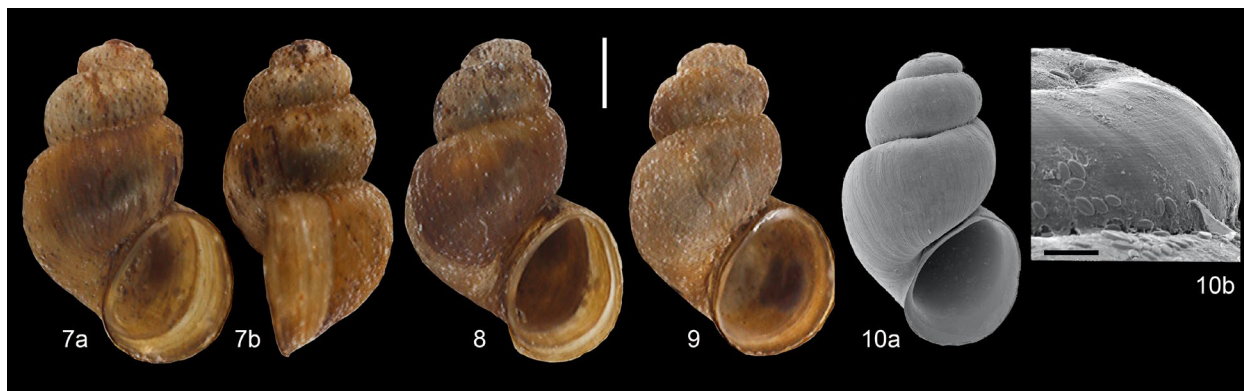
Type series. — Holotype (NBCB1417), paratypes (NBCB1418/9 + 6 in ethanol 70%; RMNH.MOL.176925/3), Bhutan, Thimphu Dzongkhag: [S121] temperate conifer forest between Royal Thimphu College and Serbithang, 27°24'41"N 89°39'35"E, 2583 m a.s.l., Choki Gyeltshen & Phuntsho Namgyel leg. 14.v.2022.

Description (n = 13). — Shell light brownish, elongated ovoid, with 3½-4 convex whorls, separated by a deeply incised suture. Spire relative high, subcylindrical. Protoconch with barely discernible spiral lirae (Fig. 10b). Teleoconch with dense, fine growth lines, alternating with some slightly more prominent lines. Aperture obliquely piriform with a rather broad parieto-palatal curvature, vertical in lateral view.

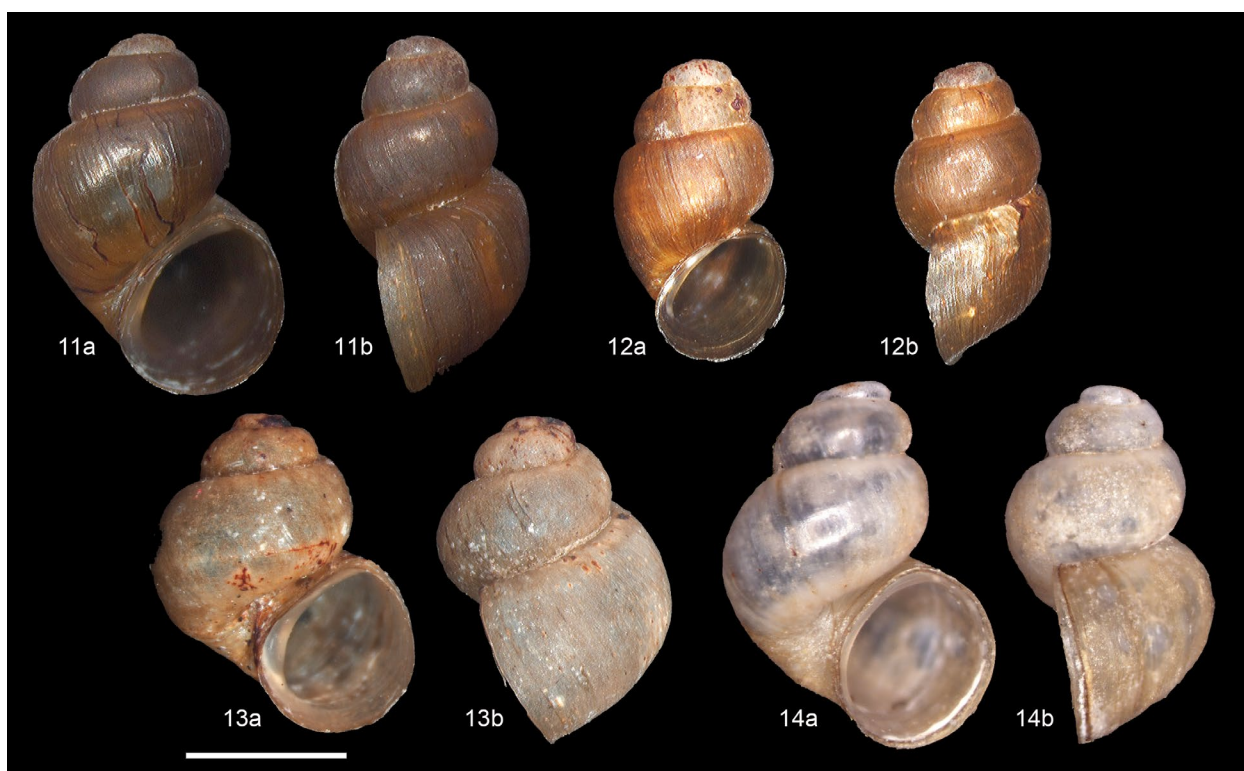
Measurements. — H 2.0-2.4 mm, B 1.2-1.4 mm.

Differentiation. — *Erhaia benjii* spec. nov. differs from the four *Erhaia* species already known from Bhutan most clearly by the relatively long, slender spire. Apart from that, *E. pelkiae* E. Gittenberger & Gyeltshen, 2020 (Fig. 12) has a more slender cylindrical shell and is smaller (H < 2 mm), *E. jannei* Gittenberger & Stelbrink, 2020 (Fig. 11) has a more globular last whorl, *E. wangchuki* E. Gittenberger, Sherub & Stelbrink, 2017 (Fig. 13) is depressed conical, with an oblique aperture in lateral view, and *E. norbui* E. Gittenberger, Gyeltshen & Stelbrink, 2022 (Fig. 14) differs by a roundish aperture.

DNA data. — The DNA of one crushed specimen was sequenced, i.e. GiMaRIS isolate AG_6474, resulting in a 313 bp long partial sequence of COI (GenBank accession number



Figs 7-10. *Erhaia benjii* spec. nov. **Fig. 7.** holotype NBCB1417 (H 2.3 mm); **Figs 8-10.** paratypes NBCB1418 (H 2.4 + 2.2 + 2.1 mm), temperate conifer forest between Royal Thimphu College and Serbithang. Photographs 7-9 by E. Gittenberger and 10 by Ms B.J. van Heuven (RMNH). Scale bar for 7-10a = 0.5 mm, for 10b = 0.05 mm.



Figs 11-14. *Erhaia* spec. **Fig. 11.** *E. jannei* E. Gittenberger & Stelbrink, 2020, holotype NBCB1057 (H 2.2 mm), Thimphu Dzongkhag, W of Geneykha. **Fig. 12.** *E. pelkiae* E. Gittenberger & Gyeltshen, 2020, holotype NBCB1059 (H 1.9 mm), Thimphu Dzongkhag, W of Geneykha. **Fig. 13.** *E. wangchuki* E. Gittenberger, Sherub & Stelbrink, 2017 (NBCB1013) (H 2.0 mm), type locality, Wangduephodrang Dzongkhag, Gangchhu. **Fig. 14.** *E. norbui* E. Gittenberger, Gyeltshen & Stelbrink, 2022, holotype NBCB1239 (H 2.3 mm), Haa Dzongkhag, Uesu, Naychu. Scale bar 1 mm. Photographs 11-13 by J. Goud (RMNH) and 14 by E. Gittenberger. Scale bar = 1 mm.

OQ221600). The closest match found through a Blast search on GenBank, i.e. with 98.08 %, concerns GenBank accession number MT237716.1, i.e. *Erhaia* spec. J (= *Erhaia jannei* E. Gittenberger & Stelbrink, 2020, in Gittenberger et al., 2020).

Etymology. — This species is named in honour of Dasho Paljor J. Dorji (popularly known as Dasho Benji) for his immense contribution to the environmental and biodi-

versity conservation. He is a pioneer environmentalist and conservationist in Bhutan.

Note. — None of the *Erhaia* shells is encrusted with a brown layer. Although *Tricula* and *Erhaia* were found together in the same micro-habitat, we cannot explain why the shells of *T. tashiae* spec. nov. are heavily encrusted, whereas those of *E. benjii* spec. nov. are not.

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