



## A NEW SPECIES OF *DAPHNIOLA* RADOMAN, 1973 (GASTROPODA: HYDROBIIDAE) FROM GREECE

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**ABSTRACT:** A new species: *Daphniola louisi* n. sp. from the spring at Kessariani, Athens, Greece, is described. This minute valvatoid-shelled snail is the second representative of the genus known so far. The shell, protoconch macro- and microsculpture, and radula are described and illustrated with SEM photographs. The other characters dealt with are: external morphology and soft part pigmentation, ctenidium and osphradium, penis, female reproductive organs. Differences between the newly described species and *Daphniola exigua* (A. Schmidt, 1856) are listed; phenetic relationships between the valvatoid-shelled Balkan hydrobiids are presented.

**KEY WORDS:** *Daphniola*, hydrobiids, taxonomy, Greece, morphology, anatomy

### INTRODUCTION

In March 1985, at Kessariani, Athens, Greece, the senior author collected a few hundred specimens of a minute valvatoid-shelled hydrobiid gastropod. The snails inhabited an artificial basin made of stone, collecting water from the spring at the Kessariani Monastery. They occurred together with *Planorbis planorbis* (Linnaeus, 1758), crawling on stones and filamentous green algae. The hydrobiids could not be assigned to any Balkan hydrobiid species known so far (RADOMAN 1973, 1983, SCHÜTT 1980, REISCHUTZ & SATTMANN

1993, BUTOT & WELTER-SCHULTES 1994). Most of all, they corresponded to the genus *Daphniola* Radoman, 1973, so far represented by a single species: *D. exigua* (A. Schmidt, 1856) (= *D. graeca* Radoman, 1973). The hydrobiid gastropod found at Kessariani was yet in some aspects different from the latter. The material was fixed in 4% formaldehyde and stored in 70% ethanol. The anatomical and SEM techniques applied were as described in FALNIOWSKI (1987, 1989a, 1990a).

### DESCRIPTION OF THE NEW SPECIES

#### *Daphniola louisi* new species

**Holotype and paratypes:** in the collection of the Zoological Museum, Jagiellonian University.

**Locus typicus:** Spring at Kessariani Monastery, Athens, Greece.

**Known distribution:** type locality only.

**Derivatio nominis:** the species is named after LOUIS J. M. BUTOT, an old friend of the senior author; his enthusiasm for the Greek malacofauna prompted the senior author to spend seven months in Greece.

**Diagnosis:** A representative of the genus *Daphniola* Radoman, 1973, characterized by a vestigial receptaculum seminis  $rs_2$ , a relatively small bursa copulatrix which does not extend posteriorly to the accessory gland complex, and a big and rather massive penis with a long filament and relatively small and blunt lateral outgrowth on the left side.

**Description:** Shell (Figs 1–4) minute, valvatoid, of about 3.5 whorls growing rapidly but regularly, divided by a moderately deep suture; spire blunt and



Figs 1–4. Shell of *Daphniola lousi*, scale bars 500  $\mu\text{m}$

broad, variable in height; umbilicus very broad, with the earlier whorls visible inside (Fig. 4); aperture oval, nearly circular, frequently with a weakly marked angle at the upper left side; peristome continuous, with a faint lip; teleoconch macrosculpture consists of faint growth lines (Figs 1–4); shell colour yellowish; the height/width proportion of the shell and the relative height of the spire varying continuously between the two extremes illustrated in Figures 1 and 2, independent of the snail age; size (mm; n = 11): shell height: 1.093–1.452, mean 1.286, SD 0.112; shell width: 1.167–1.691, mean 1.434, SD 0.151; aperture height: 0.591–0.976, mean 0.777, SD 0.095; aperture width: 0.595–0.786, mean 0.691, SD 0.056; spire height: 0.093–0.269, mean 0.170, SD 0.053; whorl number: 3.5 (occasionally 3.6); shell height/width proportion: 78.35–101.29%, mean 90.14%, SD 7.72%, correlation coefficient  $r = 0.6378$ ; protoconch (Figs 3 and 5–8) of 1.25–1.4 whorls growing slowly, the macrosculpture composed of characteristic, dense depressions, their shape irregular (Figs 5–7); the protoconch border marked by a well visible first growth line, but the protoconch-type sculpture extended as far as to the first ca.  $30^\circ$  ( $1/12$  of the whorl) of the teleoconch; under high magnifications (20,000  $\times$ ) very fine porelets irregularly scattered on the teleoconch (Fig. 8).

Radula (Figs 9–12): taenioglossate, typically hydrobiid; the cusps on the central, lateral and inner marginal teeth prominent, long and sharp; the central tooth trapezoid (Figs 9 and 11–12) with one pair of big basal cusps arising from the tooth face and numerous long cusps along the cutting edge, the basal tongue broadly V-shaped and about equal in length to the lateral margins; the central tooth formulae are:

$$\frac{(6)5-1-5(6)}{1-1} \text{ or } \frac{5-1-5}{1-1} \text{ or (rarely) } \frac{6-1-7}{1-1}$$

(figures in brackets: cusp numbers including not fully developed, rudimentary cusps). The lateral tooth (Figs 10–12) with long cusps, one of which may be distinctly longer than the adjacent ones (Figs 11–12) or the difference is inconspicuous (Fig. 10), the face of the tooth taller than wide; the lateral tooth formulae: 4–1–6 or 5–1–6; the inner marginal tooth (Figs 10–12) with 18–21 long and sharp cusps; the outer marginal tooth (Figs 10–12) with about 14 cusps smaller than those of the inner marginal tooth.

Pigmentation of soft parts (Figs 13–14) very slightly marked, limited to the delicate spots on the visceral sac which covers the digestive gland and gonad (Fig. 14); faecal pellets within the loop of the rectum well visible through the brightly yellowish,



Figs 5–12. *Daphniola lousi*. 5–6. Protoconch habitus (scale bars 50  $\mu\text{m}$ ). 7. Protoconch sculpture (scale bar 5  $\mu\text{m}$ ). 8. Teleoconch surface with very fine porelets (scale bar 1  $\mu\text{m}$ ). 9–12. Fragments of radulae (scale bars 5  $\mu\text{m}$ ): 9. Central teeth. 10. Lateral and marginal teeth 11–12. Central, lateral and marginal teeth



Figs 13–29. *Daphniola lousi*, soft part morphology and anatomy, scale bar 1 mm. 13–14. Male specimen without shell, visible operculum, contracted foot, mantle, unpigmented head with big eyes, penis, prostate, stomach, rectum with faecal pellets, weakly pigmented visceral sac, ctenidium and osphradium. 15. Head of male, with penis. 16. Head of female. 17. Ctenidium and osphradium. 18–25. Penis. 26–29. Renal and pallial section of female reproductive organs, visible gland complex, loop of oviduct, bursa copulatrix, receptaculum seminis and rectum

translucent body; head (Figs 15–16) unpigmented but with large eyes.

Ctenidium (Fig. 17) with short and broad lamellae, 12–16 (mean 14.6, SD 1.497,  $n = 5$ ) in the males and 14–16 (mean 14.8, SD 0.748,  $n = 5$ ) in the females. Osphradium (Fig. 17) lying opposite to the centre of ctenidium, elongate, three times longer than wide, somewhat irregular in shape, its length about 25% ctenidium length.

Male reproductive system (Figs 13–15 and 18–25): prostate rather big (Figs 13–14); penis (Figs 15 and 18–25) about 0.5 mm long (up to 0.7 mm when straightened), broadly triangular, characteristically bent, with a sharp, long and narrow filament and a

small and blunt lateral outgrowth on the left side (Figs 19, 21 and 23); vas deferens (penial duct) is seen through the filament running in thick, compact knobs all along the central portion of the penis.

Female reproductive system (Figs 26–30): oviduct forming a single, thickened U-shaped loop; two seminal receptacles present:  $rs_1$  small, elongate, irregular in shape, situated on the oviduct not far from the outlet of a long and narrow bursal duct,  $rs_2$  very small, almost vestigial (Fig. 30); bursa copulatrix big, bulky, oval (Figs 26 and 28) or irregular in shape (Figs 27 and 29), embedded in a short and wide albuminoid gland.

Fig. 30. *Daphniola lousi*, loop of oviduct with the vestigial  $rs_2$

## DISCUSSION

*Daphniola lousi* is the only hydrobiid inhabiting the spring at the Kessariani Monastery. It has been totally overlooked, despite the fact that the site has been well known since the Roman emperor Hadrian made use of the spring, to supply Athens with water. The shell proportions vary continuously within quite a wide range, which is reflected by the relatively low value of the correlation coefficient for the height and width of the shell. The shell variability is not correlated with any variation in soft part morphological/anatomical characters, thus all the specimens seem to belong to one species.

The characteristic macrosculpture of the protoconch does not represent any of the patterns portrayed in FALNIOWSKI (1989b, 1990a, b), FALNIOWSKI & SZAROWSKA (1991, 1995a, b) and FALNIOWSKI et al. (1996). It resembles the protoconch sculpture of “*Hauffenia kerschneri*” photographed by HAASE (1990), *Kerkia brezicensis* described by BODON & CIANFANELLI (1996), or “*Alzoniella manganellii*” (BODON et al. 1996). The very small pits visible at higher magnifications resemble the “extremely fine pores” (character 1, character state 1) of FALNIOWSKI & SZAROWSKA (1995c), found also in *Marstoniopsis* and *Dianella*.

Many of the radular character states found in *D. lousi* are accounted primitive among hydrobiids (HERSHLER & PONDER 1998). This concerns the central tooth (the trapezoidal shape, the couple of basal cusps, the V-shaped basal tongue), the lateral teeth

(their face taller than wide), the marginal teeth (cusps on the inner marginals longer than cusps on the outer marginals). The “normal” and big eyes and the weak pigmentation suggest that the snail is not a troglobiont.

*Daphniola lousi* differs from *D. exigua* (A. Schmidt, 1856) [= *D. graeca* Radoman, 1973] in several respects. The shell is flatter in *D. lousi*, but the shell variability in hydrobiids is so wide that no taxonomy can be constructed based entirely on shell characters (e.g. FALNIOWSKI 1987). The bursa copulatrix in *D. exigua* is much bigger and located completely behind the accessory gland complex, while in *D. lousi* it is smaller and situated typically on the ventral side of the gland complex; the bursa duct is shorter as well.  $rs_1$  is usually bigger in *D. lousi*, although the variability range overlaps that in *D. exigua*. On the other hand, the  $rs_2$ , well developed in *D. exigua*, in *D. lousi* is almost vestigial. The penes of the two species are also different: that of *D. lousi* is relatively bigger and more massive, more triangular in outline; its left-side lateral outgrowth is relatively smaller and blunter. The differences seem profound enough to prove the species distinctness of *D. lousi*.

The species is undoubtedly more similar to *D. exigua* than to any other known Balkan hydrobiid genus. However, the hydrobiid taxonomy at the generic level is still unclear, and the phylogenetic relationships between the hydrobiid genera are not yet established (KABAT & HERSHLER 1993). According to DNA



Fig. 31. Parsimony-based analysis of phenetic similarities (based on character states listed in Table 1) – majority rule (set at 50%) consensus tree (based on 185 trees of length 31), computed with PAUP and graphically presented by MACCLADE, per cent of the trees supporting a branch also given

Table 1. Character state distribution in the valvatoid-shelled Balkan and Italian hydrobiids

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>Horatia</i>	0	0	1	0	0	1	1	1	3	0	0	1	0	1
<i>Bracenicia</i>	1	0	1	0	1	1	1	2	3	0	0	1	0	1
<i>Prespolitorea</i>	0	0	1	0	1	1	1	0	3	0	0	1	2	3
<i>Ohridohauffenia</i>	0	0	1	0	0	1	1	1	2	0	0	1	1	1/2/3
<i>Ohrigocea</i>	0	0	1	0	0	1	1	1	3	0	0	1	0	3
<i>Pseudoislamia</i>	0	0	1	0	0	1	1	0	1	0	0	1	1	3
<i>Daphniola exigua</i>	0	0	1	0	0	1	1	1	4	0	0	1	0	1
<i>Daphniola lousi</i>	0	0	1	0	0	1	1	1	3	0	0	1	2	1
<i>Kerkia</i>	0	0	1	0	1	1	0	2	3	0	0	1	0	0
<i>Pseudohoratia</i>	2	0	1	0	1	1	1	2	1	0	0	1	0	3
<i>Zaumia</i>	0	0	1	0	0	0	1	0	2	0	0	0	0	0/3
<i>Hauffenia</i>	2	0	1	0	1	0	1	0	1	0	0	1	0	0
<i>Erythropomatiana</i>	0	0	1	0	1	0	1	0	1	0	0	1	3	0
<i>Islamia</i>	0	0	1	0	1	1	1	0	0	1	1	0	3	1/2/3
<i>Dabriana</i>	0	1	0	0	1	0	1	0	1	2	0	0	0	0
<i>Arganiella</i>	0	0	1	0	0	1	0	0	3	0	0	0	2	0

Characters and their states: 1 – outgrowth on the inner side of the operculum (0 – absent; 1 – present, not conspicuous; 2 – present, in a form of big spiral); 2 – caudal tentacle on the foot (0 – absent, 1 – present); 3 – basal cusps on the central tooth of the radula (0 – absent, 1 – present); 4 – caecal appendix on the oesophageal end of the stomach (0 – absent, 1 – present); 5 – pleuro-subintestinal connectives in the central nervous system (0 – long, 1 – short); 6 – receptaculum seminis located as  $rs_1$  of RADOMAN (0 – absent, 1 – present); 7 – receptaculum seminis located as  $rs_2$  of RADOMAN (0 – absent, 1 – present); 8 – loop of the oviduct (0 – narrow, approximately as broad as the oviduct along accessory gland complex; 1 – moderately thickened; 2 – thick); 9 – bursa copulatrix (0 – absent; 1 – small or very small, with hardly discernible duct; 2 – big, with well discernible duct; 3 – very big, with well discernible duct; 4 – extremely big, located completely behind the accessory gland); 10 – oviduct gland complex (0 – typical of nearly all hydrobiids, 1 – with strongly developed posterior part, 2 – divided into three parts); 11 – second arm of penis (0 – absent, 1 – present); 12 – one or more outgrowths on the left side of penis (0 – absent, 1 – present); 13 – shape of penis (0 – narrow and slender, sharply pointed; 1 – narrow and slender, blunt; 2 – broad and stout, sharply pointed; 3 – broad and stout, blunt); 14 – habitat (0 – subterranean, 1 – spring, 2 – river, 3 – lake); character 14 excluded from the analysis; character 8 ordered, all other characters unordered

Fig. 32. Parsimony-based analysis of phenetic similarities (based on character states listed in Table 1) – one of the MPRs, of length 31, computed with PAUP and graphically presented by MACCLADE, as a “phylogram” with branch lengths proportional to the amount of change (each bar denotes one unambiguous change)

molecular data, the Hydrobiidae are not monophyletic as a family (HERSHLER, WILKE, personal communication). Hence, although *D. lousi* could as well be included in some other genera, we have found it justified to consider the new species as belonging to the genus *Daphniola*. However, it has been impossible to trace the phylogenetic relationships of *Daphniola* as yet.

Phylogenetic analysis requires homologies to be established and the character evolution understood to the minutest degree. In hydrobiids, a number of details of the soft part anatomy and histology are called for. Very little is known in this respect when the Balkan hydrobiids are concerned. This has allowed us to apply nothing more than strictly phenetic analysis. Table 1 contains 14 characters used for such analysis, for *Daphniola lousi*, *D. exigua*, and 14 other valvatoid-shelled Balkan and Italian hydrobiids. We have not included *Pezzolia* because its anatomy is markedly different (BODON & GIUSTI 1986). It should be noted that this is the set of characters that served as a basis for the taxonomy of the Balkan hydrobiids. The data have been analyzed using the MACCLADE (MADDISON & MADDISON 1992) and PAUP (SWOFFORD 1991) packages. It must be stressed that, despite the analysis being

parsimony-based, the results are strictly phenetic, i.e. derived from nothing more than overall similarity, and as such they cannot be interpreted as a phylogeny reconstruction (FALNIOWSKI & SZAROWSKA 1995c).

All the characters except one (character 8) were regarded as unordered, and the same weight was assigned to each of them. The branch-and-bound technique resulted in 185 trees (MPRs), each 31 steps long. A majority-rule (set at 50%) consensus tree is presented in Figure 31, and one of the MPRs, constructed as a phylogram (MADDISON & MADDISON 1992), its branch lengths made proportional to the amount of evolution, is presented in Figure 32. As it can be seen, even this phenetic approach, based on characters that are commonly used in the hydrobiid taxonomy, has resulted in numerous unresolved polytomies, thus illustrating the weakness of the characters the taxonomy is based on. In fact, the structure of the data is not hierarchical, and any further taxonomy of the group may not be based on the characters used so far. However, the tree illustrates phenetic differentiation, and it is clearly seen that there are many genera whose overall similarity to *D. lousi* is no less pronounced than in the case of *D. exigua*.

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