A NEW SPRING SNAIL OF THE GENUS *GRAZIANA* (CAENOGASTROPODA: HYDROBIIDAE) FROM SWITZERLAND

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ABSTRACT

This paper describes a new species of hydrobiid spring snail, *Graziana quadrifoglio*, occurring in southern Switzerland and probably also in northern Italy. Populations of this species have been known since the middle of the twentieth century, but they were not recognized as belonging to a separate species. The description of the new species is based on detailed morphological and anatomical investigations, including electron microscopy and computer-aided reconstructions of histological serial sections. The presence of a single, distal seminal receptacle, the course of the bursal duct running between albumen gland and bursa copulatrix, the histology of the pallial oviduct, the shape of the penial lobe, together with the presence of the shield caecum arising from the posterior chamber of the stomach, characterize the new species as a member of *Graziana* Radoman, 1975. The wide, ovate-conical shell shape, the penial lobe pointing anteriorly and the complex coiling of the renal oviduct differentiate *G. quadrifoglio* from its congeners.

INTRODUCTION

In Switzerland, hydrobiid spring snails are, with few exceptions, restricted to the regions bordering France and Italy, and to areas that remained ice-free during the Pleistocene, totalling about 15% of the territory. Only the genus *Bythiospeum* Bourguignat, 1882 has a somewhat wider distribution, probably because its representatives mostly inhabit groundwaters. In addition, spring snails rarely occur above elevations of 1000 m (Turner, Kuiper, Thew, Bernasconi, Rüetschi, Wüthrich & Gosteli, 1998). These colline to montane heights are heavily influenced by human activities, such as agriculture, forestry, industry and housing development. The flows of many springs have been controlled during the last 100 years, especially so in northern Switzerland (Zollhöfer, 1997; M. Haase, personal observation) and thus the natural habitats of several groups of organisms, not only snails (cf. Illies, 1978), have been destroyed. Unfortunately, springs have received little recognition as sites deserving special attention by conservation biologists (Zollhöfer, 1997). Against this background of a dramatically declining number of suitable habitats a taxonomically accurate record of crenobiontic organisms should have high priority from the point of view of conservation biology.

Since the middle of the twentieth century, several populations of a hydrobiid spring snail allocated recently to *Graziana lacheineri* (Küster, 1853) and before that under the same (sub)specific name to a number of other genera (Toffoletto, 1960; Bernasconi, 1962, 1984; Turner *et al.*, 1998), have been reported in the canton of Ticino in southern Switzerland. A first anatomical description was provided by Bernasconi (1984). However, this species has the centre of its distribution in the foothills of the southeastern Alps. The suspicion (Turner *et al.*, 1998) that the Swiss populations might belong to either another, already known species with a more western distribution than *G. lacheineri* (cf. Haase, 1994), or even represent a separate species, has now been confirmed. This paper introduces and describes in morphological and anatomical detail the new species *G. quadrifoglio*.

MATERIAL AND METHODS

At two localities (see below) snails were picked from the substratum of stones and leaves, and immediately fixed in 70% ethanol on 14 October 2001. Shell measurements were taken under a dissecting microscope equipped with an ocular micrometer. The number of whorls was estimated to the nearest eighth of a whorl. Morphometric comparisons with other species (data taken from Haase, 1994) by principal component analysis (PCA) were based on the means of five shell parameters (shell height and width, aperture height and width, shell height/shell width). Unfortunately, data on species described by other authors were not appropriate for this comparison. The PCA started from a correlation matrix and was performed by the programme SYN-TAX 5.2 (Podani, 1993). With the same programme, a minimum-spanning tree based on Euclidean distances computed from *z*-standardized means of the five characters was calculated. Twelve specimens from the type locality were decalcified in Bouin's solution. Three males and three females were embedded in paraplast, serially sectioned at 7 μm and stained with Heidenhain's azan in order to reconstruct the anatomy. The reconstructions of one male and one female were made with the programme SURFdriver 3.5.2 (Moody & Lozanoff, 1997). The remaining six individuals were dissected. The radulae of three females were dissected and, as well as four shells, cleaned in 5% sodium hypochlorite. These hard parts and the critical point dried cephalopodia of three males, from which the mantle had been removed, were sputter-coated with gold and examined with a Philips ESEM XL30 (Environmental Scanning Electron Microscope). Whenever appropriate the terminology of Hershler & Ponder (1998) was used for morphological characters and their states. The material was deposited in the following institutions: Naturhistorisches Museum Basel (NMB), Naturhistorisches Museum Wien (NHMW) and Zoologische Staatssammlung München (ZSM).

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SYSTEMATIC DESCRIPTION

Family Hyrobiidae Troschel, 1857
***Graziana* Radoman, 1975**

Type species: Paludina lacheineri Küster, 1853

Diagnosis: Shell small, conical to pupiform with moderately convex whorls. Protoconch pitted, with or without spiral ridges. Corneous operculum paucispiral with excentric nucleus. Stomach with shield caecum. Testis a lobate sac. Penis with muscular lobe on left side. Ovary a simple sac. Renal oviduct with a single, distal seminal receptacle lying below or behind bursa copulatrix. Bursal duct running between albumen gland and bursa. Pallial oviduct consisting of bipartite capsule gland with small, anterior, non-staining part and large posterior portion and albumen gland.

Graziana quadrifoglio new species
(Figures 1–9)

Bythinella lacheineri—Toffoletto, 1960: 107 (not Küster, 1853).
Microna saxatilis lacheineri—Boeters, 1970: 123 (not Küster, 1853).
Graziana lacheineri—Turner *et al.*, 1998: 82 (not Küster, 1853).
Bythinella lacheineri minutissima—Bernasconi, 1962: 74 (not Küster, 1853).
Belgrandiella saxatilis—Giusti & Pezzoli, 1980: 33, fig. 13 (not Reyniés, 1844).

Types: Holotype NMB 11482a (Fig. 1A). Paratypes: NMB 11482b (nine specimens), NHMW Moll 101.430 (five specimens), ZSM 20013711/1–20013711/6 (six series of histological sections), all from the type locality.

Type locality: Spring emerging below the Grotta del Mago di Cantone, a small cave, which was the legendary refuge of a bandit in the seventeenth century, near Riva San Vitale, Ticino, Switzerland, 45° 53.2′ N, 8° 58.3′ E, 330 m above sea level (asl) (Fig. 8). Additional material: small spring near Spinirolo, *c.* 1 km northwest of Meride, Ticino, Switzerland, 45° 53.9′ N, 8° 56.4′ E, 620 m asl, NMB 11482c (eight specimens).

Etymology: Quadrifoglio, used as noun in apposition, is Italian and means four-leafed clover. It is also the name of a vocal quartet based in Basle. The new species is named after this quartet, and dedicated to Maja Liebendörfer (soprano), Maya Amrein (alto) and Stephan Götzö (tenor), with whom I (bass) had the pleasure of founding this ensemble.

Diagnosis: *G. quadrifoglio*, new species is characterized by the following combination of character states: proportion of shell height/shell width lowest among known *Graziana* species; protoconch only weakly pitted; lobe on the left side of penis hook-shaped pointing anteriorly; renal oviduct making two loops each comprising 270° in opposite directions.

Shell (Figs 1, 2): Colourless shell ovate-conical with up to 3.88 whorls, of which protoconch is 0.75 whorl. Measurements are given in Table 1. Protoconch only weakly pitted. Teleoconch has convex whorls and no special sculpture. Peristome simple and outer lip with shallow adapical sinus. Umbilicus narrow.

Operculum: Corneous, dark red to brown, elongate-ellipsoidal, paucispiral, with submarginal nucleus.

Radula (Fig. 3): Radula forms a U-shaped loop behind buccal mass and enters buccal mass ventrally. Radular bolsters contain black pigment. Central tooth trapezoidal, with the basal tongue as long as lateral margins and broadly V-shaped with curved edges; lateral margins give rise to a prominent pair of basal

Table 1. Shell morphometry of *G. quadrifoglio* new species. Measurements are in mm.

		sh	sw	ah	aw	sh/sw	w
Grotta del Mago	Holotype	1.60	0.98	0.64	0.64	1.63	3.63
$n = 14$	Min	1.38	0.86	0.55	0.56	1.49	3.50
	Max	1.60	0.98	0.64	0.64	1.70	3.88
	x	1.50	0.93	0.60	0.59	1.62	3.65
	SD	0.07	0.03	0.02	0.02	0.06	0.12
	CV	4.85	3.55	3.84	3.66	3.53	3.40
Spinirolo	Min	1.44	0.90	0.55	0.54	1.60	3.75
$n = 3$	Max	1.56	0.94	0.65	0.56	1.66	4.00
	x	1.49	0.92	0.59	0.55	1.62	3.92
	SD	0.06	0.02	0.05	0.01	0.03	0.14
	CV	4.68	2.46	9.72	1.97	2.22	4.00

Abbreviations: ah, aperture height; aw, aperture width; CV, coefficient of variation corrected for sample size (Sokal & Rohlf, 1995); Max, maximum; Min, minimum; *n*, number of specimens; SD, standard deviation; sh, shell height; sw, shell width; w, number of whorls; *x*, mean.

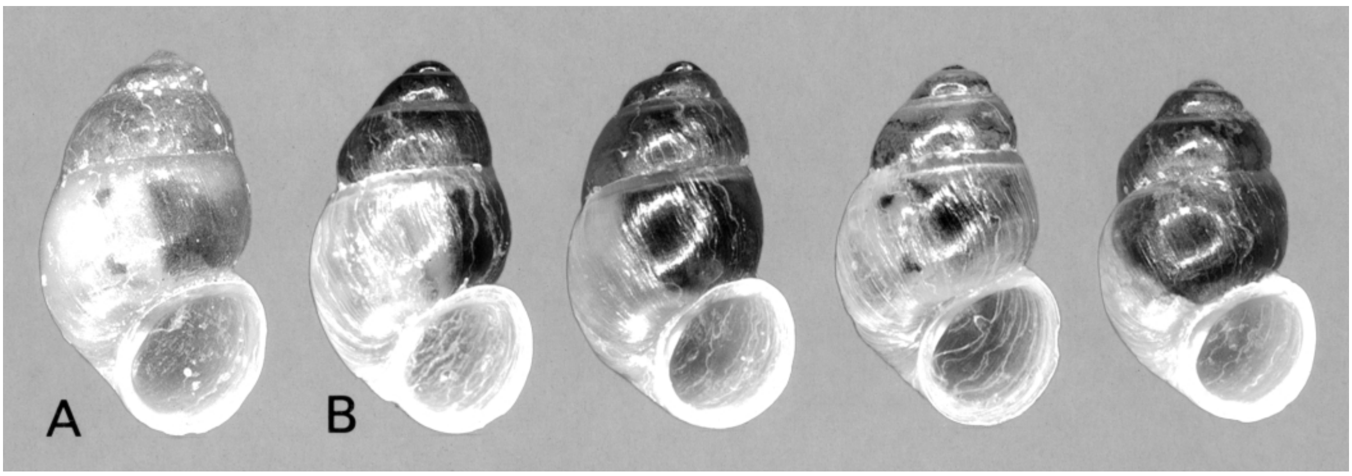

Figure 1. Shells. **A.** Holotype of *Graziana quadrifoglio* new species. **B.** Four paratypes. The paratypes were cleaned in sodium hypochlorite. Note that the pigmentation of the animals is visible through the shells. The holotype is 1.60 mm high.

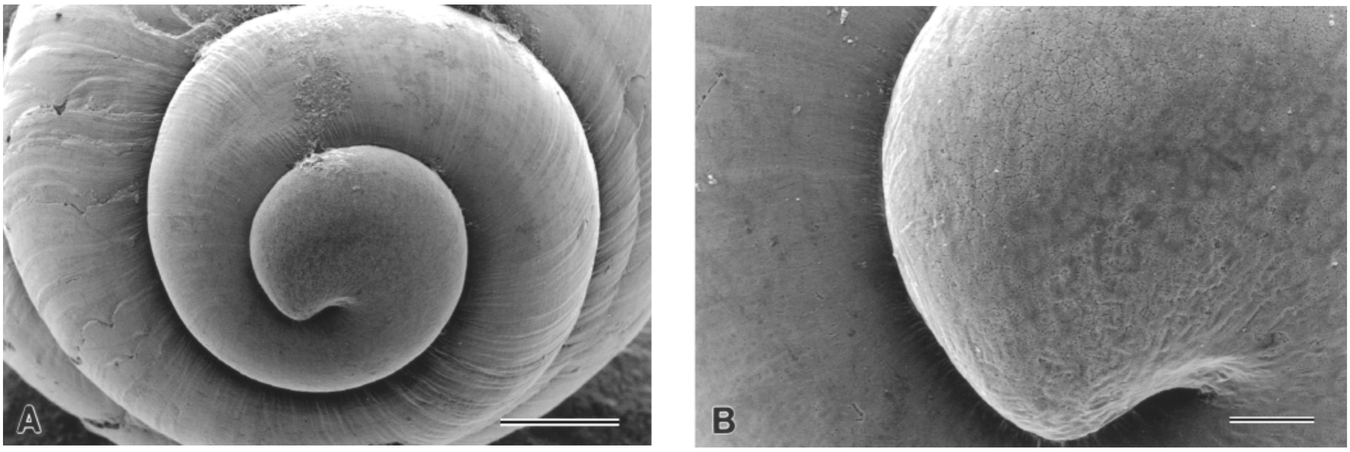

Figure 2. Apical view of shell. **A.** Protoconch. **B.** Structure of protoconch. Scale bars: **A** = 100 μm; **B** = 20 μm.

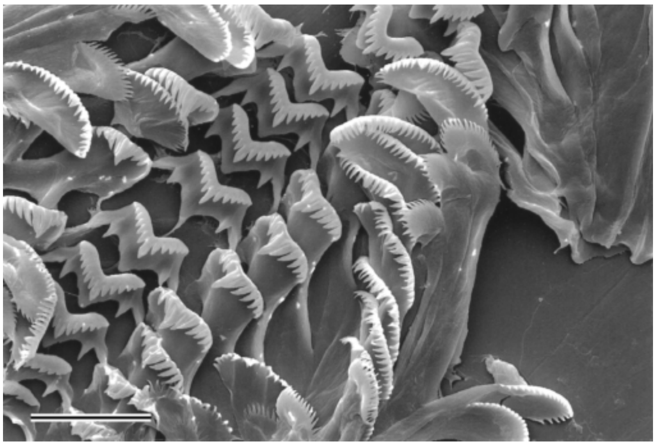

Figure 3. Radula. Scale bar = 10 μm.

Table 2. Principal component analysis based on a matrix of correlations of five shell parameters

	PC1	PC2	PC3	PC4	PC5
E	3.4060	1.2130	0.3464	0.0339	0.0006
v	68.12	24.26	6.93	0.68	0.01
cuv	68.12	92.38	99.31	99.99	100
	l	l	l	l	l
sh	0.5182	0.3831	0.5329	0.4763	0.2717
sw	–0.0952	–0.6288	0.0112	0.1669	0.7534
ah	–0.4602	–0.2275	0.1658	0.7338	–0.4130
aw	–0.1579	–0.2630	0.8238	–0.4520	–0.1516
sh/sw	–0.6969	0.5805	0.0986	–0.0508	0.4062

For abbreviations see Table 1: cuv, cumulative variance in %; E, Eigenvalue; l, loads of parameters in Eigenvectors; PC, principal component; v, variance in %.

cusps. Face of lateral teeth taller than wide, with a conspicuous basal projection. Lateral wing of the lateral teeth much longer than cutting edge. Denticles of inner marginal teeth somewhat larger than those on outer marginal teeth. Radular formula, R: 5–7 1 5–7/1 1, L: 3–4 1 6, M1: 26–30, M2: 20–25.

Non-genital anatomy: Epidermis of head, foot and mantle collar colourless (Fig. 1). 'Head' pigmentation stems from black granules in pharynx, radular bolsters and connective tissue in the base of the tentacles, where there are large, greyish spots in front of the black eyes. Black granules also present in connective tissue under operculum. Behind collar the mantle is black. On visceral sac, the uniform pigmentation fades to grey. Whether spots lying over the stomach and web-like pigmentation further proximal are in epidermis or connective tissue could not be determined unambiguously. Intensity of pigmentation varies among specimens.

Snout approximately as long as wide, distally slightly lobate. Mantle collar and tentacles simple without special ciliation. Ctenidium of 7–13 filaments (7–10 in males, 9–13 in females, $n = 3$ of each sex) and extending through more than 75% of mantle cavity. Osphradium ellipsoid and more than twice as long as wide, lying somewhat behind the middle of ctenidium.

A shield caecum (Fig. 4) arises from proximal end of stomach, protruding between large, distal lobes of digestive gland. Proximally, digestive gland is a simple sac or tube. Intestine coiling around style sac and then bending towards mantle cavity, where it runs slightly detached from prostate in males (Fig. 5) or closely against pallial oviduct in females. Anus does not reach mantle edge.

Male reproductive system (Figs 4–6): Testis a peripherally lobate sac comprising more than one whorl, but not reaching stomach. Sperm, which are stored in coiling seminal vesicle, enter prostate at its proximal end through vas deferens. Muscular, but not very thick, ejaculatory duct leaves prostate at distal end. Penis with blunt, hook-shaped lobe on left side pointing anteriorly. Black spot formed by granules in connective tissue extends over slightly widened part of penis distal to lobe.

Female reproductive system (Fig. 7): Ovary a simple sac consisting of somewhat less than one whorl and distally almost reaching stomach. Renal oviduct makes two loops of 270° in opposite directions before entering ventral channel of pallial oviduct. Seminal receptacle originating before this junction and extending behind globular bursa copulatrix. Bursal duct branches off at above junction, coiling on ventral side of bursa. It runs between albumen gland and bursa before entering this gametolytic gland. Pallial oviduct consisting of bipartite capsule gland with small, anterior, non-staining part and large posterior portion appearing dark blue in azan-stained sections, and albumen gland staining light blue.

Morphometric comparison: The PCA yielded five principal components with positive Eigenvalues. The first three components (PC) explain 99.31% of the total variance (Table 2), so that the three-dimensional ordination in Figure 9 represents the fivedimensional morphospace accurately. The shape parameter shell height/shell width has the highest load on PC1 followed by shell height and aperture height. PC2 represents mainly shell width and, again, the shell shape variable, but also the remaining three variables contribute considerably. Aperture width and shell height dominate PC3 (Table 2). PCA and the superimposed minimum-spanning tree (Fig. 9) clearly show that *G. quadrifoglio* has a morphologically exceptional position among its congeners.

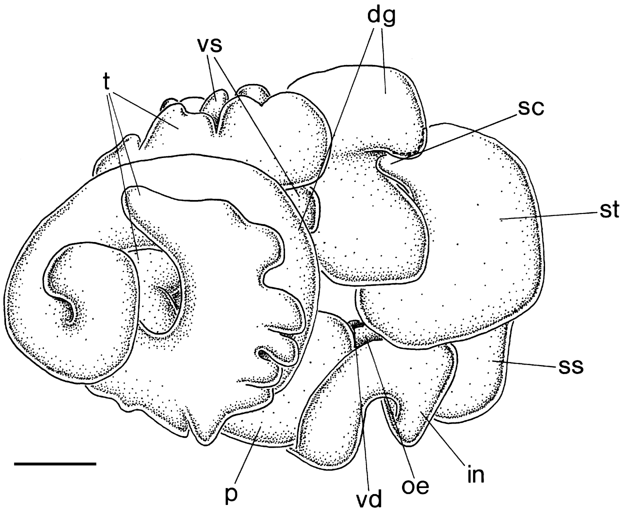

Figure 4. Visceral sac of male. Abbreviations: dg, digestive gland; in, intestine; oe, oesophagus; p, prostate; sc, shield caecum; ss, style sac; st, stomach, t, testis; vd, vas deferens; vs, seminal vesicle. Scale bar = 100 μm.

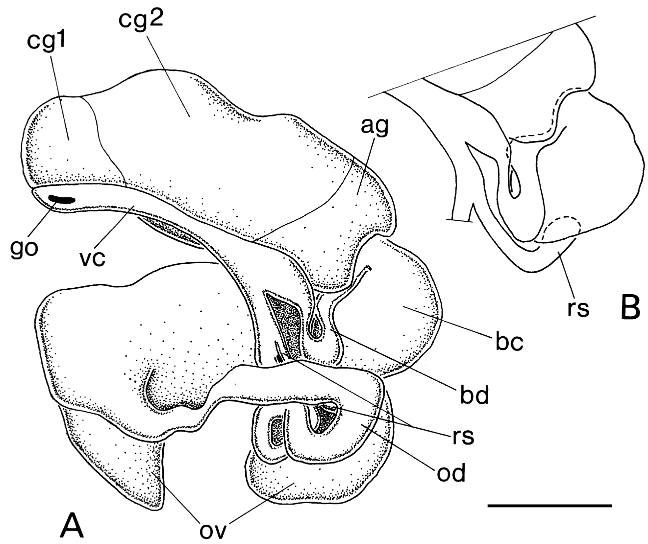

Figure 7. Female genital system. **A.** Entire system. **B.** Detail of distal genitalia showing bursa copulatrix and receptaculum seminis. Abbreviations: ag, albumen gland; bc, bursa copulatrix; bd, bursal duct; cg, capsule gland; go, genital opening; od, oviduct; ov, ovary; rs, seminal receptacle; vc, ventral channel. Scale bar = 100 μm.

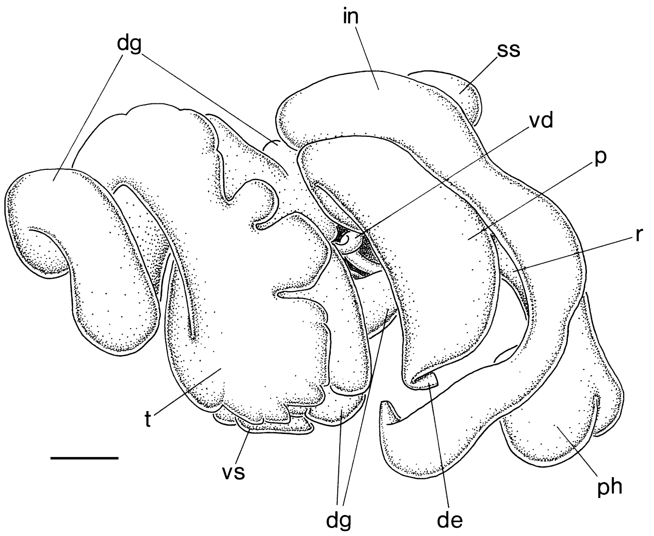

Figure 5. Digestive and genital systems of male (except salivary glands and penis, ejaculatory duct in part). Abbreviations: de, ejaculatory duct; dg, digestive gland; in, intestine; p, prostate; ph, pharynx; r, radula sheath; ss, style sac; t, testis; vd, vas deferens; vs, seminal vesicle. Scale bar = 100 μm.

Figure 8. Type locality. Spring (arrow) emerging beneath Grotta del Mago di Cantone (arrowhead).

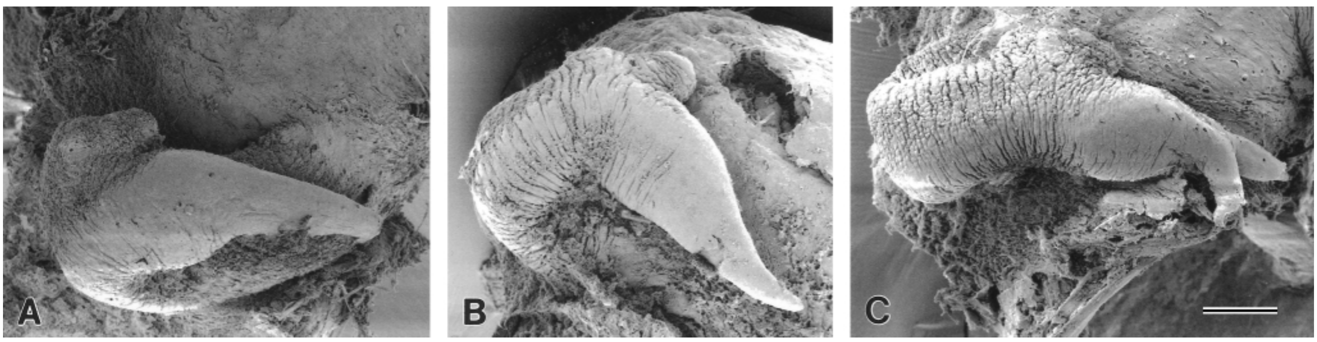

Figure 6. Penes. **A**, **B**. Normal. **C**. Teratological penis with doubled tip. Scale bar = 50 μm.

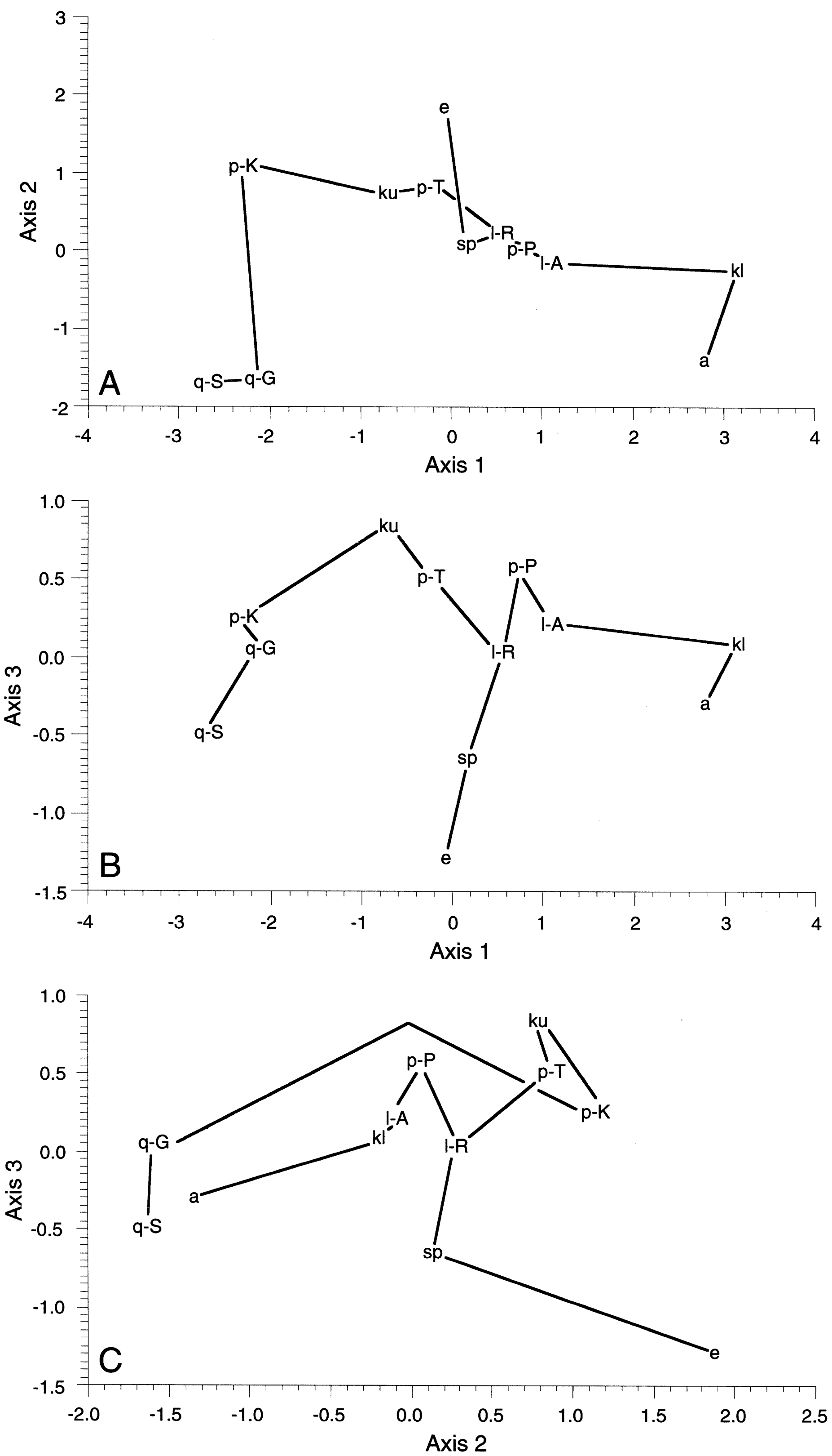

Figure 9. Principal component analysis of 12 populations belonging to seven species of *Graziana* based on a correlation matrix of five shell characters. Three-dimensional ordination with minimum spanning tree based on Euclidean distances computed from z-standardized means superimposed. For graphical clarity, the edge connecting p–K with q–G was drawn around the 'central' populations in C; the vertex does not represent a population. Abbreviations: a, *G. alpestris* (Frauenfeld, 1863); e, *G. exilis* (Paulucci, 1880); kl, *G. klagenfurtensis* Haase, 1994; ku, *G. kuesteri* (Boeters, 1970) [synonym of *G. pupula* (Westerlund, 1886), see Haase (1994)]; l–A, *G. lacheineri* from Andritz-Ursprung; l–R, *G. lacheineri* from Rabenstein; p–P, *G. pupula* from Pipan; p–T, *G. pupula* from Trögern; q–G, *G. quadrifoglio* new species from Grotta del Mago di Cantone; q–S, *G. quadrifoglio* new species from Spinirolo; sp, *Graziana* sp. from San Vigilio.

DISCUSSION

The presence of a single, distal elongate seminal receptacle, the bursal duct running between albumen gland and bursa copulatrix, the histology of the pallial oviduct with a non-staining anterior capsule gland, the shape of the penial lobe, together with the presence of the shield caecum arising from the posterior chamber of the stomach indicate the position of the new species within the genus *Graziana* Radoman, 1975 (Haase, 1994). The shield caecum has up to now only been found in this genus and thus is a defining apomorphy. The wide, ovate-conical shell shape, the penial lobe pointing anteriorly and the complex coiling of the renal oviduct differentiate *G. quadrifoglio* new species from its congeners. The anatomical account given by Bernasconi (1984) for species now classified in *Graziana* is largely in accordance with the present description; the differences are probably due to the more detailed examination carried out in the present study. Interestingly, from a biogeographic point of view, it shares two character states with *Graziana* sp. from San Vigilio on the eastern bank of the Lago di Garda, which is the geographically closest species so far described anatomically: the long duct of the seminal receptacle reaching behind the bursa copulatrix and the coil of the bursal duct on the ventral side of the bursa (Haase, 1994).

Whether all Swiss populations ascribed to *G. lacheineri* (cf. Turner *et al.*, 1998, and literature cited therein) really belong to the new species needs to be verified through shell measurements and anatomical investigations. However, it appears likely that *G. quadrifoglio* occurs throughout the southern Ticino and probably also in northwestern Italy. *G. quadrifoglio* is the westernmost *Graziana* known so far. The allocation of two species from the French Alpes Maritimes to *Graziana* based primarily on shell morphology is doubtful (Boeters, 2000, 2001). The genus thus occurs along the southern Alps from southern Switzerland, and northwestern Italy to Slovenia, Croatia and Austria in the east (Radoman, 1983; Haase, 1994; Fischer & Reischütz, 1995). How far it reaches south onto the Balkan peninsula needs to be clarified.

Hydrobiids were represented by 330 entries in the 'Animals of the World Database' of endangered species (UNEP-WCMC, 2001). The main source of threat for spring snails is certainly the vulnerability of their habitats. An evaluation of the threat to *G. quadrifoglio* would be premature considering our lack of knowledge of the actual distribution of the species (see above). At present, the new species might be categorized as 'data deficient' (IUCN, 1994).

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REFERENCES

BERNASCONI, R. 1962. Über einige für die Schweiz neue oder seltene höhlenbewohnende Insekten, Crustaceen und Mollusken. *Revue Suisse de Zoologie*, **69**: 67–76.

BERNASCONI, R. 1984. Hydrobiiden aus dem Tessin: *Belgrandiella saxatilis* und *Bythinella schmidti* (Gastropoda Prosobranchia Hydrobioidea). *Akten des 7. Nationalen Kongresses für Höhlenforschung, Schweizerische Gesellschaft für Höhlenforschung, Schwyz, 24-26.* September 1982: 35–48.

BOETERS, H.D. 1970. Die Gattung *Microna* Clessin, 1890 (Prosobranchia, Hydrobiidae). *Archiv für Molluskenkunde*, **100**: 113–145.

BOETERS, H.D. 2000. Unknown West European Prosobranchia, 11. Three new species of Hydrobiidae from France. *Heldia*, **3**: 1–5.

BOETERS, H.D. 2001. A contribution to the knowledge of *Alzoniella* Giusti & Bodon 1984 in France. Unknown West European Prosobranchia, 13. *Archiv für Molluskenkunde*, **129**: 149–156.

FISCHER, W. & REISCHÜTZ, P.L. 1995. Zwei neue Taxa von Quellschnecken aus den Gattungen *Graziana* Radoman 1975 und *Belgrandiella* A. J. Wagner 1927 (Gastropoda: Prosobranchia: Hydrobiidae). *Nachrichtenblatt der Ersten Vorarlberger Malakologischen Gesellschaft*, **3**: 3–7.

GIUSTI, F. & PEZZOLI, E. 1980. Gasteropodi, 2 (Gastropoda: Prosobranchia: Hydrobioidea, Pyrguloidea). In: *Collana del progetto finalizzato 'Promozione della qualità dell'ambiente', AQ/1/47. Guide per il riconoscimento delle specie animali delle acque interne italiane*, **8**: 1–67. Consiglio Nazionale delle Ricerche, Verona.

HAASE, M. 1994. Differentiation of selected species of *Belgrandiella* and the redefined genus *Graziana* (Gastropoda: Hydrobiidae). *Zoological Journal of the Linnean Society*, **111**: 219–246.

HERSHLER R. & PONDER, W.F. 1998. A review of morphological characters of hydrobioid snails. *Smithsonian contributions to Zoology*, **600**: 1–55.

ILLIES, J. 1978. *Limnofauna Europaea: eine Zusammenstellung aller die europäischen Binnengewässer bewohnenden mehrzelligen Tierarten mit Angaben über ihre Verbreitung und Ökologie.* Gustav Fischer, Stuttgart.

IUCN 1994. *IUCN Red List Categories.* IUCN, Gland.

KÜSTER, H.C. 1852–1853. Die Gattungen *Paludina*, *Hydrocena* und *Valvata*. In: Systematisches Conchylien Cabinet von Martini & Chemnitz (H. C. Küster, ed.), I (21): 1–56 (1852), 57–96 (1853). Bauer & Raspe, Nürnberg.

MOODY, D. & LOZANOFF, S. 1997. *SURFdriver version 3.5.2.* SURFdriver Software, Kailua.

PODANI, J. 1993. *SYN-TAX Version 5.1. Computer Programs for Multivariate Data Analysis in Ecology and Systematics.* Science Publishing, Budapest.

RADOMAN, P. 1983. *Hydrobioidea, a Superfamily of Prosobranchia (Gastropoda), I. Sistematics.* Serbian Academy of Sciences and Arts, Monographs 547. Department of Sciences 57, Belgrade.

REYNIÉS, P. DE 1844. Lettre à M. Moquin-Tandon, Président de l'Académie Royale des Sciences, Inscriptions et Belles-Lettres de Toulouse, sur quelques mollusques terrestres et fluviatiles. Toulouse.

SOKAL, R.R. & ROHLF, F.J. 1995. *Biometry. The Principles and Practice of Statistics in Biological Research*, Edn 3. Freeman, New York.

TOFFOLETTO, F. 1960. La *Bythinella lacheineri* (Charp.) in alcune sorgenti della provincia di Como. *Natura*, **51**: 106–110.

TURNER, H., KUIPER, J.G.J., THEW, N., BERNASCONI, R., RÜETSCHI, J., WÜTHRICH, M. & GOSTELI, M. 1998. *Fauna Helvetica 2. Atlas der Mollusken der Schweiz und Liechtensteins.* Centre Suisse de Cartographie de la Faune & Schweizerische Entomologische Gesellschaft, Neuchâtel.

UNEP-WCMC 2001. *Animals of the World Database.* Available at: http://www.unep-wcmc.org/species/animals/animal_redlist.html.

ZOLLHÖFER, J.M. 1997. *Quellen, die unbekannten Biotope: erfassen, bewerten, schützen. Bristol-Schriftenreihe* **6**. Bristol-Stiftung, Zürich.