

Research article

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Arganiella Giusti & Pezzoli, 1980 (Caenogastropoda: Truncatelloidea: Hydrobiidae): a widespread genus or several narrow-range endemic genera?

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Abstract. Most valvatiform genera of the gastropod family Hydrobiidae are narrow-range taxa. One exception is the genus *Arganiella*, which is comprised of three congeners: the type species *A. pescei* from the Apennine Peninsula, *A. wolfi* from the Iberian Peninsula and *A. tabanensis* from the Balkans. The genus assignment of the latter two species was based on morphological similarities with *A. pescei* in the shell, operculum, radula and genitalia. Given that the morphology of hydrobiids is sometimes susceptible to convergence, this study re-evaluates the taxonomic status of species of *Arganiella* by analysing mitochondrial (mtCOI) and nuclear (18S rRNA) sequences of topotypes or near topotypes to infer their phylogenetic position. Our phylogenetic analyses depicted *Arganiella* as a non-monophyletic group within Hydrobiidae, and sequence divergence among the three species ranged from 14.5 to 16.7% for mtCOI and 2.0 to 3.8% for 18S. We also re-examined the extent of morphological variation among species of *Arganiella* and found a few differences among them and other valvatiform genera. Consequently, we propose two new genera for *A. wolfi* and *A. tabanensis*. Our results conflict with the classification of valvatiform hydrobiid species solely based on traditional phenotypical methods and suggest further taxonomic evaluation within a molecular framework.

Keywords. Springsnails, valvatiform hydrobiids, phylogeny, morphology, Mediterranean region.

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Introduction

The gastropod genus *Arganiella* Giusti & Pezzoli, 1980 (family Hydrobiidae Stimpson, 1865) comprises three narrow-range endemic species, each living on a different Mediterranean peninsula. These tiny springsnails (1.0–1.5 mm) have a depressed trochiform to valvatiform shell and simplified anatomical structures. The type species, *A. pescei* Giusti & Pezzoli, 1980, was described from several wells and springs located in the central-eastern Apennine Peninsula (Giusti & Pezzoli 1980). A proposed representative of the genus *Boetersiella* Arconada & Ramos, 2001, *B. wolfi* Boeters & Glöer, 2007, which is endemic to the southwestern Iberian Peninsula, was transferred to *Arganiella* by Arconada & Ramos (2007a, 2007b). Based on morphological and geographical evidence, these authors also synonymized the species *A. tartesica* Arconada & Ramos, 2007 with *A. wolfi* (Arconada & Ramos 2007b). The third species, *A. tabanensis* Boeters, Glöer & Pešić, 2014, was recently erected for a hydrobiid population living in a small rheocene spring in Montenegro, in the Balkan Peninsula (Boeters *et al.* 2014).

The assignment of *A. wolfi* and *A. tabanensis* to *Arganiella* was based on morphological similarity with the type species in shell, penis and female distal genital features (Arconada & Ramos 2007a, 2007b; Boeters *et al.* 2014). However, such characters are susceptible to convergent evolution in valvatiform hydrobiid genera (Bodon *et al.* 2001) and, therefore, the systematics of the known species of *Arganiella* needs to be re-evaluated through molecular analyses. To date, DNA sequence data are available only for *A. wolfi*, and phylogenetic relationships inferred from a multilocus dataset of valvatiform hydrobiid genera from the Iberian Peninsula resolved *A. wolfi* as the sister taxon of the Iberian genus *Iberhoratia* Arconada & Ramos, 2007 (Delicado *et al.* 2019). Sequence data from the other two species of *Arganiella* are needed to confidently assess the taxonomic composition of this wide-ranging genus.

As might be expected in taxa with poor dispersal capabilities, we hypothesize that phylogenetic analyses would depict species of *Arganiella* as three unrelated, narrow-range lineages rather than as a widely distributed monophyletic group. This result would challenge the genus assignment of these valvatiform hydrobiids, which was based on morphology, and limit the known geographic distribution of *Arganiella* to the Apennine Peninsula. To test these assumptions, we analyse new mitochondrial and nuclear DNA sequence data from the type species *A. pescei* and from *A. tabanensis* with previously published molecular datasets of valvatiform hydrobiids that also include sequences of *A. wolfi* (Delicado *et al.* 2019) in order to infer the phylogenetic relationships of the species of *Arganiella* and to quantify the degree of divergence among them. We also provide previously missing descriptions of the radular features of *A. tabanensis* for a morphological comparison. Finally, the taxonomic status of species of *Arganiella* is discussed in light of the evidence from the phylogenetic analyses and morphological comparisons of these taxa with other valvatiform hydrobiid genera recorded from the same Mediterranean regions.

Material and methods

We assessed the taxonomic status of the three recognized species of *Arganiella* using DNA sequence and morphological information from these and other valvatiform genera occurring in the Apennine, Balkan and Iberian peninsulas. One individual of *A. pescei* was used for the DNA assessment. This specimen was collected from Susanna Springs, in the region of the type locality (i.e., central-eastern Apennine Peninsula), and deposited in the collection of the University of Giessen Systematics and Biodiversity (UGSB) (Diehl *et al.* 2018) in Germany (UGSB 10365). Following an exhaustive morphological examination, Bodon *et al.* (2001) had assigned the hydrobiid populations living in these springs to *A. pescei*. Morphological characters from *A. pescei* were scored using the original description by Giusti & Pezzoli (1980) and the re-description by Bodon *et al.* (2001). For the Iberian *A. wolfi*, we used the morphological description by Arconada & Ramos (2007a) and Boeters & Glöer (2007), and the sequences of a topotype used by Delicado *et al.* (2019) for the molecular analyses. The shell morphology and anatomy of the Balkan *A. tabanensis* are as illustrated by Boeters *et al.* (2014). Additional data on

radular and opercular features of this species, as well as partial sequences of the studied DNA markers, were also collected for the present study. We collected ca 50 topotypes from the *A. tabanensis* type locality (Taban Spring, Montenegro; 42.52795° N, 19.21921° E) in 2015 and preserved them in 80% ethanol in the field. Shells and opercula were photographed using a Keyence VHX 2000 3D Digital Microscope. Six adults were dissected, and their buccal mass extracted, under an Olympus SZX12 stereo microscope. Radulae were extracted, cleaned and prepared as described by Delicado *et al.* (2016) for imaging on a field emission scanning electron microscope (FESEM) DSM982 Gemini (Carl Zeiss GmbH, Germany). The collected specimens were then deposited in the UGSB collection (UGSB 18847).

We isolated total DNA from one individual per species (for *A. pescei* and *A. tabanensis*) following the CTAB protocol performed by Wilke *et al.* (2006). Fragments of the mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear ribosomal 18S rRNA (18S) were amplified and sequenced using the primer pairs LCO1490 (Folmer *et al.* 1994) and COR722b (Davis *et al.* 1998) for COI and the universal primers for metazoan 18S (Holland *et al.* 1991). Amplification conditions for both gene fragments were as previously described by Delicado *et al.* (2012). The annealing temperature used was 52°C. Samples were sequenced in an ABI 3730 XL sequencer (Life Technologies, Carlsbad, CA, USA) using a BigDye Terminator Kit ver. 3.1 (Life Technologies). The new sequences were deposited in GenBank (Table 1).

We assessed the taxonomy of the three species of *Arganiella* by analysing the COI and 18S sequences of these species along with those of other European valvatiform and (closely related) non-valvatiform genera available from GenBank (Table 1). Forward and reverse sequences were aligned and edited in Sequencher ver. 5.4.6 (Gene Codes, Ann Arbor, MI). MEGA ver. 7.0.14 (Kumar *et al.* 2016) was used to assemble the gene-partition datasets and to calculate genetic distances (uncorrected *p*-distances). The COI dataset was manually aligned also using MEGA. The rRNA 18S fragment was aligned using MAFFT ver. 7.402 (Kato & Standley 2013), with default settings for gap penalties. According to the corrected Akaike's information criterion (AICc; Akaike 1974; Sugiura 1978; Hurvich & Tsai 1989), jModelTest ver. 2.1.7 (Darriba *et al.* 2012) selected TrN (Tamura & Nei 1993) + I (invariable sites) + G (rate variation among sites) and TrNef (Tamura-Nei model with equal base frequencies; Tamura & Nei 1993) + I + G models of nucleotide evolution for the COI and 18S datasets, respectively. We used DAMBE ver. 7 (Xia 2018) and the proportion of invariant sites ($P_{inv} = 0.47$) obtained in jModelTest to conduct the saturation test (Xia *et al.* 2003; Xia & Lemey 2009) on the COI partition. The observed saturation ($I_{ss} = 0.39$) was significantly lower than the critical values ($I_{ss,c} = 0.71$; $p < 0.001$), suggesting little saturation in our COI dataset.

Phylogenetic analyses based on maximum likelihood (ML) methods were conducted using the RAxML BlackBox web-server [<https://raxml-ng.vital-it.ch/#/>; Kozlov *et al.* 2019] with 10 random starting trees and the optimal substitution models for each gene partition selected in jModelTest. Bayesian inference (BI) analyses were run with mixed substitution models in MrBayes ver. 3.2.6 (Ronquist *et al.* 2012) for 5 million generations with a sampling frequency of 1000. After verifying convergence of the BI analysis (standard deviation of split frequencies < 0.01), the first 10% of generations were discarded as burn-in. Branch robustness was evaluated by rapid bootstrapping (BS) (Stamatakis *et al.* 2008) with an automatic cut-off for ML and by Bayesian posterior probability (BPP) for BI. Inferred topologies and branch supports were visualized in FigTree ver. 1.4.3 (Rambaut 2010).

For the morphological comparisons of species of *Arganiella* with other valvatiform hydrobiid genera from the Iberian, Apennine and Balkan peninsulas (Table 2), we scored morphological character states, including features of the shell, radula, operculum and anatomy, according to the information provided in the following studies: Arconada & Ramos (2001) for *Boetersiella* and *Chondrobasis* Arconada & Ramos, 2001; Girardi & Boeters (2012) for *Corbellaria* Girardi & Boeters, 2012; Delicado *et al.* (2019) for *Deganta* Arconada & Ramos in Delicado *et al.*, 2019; Arconada *et al.* (2007) for *Iberhoratia* Arconada & Ramos, 2007; Arconada & Ramos (2006) for *Josefus* Arconada & Ramos,

Table 1 (continued on next page). Taxon name, locality name, GenBank numbers and original references for sequences analysed in this study.

Taxon	Locality	GenBank # 18S	GenBank # COI	Original reference
<i>Agrafia wiktoria</i>	Agrafa Mountains, Sikiá, Evrytania, Greece (39.36861, 21.63139)	JF906758	JF906765	Szarowska & Falniowski (2011)
<i>Alzoniella finalina</i>	Spring at the Porra River, Molino, Liguria, Savona, Italy (44.219, 8.255)	AF367686	AF367650	Wilke <i>et al.</i> (2001)
<i>Arganiella pescei</i>	Santa Susanna Springs, Rivodutri, Latium, Italy (42.49513, 12.84604)	MW561453	MW553909	Present study
<i>Arganiella tabanensis</i>	Taban Spring, Montenegro (42.52795, 19.21921)	MW561454	MW553910	Present study
<i>Arganiella wolffi</i>	Virgen de los Ángeles hermitage, Sierra de Aracena, Huelva, Spain (37.8785, -6.66681)	MH348095	MH350192	Delicado <i>et al.</i> (2019)
<i>Avenionia brevis</i>	Spring of the fountain of St.-Victor-La Coste, Gard, France (44.057, 4.636)	AF367670	AF367638	Wilke <i>et al.</i> (2001)
<i>Belgrandia thermalis</i>	Thermal channel near S. Giuliano, Tuscany, Pisa, S. Giuliano Terme, Italy (43.751, 10.440)	AF367684	AF367648	Wilke <i>et al.</i> (2001)
<i>Belgrandiella kusceri</i>	Rakovski potok [Crab stream], near Rakovski Skocjan, Rakek, Slovenia (45.77750, 14.18556)	JX970574	KT218520	Wilke <i>et al.</i> (2013); Falniowski & Beran (2015)
<i>Boetersiella sturmi</i>	La Mata Spring, Mata Bejid, Jaén, Spain (37.69503, -3.50703)	MH348097	MH350200	Delicado <i>et al.</i> (2019)
<i>Bullaregia tunisiensis</i>	A spring in Djebba, Province Béja, Tunisia (36.47125, 9.09972)	MN575709	KX821683	Khalloufi <i>et al.</i> (2017, 2020)
<i>Chondrobasis levantina</i>	Caroche Spring, Teresa de Cofrentes, Valencia, Spain (39.09608, -0.91972)	MH348098	MH350203	Delicado <i>et al.</i> (2019)
<i>Corbellaria celtiberica</i>	Manubles River, Soria, Spain (41.60997, -1.95586)	MH348099	MH350207	Delicado <i>et al.</i> (2019)
<i>Daphniola graeca</i>	Daphne Spring, about 30 km north of Larissa, Greece (39.89111, 22.60722)	EF070624	EU047764	Szarowska (2006); Falniowski <i>et al.</i> (2007)
<i>Deganta azarum</i>	La Fontona Spring, Borondes, Asturias, Spain (43.33189, -6.01494)	MH348100	MH350208	Delicado <i>et al.</i> (2019)
<i>Fissuria boui</i>	Spring near La Prouveresse, Alpes Maritimes, France (43.64279, 6.88735)	AF367690	AF367654	Wilke <i>et al.</i> (2001)
<i>Graecoarganiella parnassiana</i>	Small spring south of Eptalofos, Parnassus mountains, Greece (38.59278, 22.5039)	JN202341	JN202348	Falniowski & Szarowska (2011a)
<i>Graziana alpestris</i>	Spring at the Porra River, Liguria, Savona, Molino, Italy (44.219, 8.255)	AF367673	AF367641	Wilke <i>et al.</i> (2001)
<i>Grossuana delphica</i>	Kastalia Spring at Delphi, Greece (38.48306, 22.50528)	EF061917	EF061922	Szarowska <i>et al.</i> (2007)
<i>Hauffenia tellinii</i>	Isonzo River near Sagrado Spring, Friuli-Venetia Julia, Gorizia, Italy (45.8743, 13.4856)	AF367672	AF367640	Wilke <i>et al.</i> (2001)
<i>Horatia klecakiana</i>	Studenci Spring, N of Kučići, in the valley of the Cetina River, Croatia (43.44481, 16.80708)	KJ159127	KJ159128	Szarowska & Falniowski (2014)
<i>Iberhoratia morenoi</i>	Prado del Rey Spring, Cádiz, Spain (36.66042, -5.45028)	MH348101	MH350210	Delicado <i>et al.</i> (2019)

Table 1 (continued).

Taxon	Locality	GenBank # 18S	GenBank # COI	Original reference
<i>Islamia piristoma</i>	Spring on right bank of Magra River near Romito, Liguria, La Spezia, Arcola, Italy (44.1042, 9.9337)	AF367671	AF367639	Wilke <i>et al.</i> (2001)
<i>Josefus aitanica</i>	Flores Spring, Requena, Valencia, Spain (39.48694, -1.12205)	MH348107	MH350231	Delicado <i>et al.</i> (2019)
<i>Kerkia kusceri</i>	Cave Krska jama, Krka, Ivančna Gorica, Slovenia (45.89, 14.77111)	KY087833	KY087867	Rysiewska <i>et al.</i> (2017)
<i>Mercuria similis</i>	La Puebla, Mallorca, Spain (39.79111, 3.10472)	AF212913	AF213346	Wilke <i>et al.</i> (2000)
<i>Milesiana schuelei</i>	Spring in Gaucin, Málaga, Spain (36.51878, -4.67703)	–	MH350247	Delicado <i>et al.</i> (2019)
	Spring in Berchul, Félix, Almería, Spain (36.87719, -2.66561)	MH348108	–	Delicado <i>et al.</i> (2019)
<i>Pauluccinella minima</i>	Lago di Piediluco, S. Egidio, Italy	JX970578	JX970612	Wilke <i>et al.</i> (2013)
<i>Sadleriana fluminensis</i>	Jadro River at Solin near Split, Croatia (43.5453, 16.48780)	AF367683	–	Wilke <i>et al.</i> (2001)
	Sava River, Slovenia (46.17344, 14.41505)	–	KF193083	Szarowska & Falniowski (2013)
<i>Spathogyna fezi</i>	Roble Spring, Yémeda, Cuenca, Spain (39.74281, -0.28383)	MH348109	MH350251	Delicado <i>et al.</i> (2019)
<i>Tarraconia gasulli</i>	La Esperanza Spring, Navajas, Castellón, Spain (39.86990, -0.50719)	MH348110	MH350254	Delicado <i>et al.</i> (2019)

2006 and *Milesiana* Arconada & Ramos, 2006; Quiñonero-Salgado & Rolán (2017) for shells of *Navalis* Quiñonero-Salgado & Rolán, 2017; Boeters *et al.* (2019) for *Salaeniella* Boeters, Quiñonero-Salgado & Ruiz-Cobo, 2019; Arconada & Ramos (2002) for *Spathogyna* Arconada & Ramos, 2002; Ramos *et al.* (2000) for *Tarraconia* Ramos & Arconada, 2000; Falniowski & Szarowska (2011a) for *Graecoarganiella* Falniowski & Szarowska, 2011; Radea *et al.* (2013) for *Isimerope* Radea & Parmakelis, 2013; Radea *et al.* (2016) for *Myrtoessa* Radea, 2016 and Bodon *et al.* (2001) for the remaining genera. Character and character states were coded following the terminology of Hershler & Ponder (1998).

Results

Molecular analyses

The data matrix constructed of COI (658 bp) and 18S (492 bp) sequences yielded an alignment with a total length of 1150 bp. Our ML and BI analyses generated similar tree topologies and branch supports (Fig. 1). In both inferences, *Arganiella* did not form a monophyletic group; instead, both *A. wolffi* and *A. tabanensis* were distantly related to the type species *A. pescei*. Also in both analyses, *A. wolffi* resolved as the sister taxon to the genus *Iberhoratia* from the Iberian Peninsula (BS = 100%, BPP = 1.00), and *A. tabanensis*, to the genus *Kerkia* Radoman, 1978 from the Balkan Peninsula and adjacent islands (BS = 91%, BPP = 0.95). The phylogenetic position of *A. pescei* was not well resolved in either of the phylogenetic analyses. Sequence divergence among the three species of *Arganiella* ranged from 14.5 to 16.7% for COI and 2.0 to 3.8% for 18S. Divergence among genera ranged from 9.1 to 22.5% for COI and 0 to 4.5% for 18S.

Overall, our results show that DNA sequence divergence values among the three species of *Arganiella* fall within the range of inter-generic variation and that both *A. wolffi* and *A. tabanensis* not only have a sister group relationship with a genus from their respective Mediterranean peninsulas (Fig. 1) but also

clear morphological differences with these sister genera, indicating the two species are representatives of distinct genera (Table 2). This evidence, altogether, demonstrates the need to revise the genus assignment of the non-type species *A. wolfi* and *A. tabanensis*.

Taxonomy

Phylum Mollusca Cuvier, 1795
 Class Gastropoda Cuvier, 1795
 Superorder Caenogastropoda Cox, 1960
 Superfamily Truncatelloidea Gray, 1840
 Family Hydrobiidae Stimpson, 1865

Genus *Aretiana* Delicado & Ramos gen. nov.

urn:lsid:zoobank.org:act:7B207B6D-47E8-4211-8139-0CF98DA353D7

Type species

Boetersiella wolfi Boeters & Glöer, 2007.

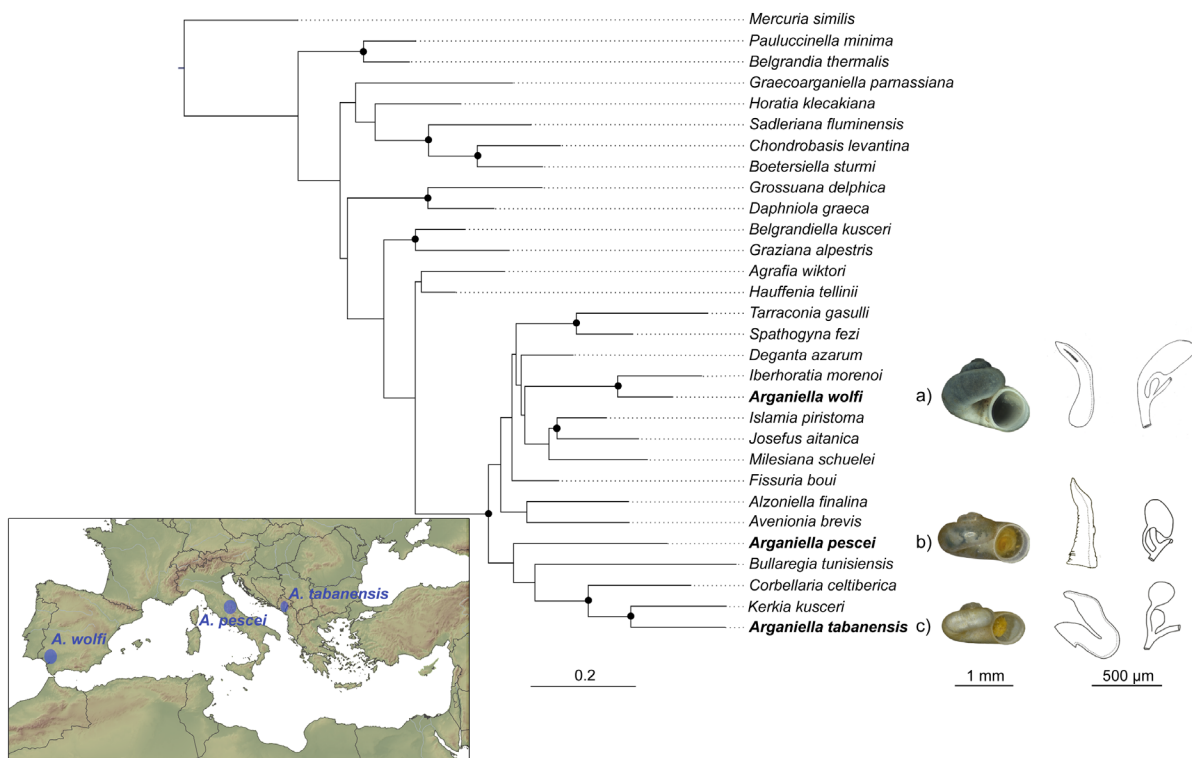


Fig. 1. Maximum likelihood tree based on the combined (COI, 18S) dataset. Statistical support of the nodes is indicated when Bayesian posterior probabilities ≥ 0.95 and bootstrap supports $\geq 75\%$ (black dots). On the right, the morphology of shell, penis and distal female genitalia is presented for: a) *Arganiella wolfi* Boeters & Glöer, b) *A. pescei* Giusti & Pezzoli, 1980 and c) *A. tabanensis* Boeters, Glöer & Pešić, 2014. Reproductive organs were re-drawn from Arconada & Ramos (2007a), Bodon *et al.* (2001) and Boeters *et al.* (2014) for the three species, respectively. Scale bar below topology: substitutions per site.

Revised diagnosis

Shell trochiform; whorls 3.5–4.0; aperture complete, rounded; outer lip narrow, straight in lateral profile; umbilicus wide. Operculum corneous, yellowish, thin, pliable, oval to rounded, paucispiral with a central nucleus, without peg. Two pairs of basal cusps on each central radular tooth. Ctenidium well developed, with approximately 14 gill filaments. Osphradium positioned opposite to approximately the middle of the ctenidium. Stomach without gastric caecum; rectum forms a gentle U-shape in the mantle cavity. Bursa copulatrix medium-sized, pyriform, pedunculated and protruding beyond the posterior edge of the albumen gland; bursal duct shorter than bursal length; unpigmented renal oviduct that makes a complete loop over the pallial gland; one elongated or pyriform seminal receptacle arising from the renal oviduct, just above the insertion point with the bursal duct. Prostate gland bean-shaped, about twice as long as wide. Penis small and simple, gradually tapering. Nervous system unpigmented.

Etymology

The genus is named after Villa Aretiana, the Roman name of the town Aracena, which gives its name to the mountain range where the genus was found (i.e., Sierra de Aracena); gender feminine.

Remarks

Aretiana Delicado & Ramos gen. nov. can be distinguished from *Arganiella* as the former has a taller shell, a more oval operculum, pigmentation on the body and eyespots, fewer gill filaments, a narrower penis, a larger and pyriform bursa copulatrix located beyond the posterior edge of the albumen gland and fewer cusps on the lateral radular teeth (for comparison, see Giusti & Pezzoli 1980; Boeters & Glöer 2007; Arconada & Ramos 2007a). The new genus differs from the closely related genus *Iberhoratia* by its taller shell with a narrower umbilicus, the absence of lobes on the inner edge of the penis and of a proximal seminal receptacle (SR2) and presence of two pairs of basal cusps on each central radular tooth (see Arconada *et al.* 2007). Mean COI divergence for *Aretiana* Delicado & Ramos gen. nov. was 15.9% with *Arganiella* and 11.2% with *Iberhoratia*.

Genus *Docleiana* Delicado & Pešić gen. nov.

[urn:lsid:zoobank.org:act:04639DB3-91D2-46B7-BFAB-FFC1EA3C27BD](https://zoobank.org/act:04639DB3-91D2-46B7-BFAB-FFC1EA3C27BD)

Type species

Arganiella tabanensis Boeters, Glöer & Pešić, 2014.

Revised diagnosis

Shell valvatiform; whorls ca 3; aperture complete, from rounded to ellipsoidal; outer lip narrow, straight in lateral profile; umbilicus wide. Operculum corneous, yellowish, thin, pliable, rounded, paucispiral with a central nucleus, without peg. Two pairs of basal cusps on each central radular tooth. Ctenidium well developed, with 10–11 gill filaments. Animal unpigmented. Osphradium positioned opposite approximately to the middle of the ctenidium. Stomach without gastric caecum; rectum forms a gentle V-shape in the mantle cavity. Bursa copulatrix small, globular, pedunculated and positioned beyond the posterior edge of the albumen gland; bursal duct longer than bursal length; unpigmented renal oviduct; one pyriform seminal receptacle arising at the insertion point with the bursal duct loop. Penis small and simple, gradually tapering.

Etymology

The new genus is named after Doclea, the name of the Roman city located on the site of modern Podgorica on whose municipal territory a new genus was found; gender feminine.

Remarks

Docleiana Delicado & Pešić gen. nov. can be distinguished from *Arganiella* as the former has a more ellipsoidal shell aperture, a narrower base of the penis, a smaller bursa copulatrix that is positioned beyond the posterior edge of the albumen gland, a larger seminal receptacle and more cusps on the lateral radular teeth (Fig. 2; for comparison, see Giusti & Pezzoli 1980; Boeters *et al.* 2014). The new genus differs from the closely related genus *Kerkia* by its smaller shell, absence of lobes on the inner edge of the penis, a smaller bursa copulatrix, a more globular seminal receptacle and V-shaped rectum (see Radoman 1978; Bodon *et al.* 2001). Mean COI sequence divergence for *Docleiana* Delicado & Pešić gen. nov. was 14.5% with *Arganiella* and 12.3% with *Kerkia*.

Discussion

Previous taxonomic studies of valvatiform hydrobiids have relied on morphological similarities to classify species, a practice that can be difficult for this group because of their small size and simplified morphologies. Considering this, more recent systematic revisions have incorporated molecular

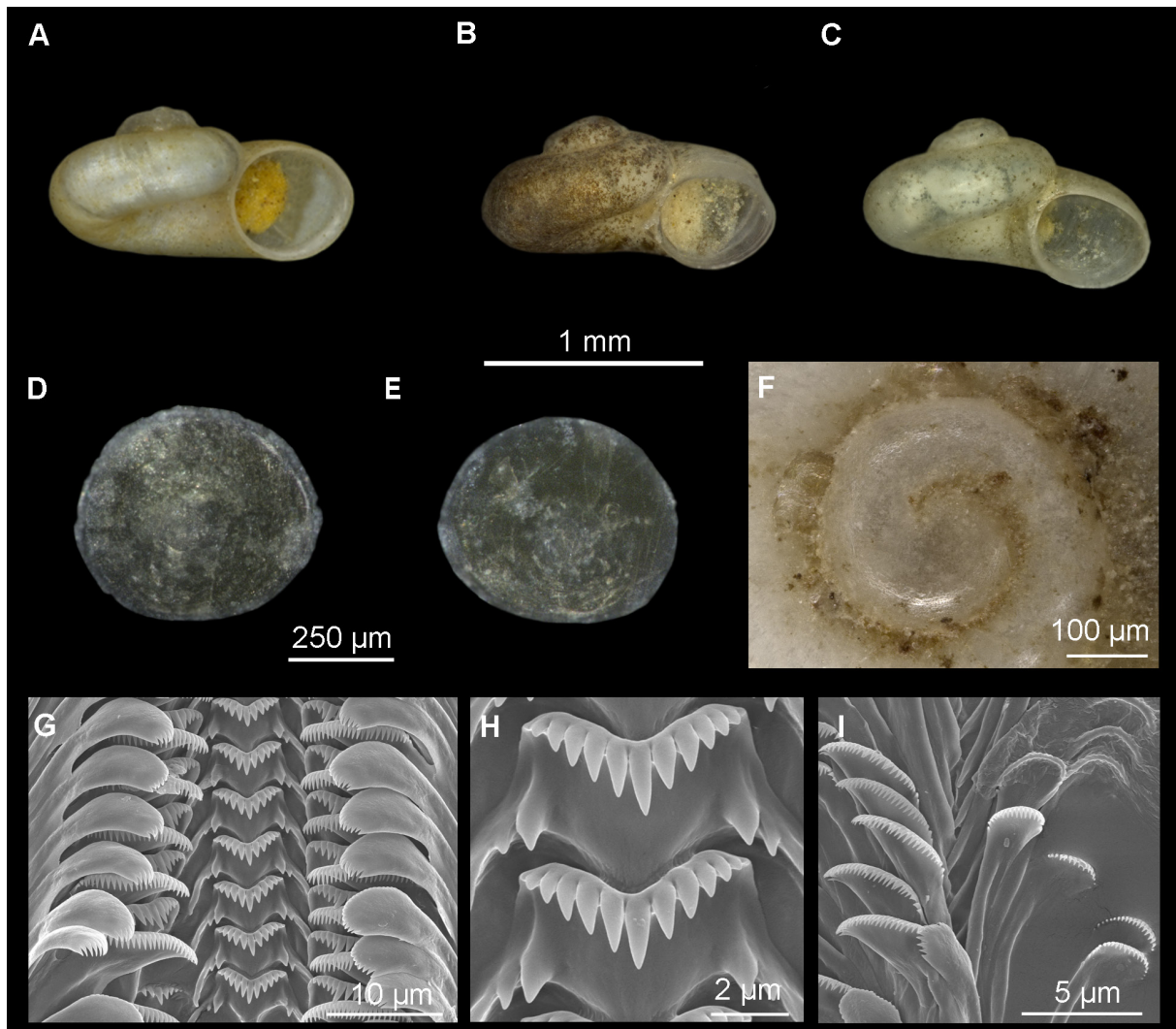


Fig. 2. Shells, operculum and radulae of *Docleiana tabanensis* (Boeters, Glöer & Pešić, 2014) (UGSB 18847). **A–C.** Shells. **D–E.** Operculum (D = inner side; E = outer side). **F.** Protoconch. **G–I.** Radulae. **G.** Portion of radula ribbon. **H.** Central radular teeth. **I.** Inner and outer marginal teeth.

methods to re-evaluate the taxonomy of valvatiform hydrobiid species and have indeed revealed genus misidentifications. Some examples are the species *Hauffenia plana* (Bole, 1961), later transferred to the genus *Bracenicica* Radoman, 1973 by Hofman *et al.* (2020); *Horatia hadei* Gittenberger, 1982 transferred to *Daphniola* Radoman, 1973 by Falniowski & Szarowska (2011b); and *Neohoratia azarum* Boeters & Rolán, 1988, later recognized as *Islamia azarum* (Boeters & Rolán, 1988) by Arconada & Ramos (2006) and currently combined as *Deganta azarum* by Arconada & Ramos in Delicado *et al.* (2019). In the case of *Arganiella*, *A. wolfi* and *A. tabanensis*, two endemic species from the Iberian and Balkan peninsulas, respectively, were classified within this genus on the basis of shell and genital similarities with the Apennine species *A. pescei* (Arconada & Ramos 2007a, 2007b; Boeters *et al.* 2014). Our survey of DNA sequence divergence within *Arganiella*, although based on a limited number of samples and gene fragments, showed the substantial divergence of these two species from the type species *A. pescei* (> 14.5% for COI). This degree of DNA sequence differentiation is comparable to those inferred between other recognized valvatiform genera (uncorrected *p*-distances 9.1–22.5% for COI). Moreover, *Arganiella* was not recovered as a monophyletic group by our phylogenetic inferences (Fig. 1).

However, incorrect systematic conclusions can be drawn from molecular phylogenies when the specimens have previously been misidentified (e.g., *Radomaniola/Horatia* in Szarowska & Falniowski, 2014). Detailed morphological examinations of the studied material are therefore needed for a more reliable systematic interpretation. Our comparative morphological study (Table 2) indicated close similarities among the, until now, considered species of *Arganiella*, especially in those characters related to the penis, ctenidium and radula and, to a lesser degree, the shell, pigmentation and distal genitalia of females. Some character states found in species of *Arganiella*, such as a simple penis without lobes, the presence of a single distal seminal receptacle and two cusps at both sides of the basis of the central radular tooth, are rarely present in other valvatiform genera (Bodon *et al.* 2001; Radea *et al.* 2016) and can, thus, lead to genus misidentification. However, differences in other characters can be observed among the three species. The most dissimilar species is *A. wolfi*: it has a larger and more trochiform shell, body pigmentation and a larger bursa copulatrix (Arconada & Ramos 2007a; Boeters & Glöer 2007). *Arganiella tabanensis* is more similar to *A. pescei* than *A. wolfi*, especially in shell shape and body pigmentation (Boeters *et al.* 2014). However, it differs from *A. pescei* in shell size, the shape of the bursa copulatrix and the bend type of the rectum. On the basis of this DNA and morphologic evidence, we assign the species *A. wolfi* and *A. tabanensis* to two distinct genera.

We base the establishment of the two new genera, *Aretiana* Delicado & Ramos gen. nov. for *Arganiella wolfi* and *Docleiana* Delicado & Pešić gen. nov. for *Arganiella tabanensis*, on the high level of DNA sequence divergence and phylogenetic position of the two species (Fig. 1), as well as on their unique combination of morphological characters (Table 2). Given the restricted geographic distribution of most valvatiform hydrobiids (Bodon *et al.* 2001), we focus our taxonomic decision on comparisons with geographically close groups. Most genera of Iberian valvatiform hydrobiids were included in our phylogenetic study, except *Navalis*, which is only known by empty shells, and *Salaeniella*, for which the females are still unknown and no well-preserved specimens are available for molecular studies. Regardless, these genera are morphologically very distinct from *Aretiana* Delicado & Ramos gen. nov. and have distant distribution ranges within the Iberian Peninsula (Quiñonero-Salgado & Rolán 2017; Boeters *et al.* 2019). According to our comparative morphological study, the most similar Iberian genus to *Aretiana* Delicado & Ramos gen. nov. is *Boetersiella*. These two genera are highly divergent genetically and display clear differences in shell and female genital features, validating them as different genera. DNA sequence data of valvatiform genera living in the Balkan springs are still scarce. Although our phylogenetic analyses recovered *Docleiana* Delicado & Pešić gen. nov. as an independent lineage sister to the Balkan genus *Kerkia*, this relationship may change as more valvatiform species are sequenced and analysed. Until then, morphological characters support the placement of *A. tabanensis* into a new genus. The Balkan genera *Hadziella* Kuščer, 1932 and *Dabriana* Radoman, 1974, which have not yet

Table 2 (continued on next page). Morphological characters recorded from the valvatiform hydrobiid genera distributed over the Iberian, Apennine and Balkan peninsulas. This data set is an extension of table 2 included in Radea *et al.* (2016).

Taxa	SL	SW	SK	UW	AS	OP	BaC	BC	SR(s)	SRP	P	PL	PP	PS	Ct	Eyes	Rectum
Iberian Peninsula																	
<i>Aretiana</i> gen. nov.	1.7	1.7	0	2	0	0	2	3	1	0	0	0	0	0	1	1	S
<i>Boetersiella</i>	1.6	1.5	0	1	1	0	1	2	1	0	0	0	0	0	0	1	U
<i>Chondrobasis</i>	1.3	1.2	0	1	1	0	1	3	1	0	1	1	0	0	0	1	U
<i>Corbellaria</i>	0.5	1.3	0	2	0	0	1	3	2	2	1	2	0	0	0	0	S
<i>Deganta</i>	1.7	1.6	0	1	0	0	2	3	1	0	2	1,2	0	0	1	1	U
<i>Iberhoratia</i>	1.5	1.8	0	2	0	0	1	1	2	2	1	2	0	0	1	1	U or S
<i>Josefus</i>	1.5	1.6	0	1	1	0	2	0	2	1	1	4	0	0	0	1	U
<i>Milesiana</i>	1.1	1.5	0	2	0	0	2	0	2	1	1	2	0	0	1	1	U
<i>Navalis</i>	0.8	1.0	1	2	0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Salaeniella</i>	1.2	1.6	0	2	1	0	-	-	-	-	1	2	0	0	1	0	S
<i>Spathogyna</i>	1.0	1.4	0	2	1	0	1	1	2	2	1	2	0	0	1	1	V
<i>Tarraconia</i>	1.8	1.8	0	2	0	0	1	3	0	0	1	2	0	0	1	1	U
Apennine Peninsula																	
<i>Arganiella</i>	1.0	2.0	0	2	0	0	2	1	1	0	0	0	0	0	1	0	S
<i>Fissuria</i> *	1.6	1.8	0	2	0	0	1	1	2	2	3	1,3	1	0	1	0	S
<i>Hauffenia</i> *	1.2	2.3	0	2	0	1	1	3	1	0	0,1	0,4	0	1	0,1	0	Z
<i>Pezzolia</i>	1.0	1.4	0	2	1	0	0	0,3	2	2	0	0	0	0	0	0	S
Balkan Peninsula																	
<i>Doctetiana</i> gen. nov.	1.1	1.5	0	2	0	0	2	2	1	0	0	0	0	0	1	0	Z
<i>Bracnica</i>	1.0	2.0	0	2	0	1	1	3	2	2	1	2	0	0	-	0	-
<i>Dabriana</i>	2.2	2.5	0	2	0	-	0	3	1	0	0	0	0	0	0	0	-
<i>Dalmatella</i>	1.6	2.2	1	2	0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Daphniola</i>	1.3	1.2	0	1	1	0	1	1	2	2	1	2	0	0	1	1	-
<i>Gocea</i>	0.9	1.6	0	2	1	1	1	3	1	0	0	0	0	0	0	0	Z
<i>Graecoarganiella</i>	1.2	1.5	0	2	1	0	1	3	2	2	1	1	1	0	0	1	-

Table 2 (continued).

Taxa	SL	SW	SK	UW	AS	OP	BaC	BC	SR(s)	SRP	P	PL	PP	PS	Ct	Eyes	Rectum
<i>Hadziella</i>	0.6	1.8	0	2	1	1	1	1	1	0	0	0	0	0	0	0	Z
<i>Horatia</i>	2.1	2.1	0	1	1	0	1	1	2	2	1	2	0	0	1	1	0
<i>Isimerope</i>	1.6	2.0	0	2	1	0	1	3	0	0	1	3	1	0	0	1	U
<i>Islamia*</i>	2.0	1.8	0	0	0	0	1	0	2	2	1	4	0	0	1	1	U
<i>Karevia</i>	1.1	1.7	1	2	2	0	1	3	2	2	1	3	0	0	–	1	–
<i>Kerkia</i>	1.4	2.3	0	2	0	1	2	1	1	0	1	3	0	0	1	0	S
<i>Lynhidia</i>	1.2	1.1	0	0	2	0	1	1	1	0	1	4	0	0	–	1	–
<i>Myrtoessa</i>	1.1	1.4	0	2	0	0	1	1	2	2	0	0	2	0	1	1	V
<i>Ohridohauffenia</i>	1.0	1.5	1	2	1	0	1	1	2	2	1	3	0	0	–	1	–
<i>Ohrigocea</i>	0.8	1.2	0	2	0	0	1	3	2	2	1	3	0	0	–	1	–
<i>Prespolitorea</i>	1.0	1.3	0	2	0	0	1	2	2	2	1	3	0	0	–	1	–
<i>Pseudohoratia</i>	1.3	1.4	0	1	0	1	1	2	2	2	1	3	0	0	1	1	0
<i>Pseudoislamia</i>	1.1	1.3	0	2	1	0	1	1	2	2	1	4	0	0	–	1	–
<i>Strugia</i>	1.6	1.7	0	2	0	0	1	1	1	0	1	3	0	0	–	1	–
<i>Zaumia</i>	1.5	1.2	0	0	0	0	1	1	1	0	1	4	0	0	–	0	–

* Genera distributed outside of the categorized regions.

Continuous (in mm) and discrete (or categorical) characters: AS = aperture shape: rounded (0), oval (1), square (2); BaC = pairs of basal cusps on the central radular tooth; BC = bursa copulatrix: absent (0), ovoid (1), globular (2), pyriform (3); Ct = ctenidium: absent (0), present (1); eyes: absent (0), present (1); OP = operculum peg: absent (0), present (1); P = penis: simple without lobes (0), with one lobe (1), with two lobes (2), with more than two lobes (3); PL = penial lobe(s): absent (0), basal lobe (1), medial lobe (2), lobe at $\frac{2}{3}$ of penis length (3), apical lobe (4); PP = penial papilla: absent (0), present (1); PS = penial stylet: absent (0), present (1); Rectum: without bend (0), S-like (S), U-like (U), V-like (V), Z-like (Z); SK = shell keels or other ornaments: absent (0), present (1); SL = shell length (maximum recorded value for the type species); SR = seminal receptacle: absent (0), one seminal receptacle (1), two seminal receptacles (2); SRP = seminal receptacle position: absent (0), close to each other (1), distantly positioned from each other (2); SW = shell width (maximum recorded value for the type species); UW = umbilicus width: very narrow (0), narrow (1), wide (2); – = no data. Continuous variables provided in mm.

been sequenced, resemble *Docleiana* Delicado & Pešić gen. nov. in penis morphology but differ in shell size and radular and female genital features; they also lack a ctenidium (Table 2).

The systematic findings suggesting erroneous assignment of the three geographically disjunct species to the same genus due to morphological similarities conflict with the use of traditional taxonomy to classify valvatiform hydrobiid taxa and highlight the need to integrate morphological and molecular data for more robust taxonomic assessments.

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