



## Research article

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# A new genus and species of uncertain phylogenetic position within the family Hydrobiidae (Caenogastropoda, Truncatelloidea) discovered in Tunisian springs

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**Abstract.** The species richness of the aquatic (non-marine) gastropod family Hydrobiidae Stimpson, 1865 reaches its peak in the European region partly because other areas, such as North Africa, remain to be extensively surveyed. Most of the hydrobiid species described in North Africa have been ascribed to the subfamilies Pseudamnicolinae, Hydrobiinae and the genus *Mercuria*. Little is known about the presence of other hydrobiid subfamilies. This study examines several specimens of gastropods collected from two springs in Tunisia. Based on a comprehensive literature review and rigorous anatomical and molecular comparisons with known species from North Africa, Europe and Asia Minor of similar morphology, we here describe a new genus, *Bullaregia* gen. nov., and new species *Bullaregia tunisiensis* gen. et sp. nov. Although the shell shape of this Tunisian species resembles that of European hydrobiid genera such as *Belgrandiella*, it differs in other anatomical structures (i.e., penis with a glandular strap-like lobe, and two seminal receptacles, SR1 pedunculate, SR2 sessile and less developed). Using maximum likelihood and Bayesian approaches, phylogenetic relationships inferred from mtCOI sequences point to an independent evolutionary lineage for this new taxon outside *Belgrandiella* and other known hydrobiid subfamilies.

**Keywords.** Freshwater snails, Western Palaearctic, North Africa, anatomy, *Belgrandiella*.

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## Introduction

The aquatic (non-marine) gastropod family Hydrobiidae Stimpson, 1865 (sensu Wilke *et al.* 2013) occurs mainly in the Western Palaearctic, Nearctic, northern Neotropic and South Africa. In Europe, a high species richness has been described for this family. However, this is likely the consequence of an underreporting of the hydrobiid fauna living in other regions such as North Africa. Based only on shell characters, Van Damme (1984) and Kristensen (1985) reported the presence in North Africa of seven European genera of Hydrobiidae *sensu lato* (i.e., *Belgrandia* Bourguignat, 1869; *Bythinella* Moquin-Tandon, 1855; *Hadziella* Kuscer, 1932; *Horatia* Bourguignat, 1887; *Hydrobia* Hartmann, 1821; *Peringia* Paladilhe, 1874 and *Pseudamnicola* Paulucci, 1878) and one endemic genus (i.e., *Heideella* Backhuys and Boeters, 1974). More recently, Ghamizi *et al.* (1999) and Bodon *et al.* (1999) added other species and genera to the Moroccan Hydrobiidae list, mostly stygobiont species. However, despite these important discoveries, most studies have provided mere descriptions of shell morphology without focusing on anatomical structures. In prior work, we confirmed that by adding anatomical and molecular data to these shell-based identifications, the reliability of generic classification could be much improved (Delicado *et al.* 2016).

In Tunisia, a few Hydrobiidae species have been shell-determined over the past centuries (Bourguignat 1860, 1864; Issel 1880; Debeaux 1863; Letourneux & Bourguignat 1887; Pallary 1921, 1923; Seurat 1921, 1934). So far, the most relevant study of this family in Tunisia was that by Boeters (1976) who listed 28 species belonging to three genera: *Pseudamnicola*, *Hydrobia* and *Mercuria* Boeters, 1971. Based on these descriptions, an extensive survey in the past ten years of freshwater superficial and subterranean malacofauna has revealed Hydrobiidae to be among the most species- and population rich families in the country. In contrast, Tunisia's subterranean aquatic fauna has recently received much less attention, although this region (especially NW Tunisia) has numerous wells and springs, and some caves. Early studies examining the general subterranean and spring fauna of Tunisia were those by Seurat (1921, 1934) and Debeaux (1863), who discovered only a few gastropod and two isopod species in these habitats. One example of Hydrobiidae is the species '*Hydrobia*' *nana* (Terver, 1939) found by Debeaux (1863) in springs of Algeria and two localities of northern Tunisia. The few more recent surveys have detected new records and new species of phreatic microgastropods (such as *Pseudamnicola*) as well as other stygobiont or eustygophilous taxa such as worms (Charni *et al.* 2004; Harrath *et al.* 2001, 2004) and crustaceans (Toumi *et al.* 2013). However, to our knowledge true stygobiont species of Hydrobiidae inhabiting Tunisian groundwaters have been rarely reported. In consequence, there are no appropriate references for the straightforward identification of Tunisian groundwater species.

In summary, we could argue that the picture of hydrobiid species richness in North Africa is still very incomplete, and that only surface-water genera of the subfamilies Pseudamnicolinae, Hydrobiinae and the genus *Mercuria* have been more intensively studied. This situation prompted a new survey of ground- and surface waters to search for possible endemic forms in Tunisia. The collection of specimens obtained contains some unknown Hydrobiidae species. We here describe one of these species and assign it to the genus *Bullaregia* gen. nov. As the shell shape of this new genus resembles that of the European *Belgrandiella* (subfamily Belgrandiellinae), we conducted a systematic review using both molecular and morphological methods. Our results indicate this taxon is a distinct hydrobiid genus characterized by a particular set of anatomical characters (not known for other subfamilies) and suggest its uncertain position within the Hydrobiidae phylogeny.

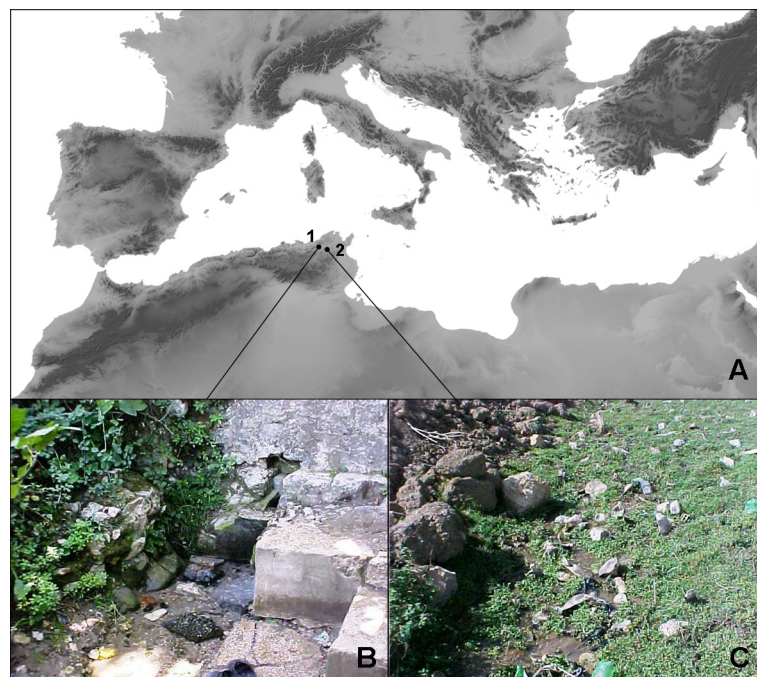
## Material and methods

The material was found at two localities in northwestern Tunisia: Aïn Gnaa (Jendouba, northwestern Tunisia), 36.5583 N, 8.74667 E, alt. 206 m a.s.l.; Djebba (Beja, northwestern Tunisia), 36.47125 N, 9.0997223 E, alt. 342 m a.s.l. (Fig. 1). Live specimens were collected within the river substrate with a

sieve of 300 µm mesh cloth. The samples were preserved in 70% ethanol and sorted in the laboratory under an optical stereo microscope (MOTIC-ST-39 Series). Anatomical characteristics (genitalia, radula, stomach and gill) were studied under the stereo microscope and compared with other described forms from North Africa, Europe and Asia Minor. Morphological characters were described based on the standard terminology of Hershler & Ponder (1998). Shell measurements were made with an eye-piece micrometer under the stereo microscope. The sex of the holotype was not examined in order to keep the shell intact. Photographs were taken with a camera system and a digital adapter. The type material is deposited in the Collection of Molluscs of the Museo Nacional de Ciencias Naturales de Madrid (MNCN).

Our phylogenetic analyses included one specimen of *Bullaregia tunisiensis* gen. et sp. nov. from a spring in Djebba (type locality) and 22 hydrobiid representatives of other European genera whose shell morphology resembles the new African hydrobiid here discovered (Table 1). Genomic DNA was extracted using the CTAB protocol of Wilke *et al.* (2006). The mitochondrial fragment of cytochrome *c* oxidase subunit I (COI) was amplified with the primers LCO1490 (Folmer *et al.* 1994) and COR722b (Wilke & Davis 2000). The cycling sequence conditions were as described in Schreiber *et al.* (2012). Final PCR products were sequenced in an ABI 3730 XL sequencer (Life Technologies, Carlsbad, CA, USA) using a Big Dye Terminator kit v. 3.1 (Life Technologies). New sequences were deposited in GenBank (Table 1).

Amplified sequences were edited in Sequencher 4.6 (Gene Codes, Ann Arbor, MI). The protein-coding mitochondrial COI sequence was unambiguously aligned in PAUP\* 4.0a123 (Swofford 2002) together with sequences of other hydrobiid species obtained from GenBank (Table 1). As Wilke *et al.* (2013) found little saturation among COI sequences of hydrobiids and other sister families, we did not test for saturation in our alignment. Prior to the species tree reconstruction, we searched for closely related taxa



**Fig. 1.** Map and photographs of the studied sampling sites. **A.** Map showing the geographic distribution of *Bullaregia tunisiensis* sp. nov. **B.** Aïn Gnaa (Bulla-régia, Jendouba). **C.** Djebba (Thibar, Beja).

on GenBank using BLASTN 2.3.1 (Zhang *et al.* 2000) against the National Center for Biotechnology Information (NCBI) nucleotide database. As no high sequence identity with other European and/or African hydrobiid was identified (one hundred contigs showed BLASTN % identity  $\leq 86\%$ , most of them corresponding to species of the American hydrobiid genus *Pyrgulopsis* Call & Pilsbry, 1886), we compared our fragments to sequences from similar species which potentially may belong to the same genus or subfamily.

Phylogenetic relationships were inferred by Bayesian-inference (BI) and Maximum Likelihood (ML). For these methods, Partition Finder 1.1.1. (Lanfear *et al.* 2012) was used to select the best-fit model of nucleotide evolution by codon position within our COI dataset, implementing corrected Akaike's information criterion (AICc; Akaike 1974; Sugiura 1978; Hurvich & Tsai 1989) for model selection and RAxML-HPC v.8 (Stamatakis 2014) for likelihood estimation. BI was performed through two independent runs of four Metropolis-coupled Markov chains in MrBayes 3.1.2 (Huelsenbeck 2000; Huelsenbeck & Ronquist 2001), using 2.5 million generations each and a sample frequency of 1000. The analysis was terminated when the average standard deviation of split frequencies was less than 0.01, indicating that the runs reached convergence. The first 10% sampled trees were discarded as burn-in. Partitioned ML analysis was undertaken using RAxML-HPC v.8 on the computer cluster of the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, [www.phylo.org](http://www.phylo.org)). Clade support for the ML phylogeny was assessed by nonparametric bootstrapping (Felsenstein 1985) using 100 pseudoreplicates, and by posterior probabilities (BPPs) for the BI.

### Abbreviations

AH = Aperture height  
 AW = Aperture width  
 H = Height  
 W = Width

### Results

The alignment of COI sequences yielded 648 bp for which Partition Finder identified TrN (Tamura & Nei 1993) + I (invariable sites) + G (rate variation among sites), F81 (Felsenstein 1981) + I and GTR (Tavaré 1986) + I + G as the best-fit models of nucleotide substitution for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> codon position, respectively. Average base frequencies were 31.2% A, 13.5% C, 12.1% G and 43.2% T. ML and BI gave rise to similar topologies and both showed *Bullaregia* gen. nov. outside the subfamilies to which this genus could belong (Fig. 2). These subfamilies, i.e., Belgrandiellinae, Belgrandiinae and Horatiinae on the one hand, and Islamiinae on the other, formed two different monophyletic groups well supported by ML and BI (bootstrap value = 70 and 72%, and BPP = 0.99 and 0.99, respectively). Though the exact phylogenetic position of *Bullaregia* gen. nov. remains unclear, both analyses showed a distant relationship between this and the European genus *Belgrandiella* (uncorrected pairwise distances of 17.1%). Morphological data also supported these distant relationships. Differences in shell and anatomical structures between *Bullaregia* gen. nov. and other hydrobiid genera mentioned above are described in the following section.

KHALLOUFI N. *et al.*, *Bullaregia tunisiensis*, a new taxon from Tunisia**Table 1.** Taxa included in the molecular analyses with their corresponding locality names, GenBank accession numbers and original references.

Taxon	Locality	GenBank # COI	Original reference
<b>Outgroup</b>			
<i>Mercuria similis</i>	Italy, Friuli-Venetia-Julia, Udine, Aquileia, Canale Panigai	AF367646	Wilke <i>et al.</i> 2001
<i>Pseudamnicola lucensis</i>	Italy, Tuscany, Bagni di Lucca, Bagni Caldi, thermal spring	AF367651	Wilke <i>et al.</i> 2001
<b>Ingroup</b>			
<i>Agrafia wiktoria</i>	Greece, Evrytania, Agrafa mountains	JF906762	Szarowska & Falniowski 2011
<i>Alzoniella finalina</i>	Italy, Liguria, Savona, Molino, spring at the Porra river	AF367650	Wilke <i>et al.</i> 2001
<i>Anagastina zetaevalis</i>	Montenegro, Vranicke, spring at the Zeta river	EF070616	Szarowska 2006
<i>Avenionia brevis</i>	France, Gard, spring of the fountain of St. Victor-La Coste	AF367638	Wilke <i>et al.</i> 2001
<i>Belgrandia thermalis</i>	Italy, Tuscany, Pisa, S. Giuliano Terme, thermal channel	AF367648	Wilke <i>et al.</i> 2001
<i>Belgrandiella kusceri</i>	Slovenia, spring of Rakek	JX970610	Wilke <i>et al.</i> 2013
<i>Boleana umbilicata</i>	Slovenia, Močilnik, the spring of the Ljubljana river	JX982795	Falniowski & Szarowska 2012
<i>Bullaregia tunisiensis</i> gen. et sp. nov.	Tunisia, Province Béja, a spring in Djebba	KX821683	present study
<i>Dalatinella fluviatilis</i>	Croatia, Jankovica Buk waterfalls, the Zrmanja River	KC344541	Falniowski & Szarowska 2013
<i>Daphniola graeca</i>	Greece, Dafne spring	EF070618	Szarowska 2006
<i>Fissuria boui</i>	France, Alpes Maritimes, Peymeinade, spring near La Prouveresse.	AF367654	Wilke <i>et al.</i> 2001
<i>Graecoarganiella parnassiana</i>	Greece, Parnassus mountains, south of Eptalofos	JN202348	Falniowski & Szarowska 2011
<i>Graziana alpestris</i>	Italy, Liguria, Savona, Molino, spring at the Porra River	AF367641	Wilke <i>et al.</i> 2001
<i>Grossuana codreanui</i>	Romania, from a spring close to the lake Techirghiol	EF061919	Szarowska <i>et al.</i> 2007
<i>Hauffenia tellinii</i>	Italy, Friuli-Venetia Julia, Gorizia, spring of the Isonzo River near Sagrado	AF367640	Wilke <i>et al.</i> 2001
<i>Horatia klecakiana</i>	Croatia, N of Kučiće, Studenci spring, in the valley of the Cetina river	KJ159128	Szarowska & Falniowski 2014
<i>Islamia piristoma</i>	Italy, Liguria, La Spezia, spring on the right bank of Magra river	AF367639	Wilke <i>et al.</i> 2001
<i>Pauluccinella minima</i>	Italy, S. Egidio, Lago di Piediluco	JX970612	Wilke <i>et al.</i> 2013
<i>Radomaniola callosa</i>	Italy, Abruzzo, Caramanico Terme, stream on the left bank of Orfento river	AF367649	Wilke <i>et al.</i> 2001
<i>Sadleriana fluminensis</i>	Croatia, Jadro River at Solin near Split	AF367647	Wilke <i>et al.</i> 2001
<i>Trichonia kephalovrissonia</i>	Greece, Termos	EF070619	Szarowska 2006



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

Genus *Bullaregia* gen. nov.

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### Type species

*Bullaregia tunisiensis* sp. nov., by present designation.

### Diagnosis

Shell ovate-conic, penultimate whorl tall relative to remaining whorls; aperture pyriform, angled on top; umbilicus closed by inner lip. Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with submarginal nucleus. One pair of basal cusps on radular central tooth. Ctenidium bearing well-developed gill filaments. Presence of bursa copulatrix; unpigmented renal oviduct with a pouch at the end of the coiled section; two seminal receptacles, SR1 elongate and pedunculate, SR2 globular, sessile and less developed. Strap-like penis attached to the neck behind the right eye, with one dorsal, strap-like glandular lobe.

### Etymology

Referring to the archeological site Bulla Regia, the region where the genus was found; gender feminine.

### Remarks

This genus differs from conchologically similar genera from Europe (e.g., *Belgrandiella*, *Terranigra* Radoman, 1978 and *Litthabitella* Boeters, 1970) and Central Asia (e.g., *Chirgisica* Glöer, Boeters & Pešić, 2015) in bearing a strap-like penis (tapered in *Belgrandiella*, *Terranigra* and *Chirgisica*, but broadly triangular in *Litthabitella*), with one dorsal glandular lobe (absent in all the aforementioned genera) (see Radoman 1983; Szarowska 2006; Glöer *et al.* 2014). *Bullaregia* gen. nov. resembles *Terranigra* and *Litthabitella* in having two seminal receptacles (none and one in *Chirgisica* and *Belgrandiella*, respectively), but they are distinguished by their globular shape (elongate in *Terranigra* and *Litthabitella*), and in the position and absence of the duct. *Bullaregia* gen. nov. conforms to *Litthabitella* in the radula features, but differs from *Belgrandiella* in the number of basal cusps on the radular central tooth (two pairs in the latter) (see Szarowska 2006).

Based on molecular and morphological data, Szarowska (2006) tentatively classified *Litthabitella* within the family Assimineidae (outside Hydrobiidae), which suggests a large genetic distance between this genus and *Bullaregia* gen. nov. Moreover, our genetic findings indicated that *Bullaregia* gen. nov. and *Belgrandiella* differ from each other by 17.1% COI p-distances.

*Bullaregia tunisiensis* sp. nov.

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Figs 3–5

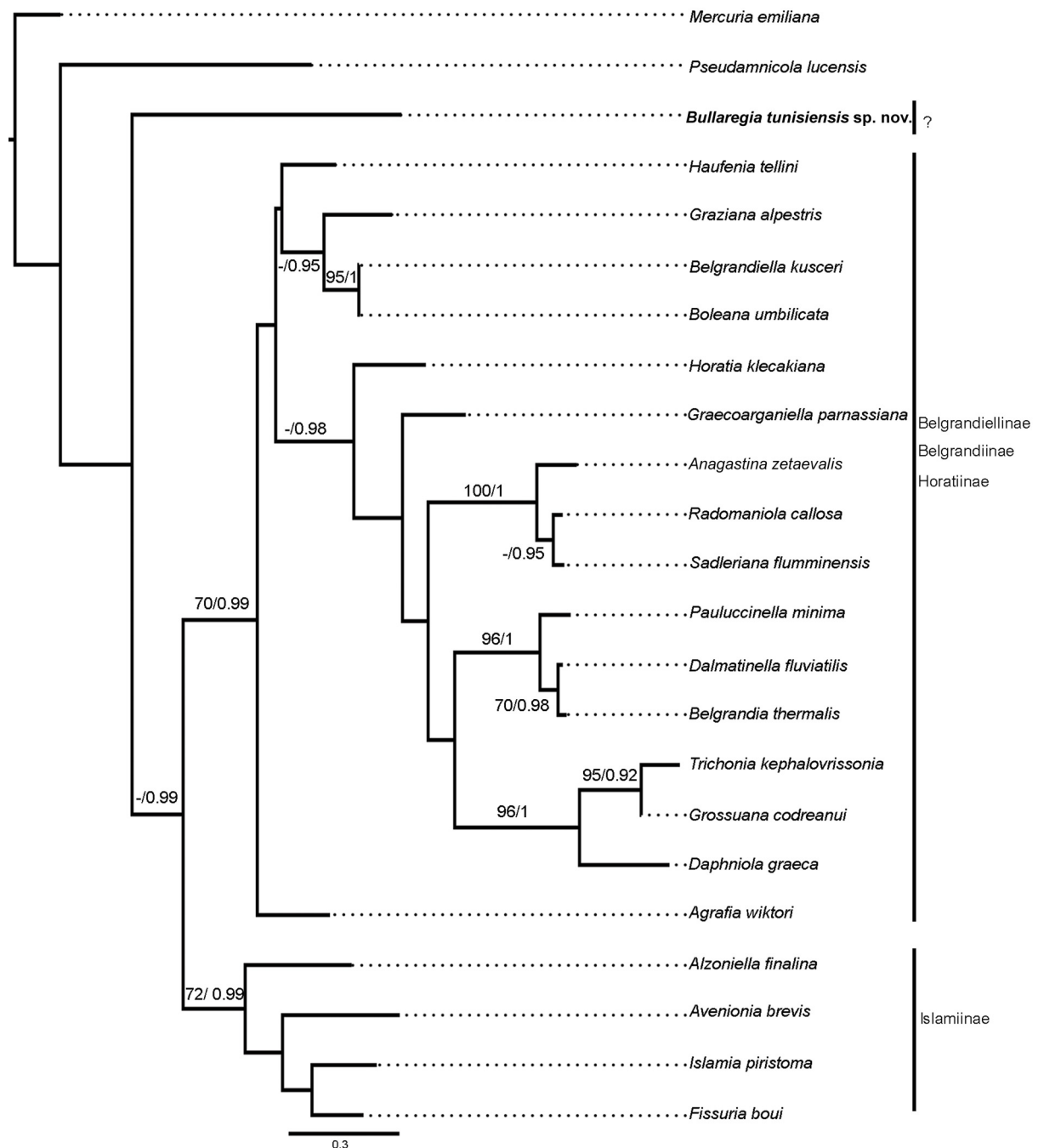
### Diagnosis

Shell 1.8 to 2.1 mm high, yellowish; central radular tooth formula 6–C–6/1–1; ovoid bursa copulatrix, with a duct longer than bursa; two seminal receptacles, SR1 elongate and pedunculate, SR2 globular

and sessile; prostate gland about 0.45 mm ovate in section, penis strap-like, with a dorsal, strap-like glandular lobe, and distal end tapered and greyish pigmented.

### Etymology

The species epithet *tunisiensis* is an adjectival toponym that refers to Tunisia, where the species was found.



**Fig. 2.** Maximum likelihood inference based on COI dataset. Bootstrap values and BPP's are given when  $\geq 70\%$  and 0.90, respectively. Black bars on the right indicate subfamily assignments. Scale bar: expected change per site.

### Type material

#### Holotype

TUNISIA: collected in Aïn Gnaa spring, Province Jendouba, 9 May 2008, leg. Khalloufi (MNCN 15.05/60177H).

#### Paratypes

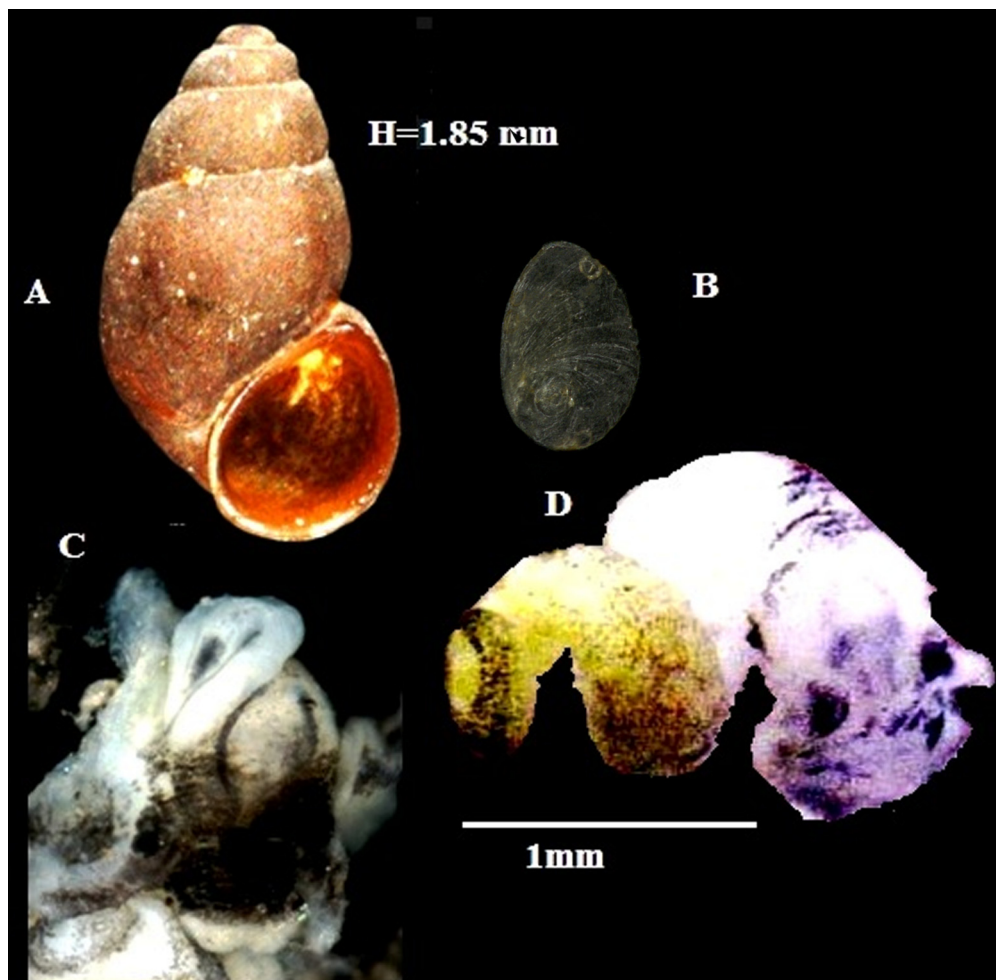
TUNISIA: Aïn Gnaa spring, 10 ex., 9 May 2008, leg. Khalloufi (MNCN 15.05/60177P); spring in Djebba, 15 Jun. 2009, leg Khalloufi (MNCN 15.05/60178).

### Type locality

TUNISIA: Aïn Gnaa spring (Bulla-régia), Province Jendouba, 36.5583 N, 8.74667 E, Alt. 206 m a.s.l.

### Other material examined

TUNISIA: 56 ex. in ethanol 95% (30 dissected), province of Jendouba, Aïn Gnaa spring (Bulla-régia), 36.5583 N, 8.74667 E, 206 m a.s.l., 9 May 2008; 15 ex. in ethanol 95% (10 dissected), province of Jendouba, spring in Djebba, 36.47125 N, 9.0997223 E, 342 m a.s.l., 2 Feb. 2009; 45 ex. in ethanol 95% (35 dissected), province of Béja, 15 Jun. 2009.



**Fig. 3.** *Bullaregia tunisiensis* gen. et sp. nov. **A.** Shell (holotype). **B.** Operculum. **C.** Head and penis. **D.** Coiled body.



## Description

MEASUREMENTS. Holotype: H = 1.85 mm, W = 1.06 mm, AH = 0.73 mm, AW = 0.79 mm

SHELL. Ovate-conic, brown-yellowish, with 4–4.5 whorls (Fig. 3A); mean SH  $1.6 \text{ mm} \pm 0.35 \text{ mm}$  and SW  $0.91 \pm 0.15 \text{ mm}$ ; maximum height is 2.1 mm; mean SL / SW  $1.75 \pm 0.2$ ; body whorl about  $\frac{2}{3}$  total length; rest of whorls slightly convex with deep sutures; aperture about 45% of shell height, complete, pyriform, slightly angled at the top and attached to body whorl; thin inner peristome but thicker than outer lip; peristome margin straight; umbilicus narrow.

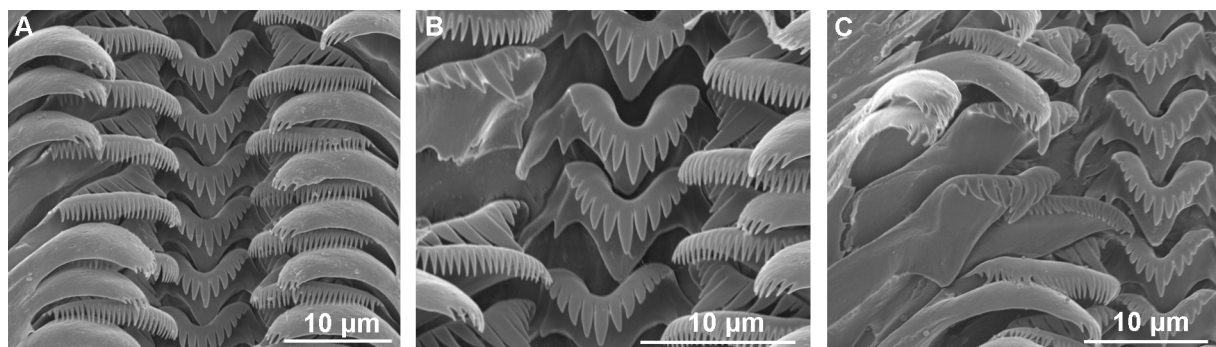
OPERCULUM. With ca 2 whorls (Fig. 3B) and muscle attachment area oval and located near the nucleus.

RADULA. Intermediate length (20% total maximum shell length), bearing about 80 rows of teeth; central tooth formula 6–C–6/1–1 (Fig. 4A–B); lateral teeth formula 5–C–5; inner marginal teeth having 30–35 sharp cusps; outer marginal teeth having 25–30 sharp cusps (Fig. 4C).

PIGMENTATION AND ANATOMY. Visceral coil yellowish, showing by transparency the gonad; traces of pigment on pallial roof, visceral coil, anterior edge of mantle, foot and light pigment between tentacles (Fig. 3D). Black eyes surrounded by brownish pigment. Snout with medium distal lobation. Tentacles parallel-side, distal end nonexpanded. Ctenidium with 9–13 gill filaments; osphradium whitish, elongate, two times longer than wide and approximate opposite middle of ctenidium. Stomach with large gastric (posterior) caecum.

MALE GENITALIA. Penis strap-like with a well-developed glandular lobe on the outer edge, 33% total penis length; glandular lobe strap-like and with a short duct; distal end of penis tapered, dark pigmented (Figs 3C, 5A). The penis has distinct basal and distal portions. The distal portion is smooth, with tapered end and variable in length. The basal portion is cylindrical and folded. Prostate gland, close, bean-shaped in outline, oval in section, and about twice as long as wide (0.45 mm/0.25 mm). Posterior vas deferens forms the seminal vesicle with many loops. It is well-developed (about 4 mm in length and 0.1 mm in section) and strongly undulating, coiling immediately after it emerges from the testis and extending over the posterior and middle part of the stomach. The pallial vas deferens is a straight and narrow tube unciliated, entering postero-ventral portion of gland and exiting from its anterior end.

FEMALE GENITALIA. Ovary simple with stalked lobes. Pallial oviduct bearing an ovoid bursa copulatrix with a duct longer than its length; unpigmented renal oviduct, with a pouch at the end of the coiled section; two seminal receptacles; SR1 elongate with a short duct, close to the insertion point of the



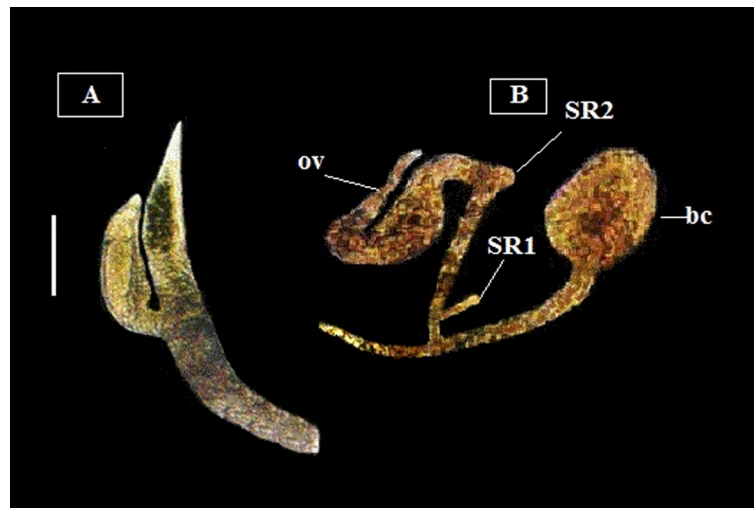
**Fig. 4.** Radula of *Bullaregia tunisiensis* gen. et sp. nov. from a spring in Djebba, Tunisia. **A.** Rows of teeth of the radula. **B.** Detail of central teeth. **C.** Detail of lateral and marginal teeth.

bursal duct to the renal oviduct; SR2 globular, less developed and sessile, positioned near antero-ventral edge of bursa (Fig. 5B).

### Remarks

Populations of this species from the Aïn Gnaa and Djebba springs vary only scarcely in terms of morphology and anatomy. In specimens from the Aïn Gnaa spring, the shell is generally more slender and has deeper sutures and a sharper protoconch. The portion of the vas deferens, forming the seminal vesicle, is more coiled and more developed. Pigmentation in the Djebba spring specimens is more intense, especially in the mantle and distal portion of the penis. Individuals from these populations are conchologically differentiated from similar regional species, such as *Pseudamnicola meluzzi* Boeters, 1976 and *P. conovula* (Frauenfeld, 1863), by their smaller and slender shell, a taller penultimate whorl relative to remaining whorls and by an aperture pyriform, angled on top.

Representatives of these two populations featured a similar shell morphology as the specimens from Algeria deposited in the Muséum national d'Histoire naturelle, Paris (MNHN). These specimens were earlier identified as *Amnicola seminium* (Morelet, 1857) by Bourguignat. However, this species, illustrated by Morelet in the original description (Morelet 1857, pl. XII fig. 10) differs from the Algerian specimens deposited in the MNHN. Based on Morelet's illustration (1857: pl. XII Fig. 10), the type of *A. seminium* has a larger body whorl, a shorter penultimate whorl and a rounder aperture (resembling *Pseudamnicola*) than the MNHN specimens. Thus, these Algerian snails could have been misidentified. Based on common shell features shared by these specimens and *Bullaregia* gen. nov., such as ovate-conic shape, tall penultimate whorl, aperture angled on top and closed umbilicus, they could be assigned to this new genus.



**Fig. 5.** Genitalia of *Bullaregia tunisiensis* gen. et sp. nov. **A.** Penis (paratype). **B.** Distal female genitalia. Abbreviations: bc = bursa copulatrix; ov = renal oviduct; SR = seminal receptacle. Scale bar 0.1 mm.

### Discussion

The freshwater gastropod diversity of some geographical regions including Europe, North America and Australasia, has been better explored than that of others (Strong *et al.* 2008). Our discovery here of *Bullaregia* gen. nov. calls for more field work in North Africa to further our understanding of global species richness, distribution and evolutionary patterns in hydrobiid clades. The new springsnail described

shows a small distribution range. This means, it could be the second Hydrobiidae genus endemic to North Africa (after *Heideella*, see Bodon *et al.* 1999). Although the total number of African hydrobiid species remains unknown (Brown 1994), taxonomic revisions of the North African Hydrobiidae *sensu lato* have recognized 13 genera, including *Bullaregia* gen. nov. discovered herein. In total, 37 species belonging to these and other undiscovered hydrobiid genera are considered threatened in North Africa (Van Damme *et al.* 2010). Pollution, habitat loss and an ever drier climate are the factors thought to have most contributed to the decline of freshwater molluscs in North Africa. In addition, numerous Tunisian freshwater wells are presently at risk of salinization (Van Damme *et al.* 2010).

However, assignments of African taxa to European genera, often based on shell descriptions only, may be erroneous. For example, the presence of *Belgrandiella* in North Africa is still uncertain. Its first record in North Africa was detected through the synonymization of the African genus *Maresia* with *Belgrandiella* (suggested by Boeters 1988). Bourguignat (1877) described the genus *Maresia* from Algeria and designated *Hydrobia dolichia* Bourguignat, 1864 as the type species. Nonetheless, Kabat & Hershler (1993) stated that Bourguignat's *Maresia* is a junior homonym, and thus invalid as a mollusc. Additionally, some *Maresia* species, including the type species, *M. dolichia*, or *M. nana* (Bourguignat, 1864), have been ascribed to the genus *Heideella* (García *et al.* 2010). There is therefore no sound evidence confirming the presence of *Belgrandiella* in North Africa.

Despite their closely resembling shell shape, the high anatomical and molecular variation observed between *Bullaregia* gen. nov. and other European hydrobiids (e.g., *Belgrandiella* or *Terranigra*) reveals these genera as different entities. Our phylogenetic analyses, which included sequences of species of the subfamilies Belgrandiellinae, Islamiinae and Horatiinae, did not cluster *Bullaregia* gen. nov. within any of these groups (Fig. 2). Hence, pending more complete phylogenies for this family, *Bullaregia* gen. nov. could be a new hydrobiid subfamily distinguishable mainly by a dorsal glandular lobe on the penis and the joint presence of seminal receptacles and a pouch in the renal oviduct. Glandular ornaments on the penis have also been observed in males of the European genus *Agrafia* Szarowska & Falniowski, 2011 (see Szarowska & Falniowski 2011) and the American subfamily Nymphophilinae (see Hershler *et al.* 2003; Hershler & Liu 2009; Hershler *et al.* 2011, among others). However, in the absence of a well supported relationship, this feature could be a synapomorphy of these clades or a result of convergent evolution. More comprehensive molecular studies will help resolve the phylogenetic relationship of this newly discovered hydrobiid clade. This type of information is indeed essential to unveil the first evolutionary events that gave rise to the major clades of the family Hydrobiidae.

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