

# A NEW SPECIES OF *BYTHINELLA* MOQUIN-TANDON, 1856 (GASTROPODA: CAENOGASTROPODA: BYTHINELLIDAE) FROM SOUTH-WESTERN FRANCE

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**ABSTRACT:** The spring-snail genus *Bythinella* is a diverse group of minute operculate gastropods, usually restricted to springs and widely distributed in Europe. Species are primarily delineated based on shell characters such as size or overall shape. Nevertheless, studies based on molecular and morphological approaches show that these characters can be highly variable and do not necessarily have taxonomic significance. In this paper we formally describe and name, according to the requirements of the International Code of Zoological Nomenclature, a species identified by previously published phylogenetic analyses and by a barcoding gap approach based on the mitochondrial COI gene. The molecular results suggest this species is a micro-endemic species currently recognized only from south-western France at its type locality. We also highlight that the barcode approach considerably reduces the number of valid species in this region, and that a strictly shell-based taxonomy probably overestimates the species richness of the genus *Bythinella*.

**KEY WORDS:** molecular species delimitation; DNA-taxonomy; new species; Padirac cave

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## INTRODUCTION

The spring-snail genus *Bythinella* Moquin-Tandon, 1856 is one of the most diverse genera of European hydrobioids, with 167 valid species (MOLLUSCABASE 2022), of which 47 occur in France (INPN 2022). *Bythinella* species are minute, with shells reaching 2–4 mm in height, with a blunt apex and an overall ovoid to conical-elongated shape, most often smooth but occasionally with spiral keels or axial varice(s) just behind the aperture. They are dioecious and oviparous. They are usually restricted to springs with low water temperatures (GIUSTI & PEZZOLLI 1977, FALNIOWSKI 1987) and occur occasionally in spring outlets and associated creeks, swamp, head-water seeps and subterranean waters. The wide range of the genus extends from the Iberian Peninsula to the Asian part of Turkey and from Poland to southern Greece (BOETERS 1973, 1998, GIUSTI & PEZZOLLI

1977, FALNIOWSKI 1987, GLÖER 2022). They also occur on many Aegean and other Mediterranean islands including Crete and Corsica (FALNIOWSKI et al. 2016).

Species of *Bythinella* are primarily delineated by non-discrete shell characters such as size and shape, and to some extent by anatomical features. Nevertheless, several studies based on both molecular and morphological approaches (e.g. FALNIOWSKI et al. 1998, MAZAN 2000, MAZAN & SZAROWSKA 2000a, b, SZAROWSKA & WILKE 2004, HAASE et al. 2007, BICHAIN et al. 2007a, b) have shown that these characters may be highly variable, are in part related to external abiotic or biotic factors, and thus are not necessarily taxonomically useful. Several species radiations have been identified (WILKE et al. 2010, SZAROWSKA et al. 2016), confirming the high species richness of the genus, but also confirming that morphological

characters overlap to a greater or lesser extent among species. Consequently, it appears necessary to evaluate a priori the morphological characters describing old and new taxa by integrating information from different types of data and methodologies. The challenge is to delineate species and thereby to identify characters that exhibit inter- and/or intra-specific variability. Application of this methodological and conceptual framework permits the proposal of more robust diagnostic tools (see for example: BICHAIN et al. 2007a, b, HAASE et al. 2007, FALNIOWSKI et al. 2009, 2016) and provides insight into the history and evolutionary processes that have generated the alpha-diversity in this taxonomic group (BENKE et al. 2009, 2011, WILKE et al. 2010, SZAROWSKA et al. 2016). Beyond these aspects, appropriate delimitation of species has important consequences both for a reliable estimate of biodiversity and for setting conservation priorities.

BICHAIN et al. (2007a, b) offered an example of three-step integrative taxonomy consisting of (i) defining primary hypotheses (current species delimitations based on phenetic characters), (ii) testing these hypotheses against criteria that are conceptually sound (Hennigian inter-nodal species concept inferred from molecular phylogenetic reconstructions), and (iii) providing a feedback to a new taxonomic hypothesis associated with new phenetic descriptors (multivariate morphometric analyses). Their results, derived from two genetic markers (rDNA ITS-1 and mtDNA COI) and continuous and discrete shell characters, allowed them to identify 11 molecular species-level lineages within a taxonomic group of 16 nominal species sampled from 35 populations mainly in south-western France. They generated an alternative species delimitation hypothesis with some newly-defined entities exhibiting distinct morphological features. Among these 11 evolutionary lineages, 8

corresponded to previous nominal species and 3 to undescribed species. These results were supported by additional sequences and phylogenetic analyses (BENKE et al. 2009, 2011, WILKE et al. 2010).

One of the undescribed species was later described as *Bythinella navacellensis* Prié & Bichain, 2009, a stygobiont species occurring within a small delimited and cohesive area of c. 300 km<sup>2</sup> around southern Larzac (PRIÉ & BICHAIN 2009). The other two putative new species identified by BICHAIN et al. (2007b) are considered to be endemic to south-western France, one to a single spring in the department of Tarn-et-Garonne and the other to the groundwater of the Padirac karstic network in the department of Lot. These taxa were named respectively ‘*Bythinella jodevidtsi*’ and ‘*B. lesuri*’ in a PhD thesis (BICHAIN 2007) that was subsequently made accessible online as a print-on-demand document (Editions Universitaires Européennes, <http://edition-eu.com>) (BICHAIN 2010). These two names are not available from the thesis nor from the print-on-demand version, according to article 9.12 of the Code of Zoological Nomenclature (ICZN 2012).

In this paper, we first test the species delimitation hypotheses of BICHAIN et al. (2007b) using a DNA barcoding approach (HEBERT et al. 2003, PUILANDRE et al. 2021) applied to a large COI dataset assembled from BOLD Systems (RATNASINGHAM & HEBERT 2007). We confirm all the previous species delineations except for ‘*Bythinella lesuri*’ from the Padirac karstic network. Therefore, we formally describe here *Bythinella jodevidtsi* n. sp. but ‘*Bythinella lesuri*’ is an unavailable name, as it was introduced in a work (the thesis) that was not a published work in the meaning of the Code (article 9.12). We also provide a correspondence between the groups delimited via our barcoding approach and the names proposed by BICHAIN et al. (2007b) and subsequent literature.

## MATERIAL AND METHODS

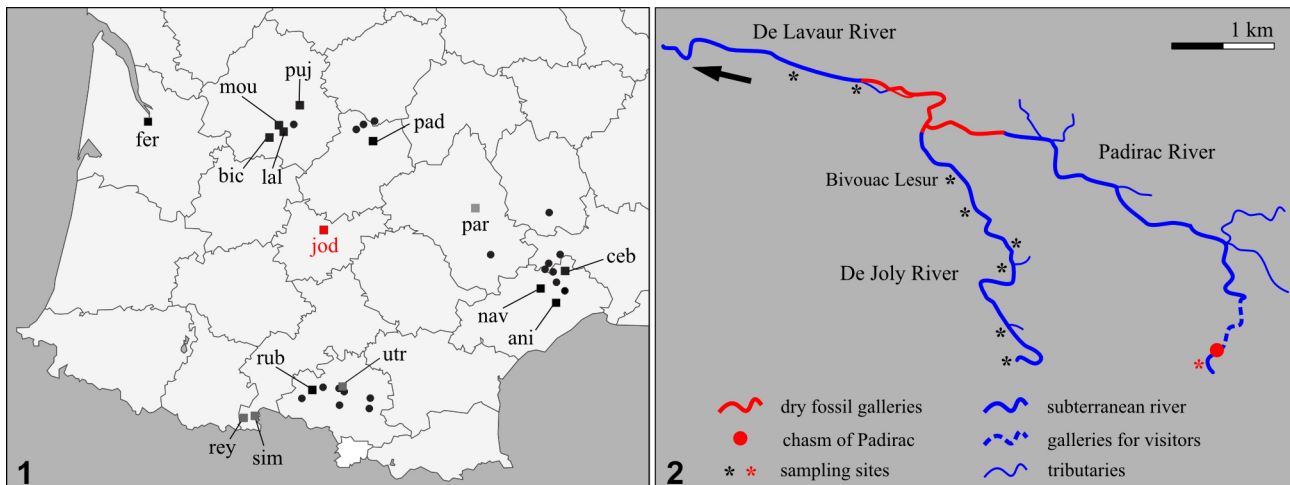
### GEOGRAPHICAL AREA AND SAMPLING METHODS

About 100 specimens were collected in the Saint-Hubert Spring (44°09'54.88"N, 01°08'52.48"E), near the village Dufort-Lacapelette (Tarn-et-Garonne, France), by hand or by washing small pebbles, aquatic vegetation and dead leaves over two sieves (2 mm and 450 µm).

In the Padirac cave (Lot, France) (Fig. 1), sampling was carried out in the deep subterranean river and in the upstream part of the Padirac river system (44°51'29.52"N, 01°45'01.26"E). Overall, ca. 60 specimens were collected from nine sampling sites (Fig. 2): six in the De Joly river, two in the De Lavaur river (N=2) and one in the “Salle de la Grande Arcade”

near the cave entrance. The latter is possibly, or located near, the type locality of *Bythinella padiraci* Locard, 1903. No specimens were collected in the upstream tourist section of the Padirac River. Details of the sampled locations are given in BICHAIN et al. (2004) and in Figure 2.

The type material of *B. padiraci* was also consulted at the MNHN (Muséum national d’Histoire naturelle, Paris) and 19 syntypes were included in the morphometric analyses. For anatomical and biometrical studies, specimens were fixed in 70% ethanol; for molecular studies, specimens with their shell were frozen alive at – 80 °C. Given the very small size of the specimens, the morphometric, anatomical and DNA sequence analyses could not each be carried out on the same individuals.



Figs 1–2. Location of *Bythinella* sampling sites in south-western France and in the Padirac subterranean karstic network: 1 – location of *Bythinella* sampling sites in south-western France after BICHAIN et al. (2007b) (filled circles correspond to sample sites and filled squares to type localities: ani – *B. anianensis* (Paladilhe, 1870); bic – *B. bicarinata* (Des Moulins, 1827); ceb – *B. cebennensis* (Dupuy, 1849); eur – *B. eurystoma* (Paladilhe, 1870); fer – *B. ferussina* (Des Moulins, 1827); jod – *B. jodevidtsi* n. sp.; lal – *B. lalindei* (Bernasconi, 2000); mou – *B. moulinsii* (Dupuy, 1849); nav – *B. navacellensis* Prié et Bichain, 2009; pad – *B. padiraci* Locard, 1903; par – *B. parvula* Locard, 1893; puj – *B. poujolensis* (Bernasconi, 2000); rei – *B. reyniesii* (Dupuy, 1851); rub – *B. rubiginosa* (Boubée, 1833); sim – *B. simoniana* (Moquin-Tandon, 1856); utr – *B. utriculus* (Paladilhe, 1874); grey squares indicate terra typica of nominal species involved in this study); 2 – location of sampling sites in the Padirac subterranean karstic network (the red stars indicate the type locality of *B. padiraci*, near the chasm of Padirac, in the «Salle de la Grande Arcade»)

## MOLECULAR SPECIES DELIMITATIONS

Sequences of the mitochondrial DNA cytochrome c oxidase subunit I (COI) and the nuclear ribosomal Internal Transcribed Spacer (ITS1) were obtained following the methods of BICHAIN et al. (2007b). In total, two COI sequences (GenBank numbers: EF016207-EF016208) were obtained from specimens sampled at the Saint-Hubert Spring. In the Padirac subterranean karstic network, four COI sequences (GenBank numbers: EF016184 to EF016187) and three ITS1 sequences (GenBank numbers: EF016148-EF016150) were obtained from specimens sampled in the “Salle de la Grande Arcade” (Fig. 2). Additionally, five COI sequences (GenBank numbers: EF016188 to EF016192) and two ITS1 sequences (GenBank numbers: EF016151-EF016152) were obtained from specimens sampled in the De Joly subterranean river at the Lesur bivouac (Fig. 2). All these sequences were incorporated in a global dataset of 95 COI sequences and 54 ITS1 sequences, which represents a taxonomic coverage of 16 nominal *Bythinella* species sampled from 35 populations mainly from south-western France (Fig. 1).

Concerning species delimitations, BICHAIN et al. (2007b) followed the Hennigian inter-nodal species concept formalized by SAMADI & BARBEROUSSE (2006). In this framework, species are considered sets of organisms that have genealogic relationships and form isolated, irreversible evolutionary lineages. Consequently, species-level taxa were delineat-

ed based on (i) the cohesiveness of haplotype networks and (ii) the monophyly criterion derived from the Phylogenetic Species Concept, in which species constitute the smallest diagnosable monophyletic groups.

In order to test the species delimitation hypotheses of BICHAIN et al. (2007b), the Assemble Species by Automatic Partitioning (ASAP) method (PUILLANDRE et al. 2021) was applied to an initial dataset of 1 338 COI sequences compiled from BOLD Systems (<https://boldsystems.org>, accessed 13 June 2022). All available BOLD data were mined from GenBank with reference to the sequence numbers and species names given in the relevant publications. We removed 13 sequences that contained too many uninformative ‘N’ nucleotides.

The sequences were aligned by the MEGA X software, version 10.1.7 (KUMAR et al. 2018) using the CLUSTAL W Multiple alignment algorithm (THOMPSON et al. 1994). In total, our dataset contains 1 325 COI sequences representing 113 nominal species with specimens from 22 countries including all specimens sequenced by BICHAIN et al. (2007b) and topotypes of 11 nominal species occurring in France. This global dataset was implemented in the online ASAP version (<https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>) with default settings and the Kimura-2-parameters (K2P) distance model (KIMURA 1980). The retained partition of species is the one with the lowest ASAP-score (PUILLANDRE et al. 2021).

The MOLD software (FEDOSOV et al. 2022) was also used, based of the BOLD final dataset and the ASAP species partition, to propose diagnostic molecular characters for the new taxon named here.

#### SHELL MEASUREMENTS

Before measurements, shells were washed in a 5% Chlorox solution for 30 minutes, then rinsed in distilled water, transferred to absolute ethanol and dried. The shells were then placed on an adhesive support in a standardized position (see BICHAIN et al.

2007a: fig. 2) and digitised with a graduated scale using a stereomicroscope associated with a digital camera. Seven parameters were recorded on each picture using the TpsDig 1.23 software package (ROHLF 2001): shell height (H), shell width (W), aperture height (Ho), aperture width (Wo), last whorl height (Hdt), last whorl width (Wdt) and number of whorls. Overall, 29 shells from the Saint-Hubert Spring, 31 shells from the deep subterranean network of the Padirac cave and 19 syntypes of *B. padiraci* were measured. All statistical analyses were performed with the software package STATISTICA 6.0 (STATSOFT 2001).

#### RESULTS

The best-scoring ASAP analysis (ASAP-score = 6.5; P-val =  $1.04 \times 10^{-2}$ ; Wrank =  $1.72 \times 10^{-6}$ ) delimited 61 species among 1,325 COI sequences with a distance threshold of 0.0271. The other partitions proposed by ASAP have much higher scores, but in all cases *B. jodevidtsi* n. sp. is considered as a separate species, with a genetic distance to the closest species of 0.057. The ASAP results also confirm that *Bythinella lancelevei* Locard, 1884 and *Bythinella carinulata* (Drouët, 1867) can indeed be considered as junior synonyms of *Bythinella viridis* (Poiret, 1801) (PRIÉ & CUCHERAT 2021).

In contrast, the specimens from the subterranean Padirac network are here assigned to only two species the applicable names of which are *Bythinella padiraci*

Locard, 1903 and *Bythinella* cf. *reyniesii* (Dupuy, 1851). All the specimens previously assigned to the putative species '*Bythinella lesuri*' (BICHAIN 2007, 2010) are clustered within the '*reyniesii*' group with K2P genetic distances with other species ranging from 0.0356 to 0.12797. Therefore, we do not recognise '*Bythinella lesuri*' from the Padirac cave as a valid species and, as the name was not introduced in a work deemed to have been published according to the Code (ICZN 2012), it is now considered unavailable.

The correspondence between the species delineated here and those reported by BICHAIN et al. (2007b) or in the subsequent literature as well as the intra- and interspecific genetic distances are given in Table 1.

#### SYSTEMATIC PART

**Class Gastropoda** Cuvier, 1795

**Subclass Caenogastropoda** Cox, 1960

**Family Bythinellidae** Locard, 1893

**Genus *Bythinella*** Moquin-Tandon, 1856

**Type species: *Bythinella viridis* (Poiret, 1801)**  
by subsequent designation (KADOLSKY 2005).

***Bythinella jodevidtsi* n. sp.**

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*Bythinella* sp. – BICHAIN et al. 2007b: fig. 2 (clade I);  
fig. 5 (*Bythinella* sp. 3)

*Bythinella* sp. 6 – WILKE et al. 2010: fig. 2 and fig. 3  
(lineage 2)

*Bythinella jodevidtsi* Bichain, 2007 – GLÖER 2022: p.  
257, fig. 410 [name not made available, unpublished thesis, BICHAIN 2007].

*Bythinella jodevidtsi* – BICHAIN 2010: p. 176, fig.  
9 [name not available, thesis published as a  
print-on-demand book (Editions Universitaires

Européennes, <http://edition-eu.com>), which does not constitute a published work according to the Code (ICZN 2012) article 9.12.]

**Type material.** Holotype (empty shell; MNHN-IM-2000-9536; Fig. 3), 10 paratypes (empty shells; MNHN-IM-2000-9537) deposited in the Muséum National d'Histoire Naturelle (Paris, France) and 10 paratypes (empty shells; MHNE.2022.2.1) deposited in the Musée d'Histoire Naturelle et d'Ethnographie de Colmar (France).

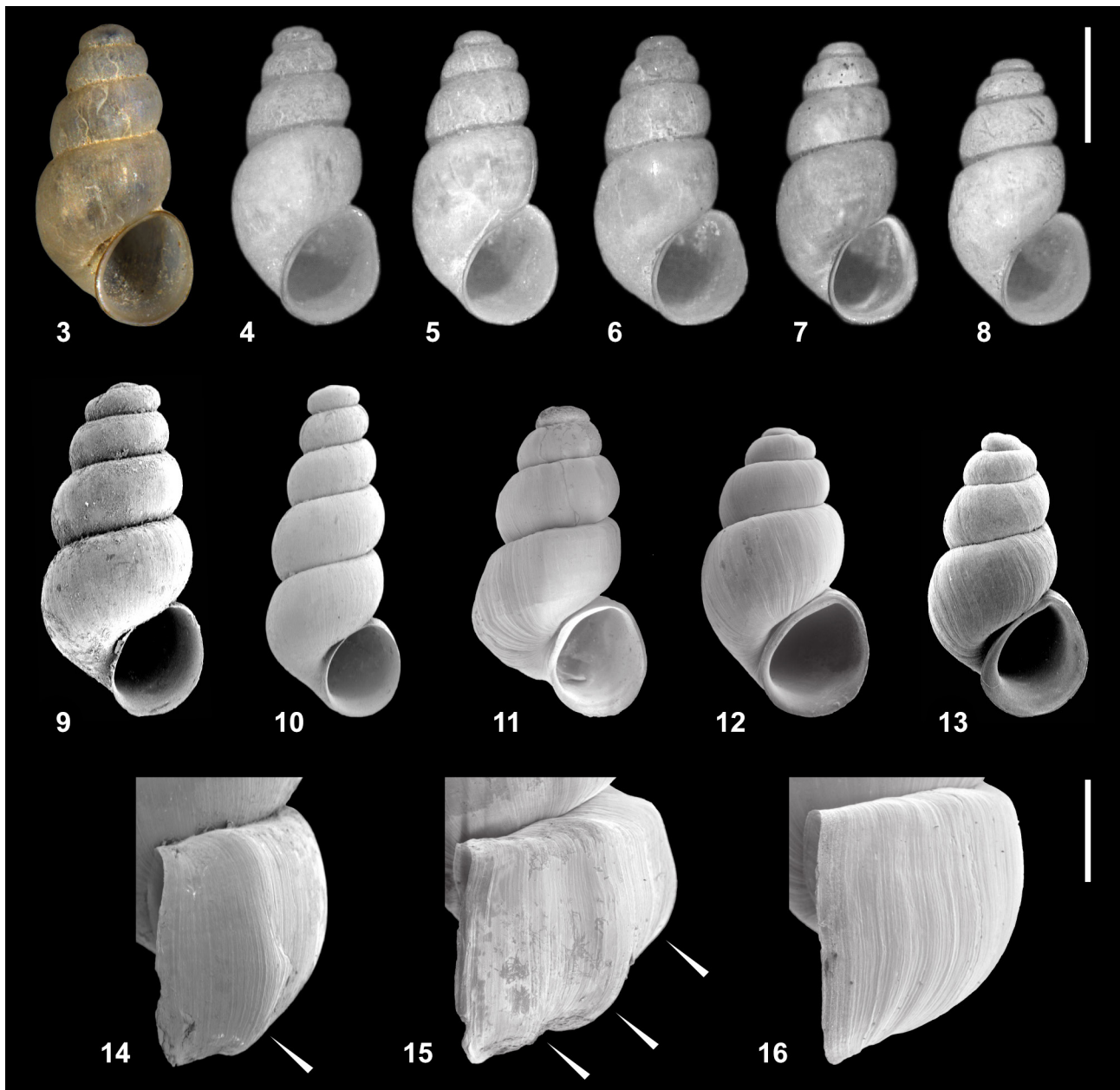
**Type locality.** Saint-Hubert Spring (44°09'54.88"N, 01°08'52.48"E) near the Dufort-Lacapelette Village (Tarn-et-Garonne, France).

**Other material.** Two sequences of the mitochondrial DNA cytochrome c oxidase subunit I (COI, GenBank numbers: EF016207-EF016208), from specimens sampled at the type locality and 31 specimens used for shell measurements also from the type locality (Table 2).



Table 1. Correspondence between monophyletic groups based on COI, species delimitation based on barcoding approach (this article); the species names applied to these clades and proposed synonymies. Abbreviations used: Cl – clade (BICHAIN et al. 2007b: figs 2 & 3); intrasp. K2p-dist – intraspecific K2P-distance; intersp. K2P-dist – interspecific K2P-distance (minimum and maximum values); nc – not concerned.

| Cl  | Valid name  | Junior synonymym  | Status of specimens studied                 | Bold sequences | Authors  | intrasp. K2p-dist | intersp. K2P-dist   |
|-----|---|---|---|----------------|--|-------------------|---------------------|
| E   | <i>viridis</i> Poiret, 1801 [ <i>Bulimus</i> ]                      |   | topotype                                    | 29 COI         | BICHAIN et al. (2007b),<br>PRIÉ & BICHAIN (2009) | 0.009             | 0.105 < K2p < 0.119 |
| A   | <i>bicarinata</i> Des Moulins, 1827<br>[ <i>Paludina</i> ]          | <i>carinulata</i> Drouët, 1867 [ <i>Hydrobia</i> ]                          | ergonymotope (sensus<br>FRÉTEY et al. 2018) | 3 COI          | PRIÉ & CUCHERAT (2021)                           |                   |                     |
|     |   | <i>lanceleveii</i> Locard, 1884 [ <i>Bythinella</i> ]                       | topotype                                    | 3 COI          | PRIÉ & CUCHERAT (2021)                           |                   |                     |
|     |   | <i>moulinii</i> Dupuy, 1849 [ <i>Bitinia</i> ]                              | topotype                                    | 3 COI          | BICHAIN et al. (2007b)                           | 0.002             | 0.041 < K2p < 0.138 |
|     |   | <i>dumkeri</i> Frauenfeld, 1857<br>[ <i>Paludina</i> ]                      | topotype                                    | 11 COI         | BICHAIN et al. (2007b)                           |                   |                     |
|     |   | <i>lalindei</i> Bernasconi, 2000<br>[ <i>Bythinella</i> ]                   | topotype                                    | 37 COI         | BICHAIN et al. (2007b),<br>BENKE et al. (2009)   |                   |                     |
| J   | <i>rubiginosa</i> Boubée, 1833 [ <i>Paludina</i> ]                  | <i>pujolensis</i> Bernasconi, 2000<br>[ <i>Bythinella</i> ]                 | topotype                                    | 3 COI          | BICHAIN et al. (2007b)                           |                   |                     |
|     |   | <i>simoniana</i> Moquin-Tandon, 1856<br>[ <i>Bythinia</i> ]                 | topotype                                    | ITS1           | BICHAIN et al. (2007b)                           | nc                | nc                  |
|     |   | <i>anianensis</i> Paladilhe, 1870<br>[ <i>Paludina</i> ]                    | terra typica                                | ITS1           | BICHAIN et al. (2007b)                           | nc                | nc                  |
| H   | <i>cebenensis</i> Dupuy, 1851 [ <i>Bitinia</i> ]                    | <i>compressa</i> Frauenfeld, 1857<br>[ <i>Paludina</i> ]                    | topotype                                    | 6 COI          | BICHAIN et al. (2007b)                           | <0.001            | 0.039 < K2p < 0.128 |
|     |   | <i>parvula</i> Locard, 1893 [ <i>Bythinella</i> ]                           | topotype                                    | 2 COI          | BICHAIN et al. (2007b)                           |                   |                     |
| D   | <i>reyniesii</i> Dupuy, 1851 [ <i>Hydrobia</i> ]                    | <i>parvula</i> Locard, 1893 [ <i>Bythinella</i> ]                           | topotype                                    | 9 COI          | BICHAIN et al. (2007b)                           | 0.010             | 0.036 < K2p < 0.127 |
|     |   | <i>rufescens</i> Küster, 1852 [ <i>Paludina</i> ]                           | topotype                                    | 19 COI         | BENKE et al. (2009)                              |                   |                     |
| F   | <i>eurystoma</i> Paladilhe, 1870<br>[ <i>Paludina</i> ]             | <i>persuturata</i> Bofill, Haas et Aguilar-Amat, 1921 [ <i>Bythinella</i> ] | topotype                                    | 5 COI          | BICHAIN et al. (2007b)                           |                   |                     |
|     |   | <i>badensis</i> Boeters, 1981 [ <i>Bythinella</i> ]                         | topotype                                    | 5 COI          | BENKE et al. (2009)                              |                   |                     |
|     |   | <i>boldi</i> Bofill, Haas et Aguilar-Amat, 1921 [ <i>Bythinella</i> ]       | topotype                                    | 2 COI          | BOLD SEQUENCE ID<br>MOLCA037-19.COI-5P           |                   |                     |
| K   | <i>utriculus</i> Paladilhe, 1874<br>[ <i>Paludina</i> ]             | <i>badensis</i> Boeters, 1981 [ <i>Bythinella</i> ]                         | topotype                                    | 18 COI         | BENKE et al. (2009)                              | <0.001            | 0.039 < K2p < 0.127 |
|     |   | <i>utriculus</i> Paladilhe, 1874<br>[ <i>Paludina</i> ]                     | topotype                                    | 19 COI         | BICHAIN et al. (2007b)                           |                   |                     |
| C   | <i>padraci</i> Locard, 1903 [ <i>Bythinella</i> ]                   | <i>utriculus</i> Paladilhe, 1874<br>[ <i>Paludina</i> ]                     | topotype                                    | 4 COI          | BICHAIN et al. (2007b)                           | 0.003             | 0.056 < K2p < 0.132 |
|     |   | <i>padraci</i> Locard, 1903 [ <i>Bythinella</i> ]                           | topotype                                    | 1 COI          | BICHAIN et al. (2007b)                           | nc                | 0.034 < K2p < 0.134 |
| G-F | <i>navacellensis</i> Prié et Bichain, 2009<br>[ <i>Bythinella</i> ] | <i>navacellensis</i> Prié et Bichain, 2009<br>[ <i>Bythinella</i> ]         | topotype                                    | 8 COI          | BICHAIN et al. (2007b),<br>PRIÉ & BICHAIN (2009) | 0.001             | 0.026 < K2p < 0.134 |
|     |   | <i>jodevidtsi</i> n. sp. [ <i>Bythinella</i> ]                              | topotype                                    | 2 COI          | this article                                     | 0                 | 0.057 < K2p < 0.138 |



Figs 3–16. Shells of *Bythinella* spp.: 3 – holotype of *Bythinella jodevidtsi* n. sp., 4–8 – *Bythinella jodevidtsi* n. sp. from type locality, 9 – *Bythinella cebennensis* (Dupuy, 1849) from Brissac, Buèges Spring (Hérault, France), 10 – *Bythinella ferussina* (Des Moulins, 1827) from the type locality at Château d'Eyrans near Bordeaux (Gironde, France), 11 – *Bythinella* cf. *simoniana* (Moquin-Tandon, 1856) from the Arguilla Spring near Engomer (Ariège, France), 12 – *Bythinella rubiginosa* (Boubée, 1833) from the type locality at Audinac-les-Bains (Ariège, France), 13 – *Bythinella eurystoma* (Paladilhe, 1870) from Saint-Julien-de-la-Nef (Gard, France), 14 – aperture of *B. ferussina*, specimen illustrated in Fig. 10, 15 – aperture of *B. cf. simoniana*, specimen illustrated in Fig. 11, 16 – aperture of *B. rubiginosa*, specimen illustrated in Fig. 12. Arrows indicate the axial varices just behind the aperture. Scale bars 1 mm

**Etymology.** Named after Joseph Devidts (1921–2003), curator of Molluscs at the Musée Zoologique de Strasbourg (France).

**Molecular diagnostic characters.** A molecular diagnosis was obtained with the MOLD software (FEDOSOV et al. 2022), which searches for single nucleotides and combinations of nucleotides that are diagnostic for a given taxon. For *Bythinella jodevidtsi* n. sp., the presence of a 'G' at position 510 of the

658 bp COI fragment (Folmer fragment) has been identified as a diagnostic nucleotide within the genus *Bythinella*, and the shortest rDNC (redundant Diagnostic Nucleotide Combination, see FEDOSOV et al. 2022) corresponds to a 'C' in position 147, a 'T' in position 483 and a 'G' in position 510 [147: 'C', 483: 'T', 510: 'G'].

**Description.** Shell distinctly elongated with relative high spire, up to 3.35 mm high and 1.35 mm wide,

Table 2. Shell measurements: shell height (H), shell width (W), aperture length (Ho), aperture width (Wo), last whorl height (Wdt), last whorl width (Hdt). The measurements are given in millimetres

| <i>B. jodevidtsi</i> n. sp.                 | H    | W    | Hdt  | Wdt  | Ho   | Wo   | W/H  | Ho/H | Hdt/H | Wo/Ho |
|---|------|------|------|------|------|------|------|------|-------|-------|
| Mean [N=31]                                 | 2.97 | 1.57 | 2.02 | 1.35 | 1.22 | 1.00 | 0.53 | 0.41 | 0.68  | 0.82  |
| Minimum                                     | 2.75 | 1.39 | 1.89 | 1.23 | 1.14 | 0.87 | 0.48 | 0.39 | 0.64  | 0.74  |
| Maximum                                     | 3.35 | 1.82 | 2.25 | 1.49 | 1.38 | 1.09 | 0.58 | 0.44 | 0.72  | 0.88  |
| Standard deviation                          | 0.16 | 0.08 | 0.10 | 0.06 | 0.06 | 0.05 | 0.02 | 0.02 | 0.02  | 0.03  |
| <i>B. cf. reyniesii</i> [Padirac cave]      | H    | W    | Hdt  | Wdt  | Ho   | Wo   | W/H  | Ho/H | Hdt/H | Wo/Ho |
| Mean [N=29]                                 | 2.23 | 1.49 | 1.65 | 1.23 | 1.05 | 0.89 | 0.67 | 0.47 | 0.74  | 0.85  |
| Minimum                                     | 1.90 | 1.30 | 1.39 | 1.09 | 0.87 | 0.74 | 0.59 | 0.40 | 0.65  | 0.78  |
| Maximum                                     | 2.70 | 1.79 | 1.96 | 1.36 | 1.26 | 1.12 | 0.73 | 0.50 | 0.79  | 0.90  |
| Standard deviation                          | 0.20 | 0.13 | 0.14 | 0.07 | 0.10 | 0.09 | 0.03 | 0.02 | 0.03  | 0.03  |
| <i>B. cf. reyniesii</i> [epigean specimens] | H    | W    | Hdt  | Wdt  | Ho   | Wo   | W/H  | Ho/H | Hdt/H | Wo/Ho |
| Mean [N=178]                                | 2.39 | 1.56 | 1.84 | 1.30 | 1.09 | 0.96 | 0.65 | 0.46 | 0.77  | 0.88  |
| Minimum                                     | 1.97 | 1.31 | 1.59 | 1.15 | 0.91 | 0.82 | 0.54 | 0.40 | 0.69  | 0.80  |
| Maximum                                     | 2.79 | 1.88 | 2.18 | 1.42 | 1.34 | 1.12 | 0.81 | 0.52 | 0.83  | 0.96  |
| Standard deviation                          | 0.17 | 0.10 | 0.10 | 0.06 | 0.07 | 0.05 | 0.04 | 0.02 | 0.03  | 0.03  |
| <i>B. padiraci</i> Locard, 1903 syntypes    | H    | W    | Hdt  | Wdt  | Ho   | Wo   | W/H  | Ho/H | Hdt/H | Wo/Ho |
| Mean [N=19]                                 | 3.28 | 1.85 | 2.11 | 1.51 | 1.24 | 1.04 | 0.56 | 0.38 | 0.64  | 0.84  |
| Minimum                                     | 2.93 | 1.65 | 1.83 | 1.38 | 1.10 | 0.95 | 0.56 | 0.37 | 0.63  | 0.86  |
| Maximum                                     | 3.82 | 2.03 | 2.29 | 1.62 | 1.34 | 1.12 | 0.53 | 0.35 | 0.60  | 0.83  |
| Standard deviation                          | 0.23 | 0.11 | 0.12 | 0.08 | 0.07 | 0.05 | 0.02 | 0.02 | 0.03  | 0.02  |

with ca. 4–4.5 whorls (Figs 3–8). Teleoconch whorls with slightly convex outline and growing regularly in width. Last whorl reaches on average ca. two-thirds ( $0.68 \pm 0.02$ ) of shell height. Aperture distinctly ellipsoid, up to 1.22 mm for the long axis, reaches less than half ( $0.44 \pm 0.02$ ) of shell height. Aperture outer lip simple, often angular in its sinusal part and widening in its lower part. Umbilicus slit-like. Teleoconch glossy, with delicate growth lines, periostracum dark brown. Operculum smooth on its inner and outer surfaces. Shell parameters for a series of specimens (N=31) are given in Table 2.

**Distribution.** Based on current information, this new species is restricted to the type locality (Fig. 1).

**Ecology.** Epigean and crenobiont species.

**Remarks.** *B. jodevidtsi* n. sp. constitutes a distinct clade, sister to the clade formed by *B. rubiginosa* (Boubée, 1833) and *B. utriculus* (Paladilhe, 1874), two Pyrenean endemic species (BICHAIN et al. 2007b, PRIÉ & BICHAIN 2009, WILKE et al. 2010).

## DISCUSSION

Our results based on a barcoding approach support the species rank for *B. jodevidtsi* n. sp. as proposed by BICHAIN et al. (2007b). This species belongs to a clade that contains genetically closely related species and has shell characters that partly overlap with those of its sister species. This situation is normal for the genus *Bythinella* in which most species do not exhibit clearly distinct morphological characters.

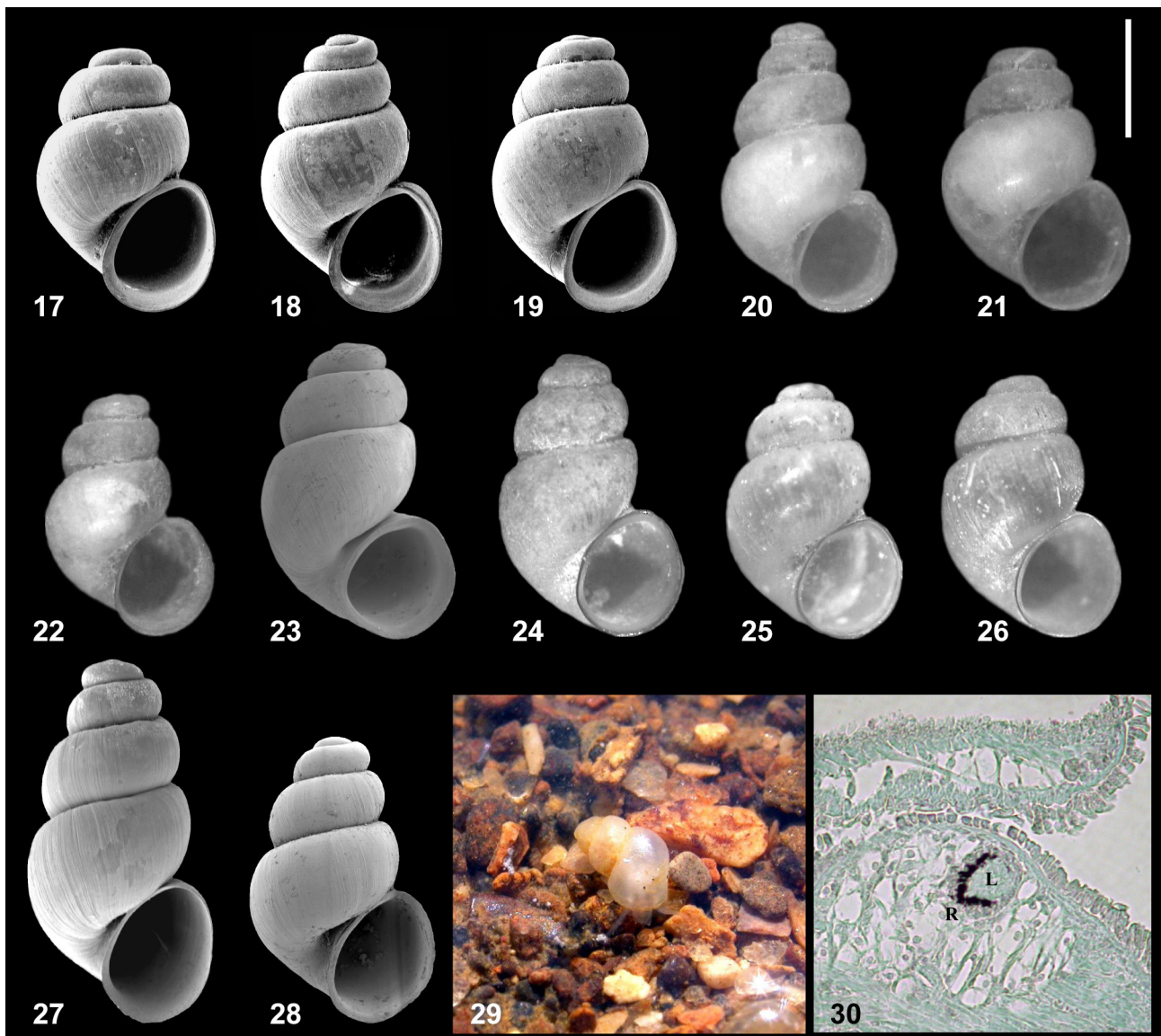
Morphologically, this new species differs clearly from *B. utriculus* (Paladilhe, 1874), which has an ovoid-globose shell shape and a smaller average shell size (height up to 3.15 mm and  $2.50 \pm 0.24$  mm on average), and from *B. rubiginosa* (sensu BICHAIN et al. 2007b: table 2) (Figs 12 & 16), which has more convex and angular whorls and a higher ratio of last whorl height to shell height ( $Hdt/H: 0.70 \pm 0.038$ ). *B. jodevidtsi* n. sp. can be distinguished from other *Bythinella* species from southern France the shells of which are also elongated, such as *B. ferussina* (Des Moulins, 1827) (Figs 10 & 14), *B. simoniana* (Moquin-Tandon, 1856) (Figs 11 & 15), *B. cebennensis* (Dupuy, 1849) (Fig. 9), and *B. eurystoma* (Paladilhe, 1870) (Fig. 13). *Bythinella cebennensis* and *B. ferussina* have shells with very convex whorls, deep sutures, round-oval aperture (Figs 9–10) or an axial varix behind the aperture (Fig. 14). *B. simoniana* have shells with distinctly angular whorls and several axial varix behind the aperture (Fig. 15). Finally, *B. eurystoma* has a patulescent aperture and conical spire (Fig. 13).

Indeed, according to WILKE et al. (2010), non-adaptive radiations seem to be the rule within *Bythinella*. Although the concept of non-adaptive radiation has been controversial in molluscs (e.g. GITTENBERGER 1991, 2004, DAVIS 1993), a non-adaptive radiation process may be suspected within a monophyletic group of species that diversified within a relatively short period, without clear differentiation of ecolog-

ical niches, and usually with a low degree of phenotypic variation and with allopatric distributions (see [RUNDELL & PRICE 2009](#) and [WILKE et al. 2010](#) for an overview). Such a radiation can be explained by the subdivision of an ancestral population into isolated but similar habitats through dispersal or vicariance. The consequence of such an event may be diversification not accompanied by adaptation to different niches.

Our results suggest also the presence of two distinct phylogenetic lineages, attributed to *B. cf. reyniesii* (Figs 17–26) and *B. padiraci* (Fig. 27), in the

upper stream of the Padirac subterranean hydrogeological system. The specimens of *B. cf. reyniesii* collected from the deep parts of the Padirac cave exhibit typical troglomorphic features ([BICHAIN et al. 2004](#)) such as the lack of tegument pigmentation (Fig. 29), eye regression (Fig. 30), small shell size (Table 2) and, moreover, genetic divergence from the other epigeal specimens belonging to the ‘*reyniesii*’ lineage. Drifting with surface waters towards subterranean habitats (or progressive backward colonisation from springs) appear as probable and recurrent ways of invasion ([BOETERS 1979, 1992](#), [GIUSTI & PEZZOLI](#)



Figs 17–30. Shells of *Bythinella* spp.: 17–26 – *Bythinella cf. reyniesii* (Dupuy, 1851) (17–22 – specimens sampled in the deep parts of the Padirac Cave (Bivouac Lesur, see map on the [Fig. 2](#)), 23 – Bousсенac, spring at the Col-de-Port (Ariège, France), 24 – Montségur, spring at Barrineuf (Ariège, France), 25 – Bousсенac, fountain at Col-de-Port (Ariège, France), 26 – Bousсенac, watering hole at Col-de-Port (Ariège, France)); 27 – syntype of *Bythinella padiraci* Locard, 1903; 28 – *Bythinella navacellensis* Prié et Bichain, 2009 from the type locality at the Folatière cave (Gard, France); 29 – living specimen of *Bythinella cf. reyniesii* (Bivouac Lesur, Padirac cave); 30 – histological section showing the eye pigimentary layer of *Bythinella cf. reyniesii* (Bivouac Lesur, Padirac cave). Abbreviations: L – lens, R – retina with pigmented layer. Scale bar 1 mm





1982, BOLE & VELKOVHRH 1986, BERNASCONI 2000, HLAVÁČ 2002).

The taxonomic status of *B. padiraci* Locard, 1903 remains unclear and requires additional data. The syntypes (MNHN; Fig. 27), sampled by LOCARD (1903) from the upstream part of the Padirac subterranean river, are highly variable in shape and size (Table 2; see also LOCARD 1903 and BERNASCONI 2000). It seems likely, as our molecular results indicate, that the type material belongs to several nominal species including *B. padiraci* in the sense of LOCARD (1903) (elongated subcylindroid shell composed of 4.5 to 5 whorls; Fig. 27) and *B. cf. reyniesii* (pupoid shell; Figs 17–26). The designation of a neotype on the basis of molecular evidence seems to be the only way to solve this taxonomic problem.

South-western France, including the Pyrenees and its foothills, as well as the southern margin of the Massif Central has been identified as a glacial refugium and a biodiversity hotspot for the genus *Bythinella* (BENKE et al. 2009, 2011) with about 32 nominal species considered as valid by INPN (2022). However, our results and those of BICHAIN et al. (2007a, b), covering 22 nominal species mainly from south-western France, suggest a 60% lower species diversity (9 against 22 Table 1). Consequently, a strictly shell-based taxonomic approach seems to overestimate the species richness of the genus and leads to a confusing picture of conservation priorities. For example, *B. eutrepha* (Paladilhe, 1867) was described from empty shells sampled in the alluvium of the Lez by PALADILHE (1867). Although its taxonomic status is still unclear (PRIÉ & BICHAIN 2009), it is categorized as Critically Endangered in the national red list of threatened molluscs in France

(UICN COMITÉ FRANÇAIS, OFB & MNHN 2021). Additionally, GLOËR (2022) considered *B. navacellen-sis* Prié et Bichain, 2009 (Fig. 28) as a junior synonym of *B. eutrepha*, without any explicit argument. This author also considers 10 species to be valid while ignoring previously published molecular data (Table 1), which sets part of the taxonomy of the genus back 15 years.

The molecular data seem to confirm that the high number of described nominal species may reflect to some extent part of the real diversity. However, we note that taxonomic changes involve more than half of the species currently recognised as valid. There are probably still new species to be discovered, but the question remains which of the currently described taxa correspond to real species. For this reason, we concur again with the plea of HAASE et al. (2007) for the application of more integrative approaches to the delimitation and/or revision of species within the genus *Bythinella*.

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