



The polymorphic top-shell puzzle: iterative taxonomy of *Calliostoma* 2 Swainson, 1840 (Gastropoda: Calliostomatidae) in the Mediterranean Sea

Giacomo Chiappa, Giulia Fassio, Carlo Smriglio, Paolo Mariottini, Paolo G. Albano, Maria Vittoria Modica, Dario Zuccon, Nicolas Puillandre, Marco Oliverio

► To cite this version:

Giacomo Chiappa, Giulia Fassio, Carlo Smriglio, Paolo Mariottini, Paolo G. Albano, et al.. The polymorphic top-shell puzzle: iterative taxonomy of *Calliostoma* 2 Swainson, 1840 (Gastropoda: Calliostomatidae) in the Mediterranean Sea. *Journal of Molluscan Studies*, 2024, 90 (3), pp.eyae026,. <10.1093/mollus/eyae026>. <hal-04987800>

HAL Id: hal-04987800

<https://hal.science/hal-04987800v1>

Submitted on 12 Mar 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

The polymorphic top-shell puzzle: iterative taxonomy of *Calliostoma* Swainson, 1840 (Gastropoda: Calliostomatidae) in the Mediterranean Sea

Giacomo Chiappa¹, Giulia Fassio¹, Carlo Smriglio², Paolo Mariottini², Paolo G. Albano³, Maria Vittoria Modica⁴, Dario Zuccon⁵, Nicolas Puillandre⁵ & Marco Oliverio¹

¹*Department of Biology and Biotechnologies “Charles Darwin”, Sapienza University of Rome, Viale dell’Università, 32, 00185 Rome, Italy; giacomo.chiappa@uniroma1.it, giulia.fassio@uniroma1.it, marco.oliverio@uniroma1.it*

²*Department of Science, University RomaTre, Viale Marconi 446, 00146 Rome, Italy; csmriglio@alice.it, paolo.mariottini@uniroma3.it*

³*Department of Marine Animal Conservation and Public Engagement, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy; pgalbano@gmail.com*

⁴*Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Via Gregorio Allegri 1, 00198 Rome, Italy; mariavittoria.modica@szn.it*

⁵*Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d’Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 51, 75005 Paris, France; dario.zuccon@mnhn.fr, nicolas.puillandre@mnhn.fr*

Running head: ITERATIVE TAXONOMY OF CALLIOSTOMA

Correspondence: G. Chiappa; email: giacomo.chiappa@uniroma1.it

ABSTRACT

Calliostoma Swainson, 1840 as currently conceived, is the most species rich genus of the order Trochida, with over 350 recognised species worldwide. The shell variability in these vetigastropods is extremely high, resulting in conflicting taxonomic attributions at both the genus and the species levels. In the Mediterranean Sea, the remarkable morphological diversity of the *Calliostoma* top-shells has led to the description of dozens of different taxa, of which nine are currently accepted. This taxonomic framework was tested using an iterative taxonomic approach. Species-delimitation (ASAP, clade monophyly, K2P distances), and phylogenetic analyses (Maximum Likelihood and Bayesian Inference) were carried out on 247 specimens from the Mediterranean and neighbouring Atlantic, including eight Mediterranean species and the Azorean *C. lividum*, spanning a large part of the morphological diversity and geographic distribution of the genus in the area. The molecular dataset comprised one nuclear (*its2*) and two mitochondrial markers (*cox1*, *16S rRNA*). Results indicate that the number of species is overestimated, as only *C. conulus*, *C. granulatum*, *C. zizyphinum* and *C. laugier* are supported by molecular data among the assessed species. It is suggested that the morphological characters commonly used to diagnose species are variable within a single taxon, as three nominal taxa, allegedly endemic to the Mediterranean Sea, are here shown to be a single genetic species (*Calliostoma laugier*). An *its2* 2D folding structure is also reported as potentially distinctive for calliostomatids, compared to known Vetigastropoda. Our study indicates that to address the taxonomy of calliostomatid top-shells, an integrative approach including molecular data is highly advisable to support species delimitation and especially new species description.

INTRODUCTION

Traditionally, taxonomic practice has been heavily dependent on the external appearance of organisms, and morphology represented a valuable source of characters for describing, cataloguing, and recognising species and for inferring phylogenetic relationships. In malacology, the shell is of critical importance, due to the richness of characters and its long-lasting nature, allowing fossil and extant specimens to be studied with the same morphological approach. Nevertheless, delimiting morphological boundaries among species often requires the ability to identify evolutionarily divergent traits in intrinsically polymorphic organisms, which is not an easy task. The relative importance of diagnostic characters can be subject to personal interpretation, and in complicated groups this could lead to biased diversity estimates and conflicting taxonomy. The top shells of the genus *Calliostoma* are a perfect example of this situation.

Calliostoma Swainson, 1840 was long included in the vetigastropod family Trochidae Rafinesque, 1815, and later reassigned to a separate family: Calliostomatidae Thiele, 1924 (1847) (Marshall, 1995a; Bouchet *et al.*, 2005). Among trochoideans, the most representative features of this family are a protoconch sculptured with a honeycomb pattern and a carnivorous diet, the latter trait being very uncommon in Vetigastropoda (Hickman & McLean, 1990; Hickman & Taylor, 1996). Species of *Calliostoma* are commonly found worldwide and the genus as currently conceived displays a high degree of morphological diversification in shell size, shape, colour, and sculpture.

Species identification based exclusively on shell morphology in Calliostomatidae can be difficult even combining larval and adult sculpture (Ikebe, 1942) with the morphology of the radular teeth (Warén, 1990). This problem extends to supraspecific classification, and due to the “bewildering

1
2
3 67 mosaic of intermediate character states” (Marshall, 1995a) among species of putatively different
4 68 taxa, most of the ~600 known recent species of the family still fall under the nominotypical genus
5 69 *Calliostoma* s.l. (Landau *et al.*, 2017; Marshall, 1995a, 1995b; Quinn, 1992). In the Mediterranean
6 70 Sea, more than a hundred names have been introduced for species and varieties of *Calliostoma*
7 71 (Ghisotti & Melone, 1971). The frustration with this puzzling genus prompted the proposition by
8 72 Cantraine (1835) to institute a single taxon, to be named “*Trochus polymorphus*” which would
9 73 include all Mediterranean calliostomatids until a truly reliable set of diagnostic characters was
10 74 found (Ghisotti & Melone, 1971; Parenzan, 1970). Currently, there are nine accepted species for
11 75 the Mediterranean area and two taxa inquirenda.
12
13
14
15 76 The assumption that different phenotypes are correlated to evolutionary divergence of
16 77 reproductively isolated lineages, and thus good proxies of species distinction, can sometimes be
17 78 misleading, resulting in (i) the overestimation of species diversity in groups that display high
18 79 plasticity (Chiappa *et al.*, 2022; Fassio *et al.*, 2019, 2020; Russini *et al.*, 2020), or (ii) the
19 80 underestimation of species diversity in groups with homogeneous or cryptic external features
20 81 (Barco *et al.*, 2013; Furfaro *et al.*, 2018, 2022; Krug *et al.*, 2013). An iterative taxonomic approach
21 82 (Dayrat, 2005; Yeates *et al.*, 2011), combining the information given by genetic markers with the
22 83 specialistic interpretation of phenotypic variability, together with the study of the biology and the
23 84 ecology of organisms, has greatly contributed to revealing and solving species delimitation issues
24 85 in problematic taxa (Duda *et al.*, 2008; Fassio *et al.*, 2022a, 2022b; Hebert *et al.*, 2003; Lee, 2004;
25 86 Puillandre *et al.*, 2009, 2014; Russini *et al.*, 2017).
26
27
28
29
30 87 Molecular data are still largely lacking in the Calliostomatidae, hampering an iterative approach on
31 88 a vast scale which could be decisive for disentangling its systematics (Callea, 2012; Gofas &
32 89 Hoffman, 2020; Landau *et al.*, 2017). In this study we have attempted a first taxonomical
33 90 reappraisal of the genus *Calliostoma* in the Mediterranean Sea and neighbouring Atlantic area
34 91 using an iterative approach, with the genetic assessment of specimens ascribed to 20
35 92 morphotypes — of which eight are currently accepted as valid species (WoRMS, accessed on 25th
36 93 March 2024) — which eventually converged into four species. We also present a first phylogenetic
37 94 reconstruction of the subfamily Calliostomatinae using all currently available data.
38
39
40
41
42 95
43
44
45

46 96 MATERIALS AND METHODS

46 97 *Dataset*
47
48 98 Our dataset consisted of 247 specimens sampled throughout the Mediterranean, the nearby
49 99 Atlantic and the Azores, including specimens identified as *C. conulus* (Linnaeus, 1758), *C.*
50 100 *funiculatum* Ardovali, 2011, *C. granulatum* (Born, 1778), *C. gualterianum* (R. A. Philippi, 1848), *C.*
51 101 *laugierii* (Payraudeau, 1826), *C. lividum* Dautzenberg, 1927, *C. spongiorum* (Bucquoy, Dautzenberg
52 102 & Dollfus, 1885), *C. virescens* Coen, 1933, and *C. zizyphinum* (Linnaeus, 1758) (Table 1, Fig. 1).
53 103 Specimens were either directly fixed in ethanol, or preprocessed in a microwave oven to facilitate
54 104 separation of soft tissue from the shell (Galindo *et al.* 2014) and then preserved in 95–98%
55 105 ethanol. Vouchers are stored in the malacological collection of the Department of Biology and
56 106 Biotechnologies “Charles Darwin”, Sapienza University of Rome (BAU), the Muséum national
57 107 d’Histoire Naturelle, Paris (MNHN), the Museo Nacional de Ciencias Naturales, Madrid (MNCN),

Stazione Zoologica Anton Dohrn (CY), and the Museo di Zoologia of the University of Bologna (MZUB).

Laboratory and Bioinformatic analyses

Upon collection, whole samples or a tissue clip were preserved in ethanol for molecular analyses. A fragment (3–30 mg) of tissue, preferably dissected from the foot, was lysed and processed for DNA extraction following a salting-out protocol from Aljanabi & Martinez (1997) as modified by Fassio *et al.* (2022c). Two fragments of the mitochondrial *cox1* and *16S rRNA* and a fragment of the nuclear *its2* were amplified via PCR (see Supplementary Material 4 for the list of used primers) using 1 µL of DNA with a 24 µL mix comprising 16.55–15.5 µL H₂O, 2.5–3 µL Reaction Buffer (10 pmol NH₄), 0.5 µL dNTP (10 pmol), 2.5–3 µL MgCl₂, 1 µL BSA 10%, 0.4 µL of each primer (25 pmol), and 0.15–0.2 µL BioTAQ™ DNA Polymerase. The open tube containing the mix was pre-emptively exposed to ultraviolet light for 15 minutes at <10 cm distance from the source before adding dNTPs, primers, and DNA polymerase in order to minimize chances of external contamination (Champlot *et al.*, 2010). Concentration of MgCl₂ was gradually decreased down to 0.75 µL for amplification of the *its2* until adequate results were obtained. For amplification of the *cox1*, jgLCO1490 and jgHCO2198 (Geller *et al.* 2013) primers were used, and the best performing cycle was a “touchdown” protocol, which started with 2 min at 94 °C, followed by 35–45 cycles with 10 sec at 94 °C, 30 sec at 60 °C decreasing by 1 °C each cycle for the first ten cycles, and 50 sec at 72 °C, and a final elongation step at 72 °C for 7 min (modified from Leray *et al.*, 2013). For *16S rRNA* the sequencing primers were 16SA (Palumbi, 1996) combined with CGleur (Hayashi, 2005) for longer sequences (~800 bp) or 16SH (Espirito *et al.* 2001) for shorter sequences (~550 bp); for *its2* the primers were 3d and 4R (Oliverio & Mariottini, 2001). PCR started with 5 min at 94 °C, followed by 35 cycles with 30 sec at 94 °C, 40 sec at 52 °C and 50–60 sec at 72 °C, and a final elongation step at 72 °C for 7 min. The two internal primers mICOLintF and mICOLintR (Leray *et al.* 2013) were paired with the external *cox1* primers for some difficult samples to amplify two non-overlapping minibarcodes, using the same PCR protocol used for *cox1* and using 1–2 µL of DNA or 1 µL of *cox1* amplicon.

PCR amplification success was checked by electrophoresis on 1.5% agarose gel stained with 3.5 µL of Midori Green Advance (Nippon Genetics Europe, Düren, Germany). Suitable PCR products were purified with 8 µL ExoSAP-IT (79 µL H₂O, 20 µL rAPid Alkaline Phosphatase, 1 µL Exonuclease I), incubated at 37 °C for 15 min and at 80 °C for 15 min, and finally sent to Macrogen Inc. (Madrid, Spain and Milan, Italy) for Sanger sequencing.

Samples collected in the MNHN CORSICABENTHOS expeditions were processed following a separate pipeline. DNA was extracted using the Macherey-Nagel NucleoSpin 96 Tissue Kit following the manufacturer’s protocol, in combination with the epMotion 5075 robot (Eppendorf). The mitochondrial cytochrome oxidase I (*cox1*) was amplified with primers LCO1490 and HCO2198 (Folmer *et al.* 1994). Amplification consisted of an initial denaturation step at 94°C for 5 min, followed by 35–40 cycles of denaturation at 94°C for 30 sec, annealing at 48°C, followed by extension at 72°C for 1 min. The final extension was at 72°C for 5 min. PCR products were loaded on 1.5% agarose gel stained with ethidium bromide and positive amplicons were then sequenced by a commercial service (Eurofins).

Forward and reverse strands were assembled, visually checked for sequencing errors, and trimmed of primers using Geneious pro 4.8.5. The *cox1* sequences were then aligned using Geneious, whereas *16S rRNA* and *its2* sequences were aligned using Mafft 7 (mafft.cbrc.jp, accessed on 27th August 2023), with the E-ins-i iterative refinement method. The *cox1* alignment (658 bp, $n = 225$), including 30 minibarcode sequences (319 bp, 5'-end, $n = 2$; 313 bp, 3'-end, $n = 28$), the *16S rRNA* alignment (827 bp, $n = 149$), and the *its2* alignment (508 bp, $n = 41$). All sequences were uploaded on GenBank (NCBI Accession Numbers: *cox1*, OR633461–OR633676 and PP528820–PP528828; *16S rRNA*, OR632437–632560 and PP52829–PP528853; *its2*, OR678225–OR678266).

The molecular dataset was integrated with 40 *cox1* sequences and 20 *16S rRNA* sequences downloaded from GenBank, including 24 non-Mediterranean species (Supplementary Material 2; Harasewych, 2006; Kano, 2007; Williams *et al.* 2008; 2010; Aktipis & Giribet, 2012; Layton *et al.* 2014; Barco *et al.* 2016; Borges *et al.* 2016; Castelin *et al.* 2016; Chet *et al.* 2017; Uribe *et al.* 2017; Merino Valencia, 2020; Mugnai *et al.* 2023). Only three sequences were retained for species from outside the area of study. *Margarella antarctica* (E. Lamy, 1906) from the subfamily Margarellinae S.T. Williams, 2013 was included as the most distant outgroup in the phylogenetic analyses, following results from Williams *et al.* (2010).

Radulae were dissected from selected specimens (Table 1) under a stereomicroscope, washed in 20% NaOH solution for ~2–3 hours, rinsed in distilled water, air dried and platinum coated with a Sputter Coater Leica EM ACE 200. Details of protoconchs and radulae were observed and photographed using a Hitachi TM3000 SEM. Plates were assembled using Adobe Illustrator 1.0 and Adobe Photoshop 14.2.

Species delimitation

An iterative approach was adopted (Yeates *et al.*, 2011), where species are considered hypotheses to be subsequently tested by independent evidence (Puillandre *et al.*, 2014, 2012, 2009). In a first step, the iterative process of species delimitation started by identifying distinguishable morphotypes, and assigning the shell of each specimen to a specific morphotype, thus defining Preliminary Species Hypotheses (PSH). Basing as far as possible on adult specimens, the shell size, shape, colour, and sculpture (with a focus on the peripheral cord) were taken into consideration. Given the complex history of this taxon, the identification process was not limited to the accepted species, and additional nominal taxa published for Mediterranean calliostomatids were employed (Russo, 2023), also benefiting from the study of type and historical museum materials, particularly that of the Monterosato collection (Museo Civico di Zoologia, Rome, Italy; MCZR: Appolloni *et al.* 2018): species and varietal names were linked to morphotypes by comparing shell features of assayed vouchers with those of the relevant types or other historical museum samples (see Fig. 7 and Supplementary material 3). After this process, additional operational names were used for remarkable morphotypes that we could not link to any available nominal taxa.

A distance-based species delimitation analysis was carried out on ASAP (bioinfo.mnhn.fr/abi/public/asap/, accessed on 15th September 2023), a hierarchical clustering program (Puillandre *et al.*, 2021), using the full *cox1* alignment, excluding the two 5' minibarcode sequences. ASAP yields a series of partition hypotheses and ranks them considering their *p-value* (statistical probability of a partition) and *W* (slope of the ranked distances curve) scores. The best

scored ASAP partition was taken as SSH (Secondary Species Hypothesis). To validate the SSH among the Mediterranean and Atlantic specimens, phylogenetic reconstructions were conducted to test monophyly of hypothetical species with Maximum Likelihood (ML) and Bayesian Inference (BI) approaches (see “phylogenetic reconstruction” paragraph for method details), and interspecific vs intraspecific K2P distances on *cox1* were computed to verify the presence of a barcoding gap (Hebert *et al.*, 2003; Meier *et al.*, 2008). The most supported hypothesis determined the final number of species, which were also characterised by a structural analysis of their *its2* 2D folding.

The pairwise genetic *cox1* divergence was computed using Hooty (available at <https://github.com/Princic-1837592/Hooty>), a newly designed tool that computes the minimum vs maximum Kimura-2-parameter (Kimura, 1980) distance matrix within and between groups in a predefined partition.

The secondary structure of the *its2* marker was analysed to determine the presence of structural differences among the SSH. Modelling of *its2* rRNA secondary structures were obtained using the program mfold (Zuker & Jacobson, 1998; Zuker, 2003). Best-supported folding models were predicted combining a thermodynamic approach (Mathews *et al.*, 1999) with a close inspection of paired conserved regions. The rRNA sequence-structures were visualised with 4Sale (Seibel *et al.*, 2008).

Phylogenetic reconstruction

The *cox1* alignment was partitioned by codon using PAUP V4.0 (Swofford, 2002). The best fitting nucleotide substitution model for each *cox1* codon, *16S rRNA* and *its2* was determined using jModelTest 2.1.6 (Darriba *et al.*, 2012) on the CIPRES portal (www.phylo.org, accessed on 27th August 2023), following the Bayesian Information Criterion. A Maximum Likelihood and a Bayesian Inference phylogenetic analyses were performed using IQTree (Trifinopoulos *et al.*, 2016) and MrBayes (Ronquist *et al.*, 2012) on the CIPRES portal. The ML analysis consisted in 1000 Ultrafast bootstrap replicates, and the BI run consisted in four independent MCMCs of 20 million generations with 25% burnin. Convergence of the MCMCs results were verified on Tracer v1.7.1 (Rambaut *et al.*, 2018) by ensuring all ESS values were above >200. Node values were considered supported above 95 likelihood score (ML) and 0.95 posterior probability (BI).

Trees were graphically edited using FigTree v1.4.4 and Adobe Illustrator 1.0.

RESULTS

Species delimitation

From the screening of the literature and the museum materials, we have identified 20 distinguishable morphotypes represented in our dataset (Figs 4–6), which we used as PSH. Only two currently accepted Mediterranean species were not represented in our dataset: *Calliostoma agrigentinum* Coen, 1936 and *Calliostoma gubbiolii* Nofroni, 1984. Of the 20 PSH, seven corresponded to currently accepted Mediterranean species:

- *C. conulus* (*Trochus conulus* Linnaeus, 1758), 32 specimens (Fig. 4A–J); shell of large size, acute, yellow to orange background, occasionally pinkish or reddish, base flat, early whorls

- always beaded, spiral cords faint and regularly spaced, peripheral cord more prominent, usually striped with white blotches, apex occasionally darker.
- *C. funiculatum* (*Calliostoma funiculatum* Ardoivini, 2011), 14 specimens (Fig. 6V–X); shell of small size, acute, orange background (one albino specimen known), base flat, early whorls always beaded, spiral cords strong and regularly spaced, peripheral cord more prominent, usually striped with white blotches.
 - *C. granulatum* (*Trochus granulatus* Born, 1778), 14 specimens (Fig. 4K–S); shell of large size, stout, slightly coeloconoid, uniformly white or cream background, occasionally pinkish, with small reddish or brown blotches, base convex, spiral cords granulated and irregular, peripheral cord more prominent and with larger granules, occasionally striped with reddish blotches.
 - *C. gualterianum* (*Trochus gualterianus* R. A. Philippi, 1848), ten specimens (Fig. 6P–Q); shell of small to medium size, very acute, uniform but variable background colour (e.g. yellow, brown, purple, white), base slightly convex, early whorls rarely beaded, spiral cords very faint or absent, peripheral cord not visible on last whorls.
 - *C. laugierii* (*Trochus laugierii* Payraudeau, 1826), 52 specimens (Fig. 6A–K); shell of small to medium, stout, brown, grey or green background, usually with white and/or reddish stripes, base flat, early whorls (sometimes very dark coloured) usually beaded, spiral cords absent or present only in early whorls, peripheral cord usually barely evident only on early whorls, with reddish or white blotches.
 - *C. virescens* (*Calliostoma virescens* Coen, 1933), eight specimens (Fig. 5Q); shell of medium sized, stout to very stout, greenish brown or grey background with white or reddish blotches, base convex, early whorls weakly beaded, spiral cords strong and irregularly spaced, peripheral cord slightly more prominent.
 - *C. zizyphinum* (*Trochus zizyphinus* Linnaeus, 1758), 34 specimens (Fig. 5A–I); shell of medium to large size, very stout, cream background, usually with reddish stripes or blotches, base flat, early whorls usually beaded, spiral cords faint to strong, regularly spaced, one to three peripheral cords prominent, with reddish blotches.

One PSH corresponded to a currently accepted Atlantic species:

- *C. lividum* (*Calliostoma conulus* var. *livida* Dautzenberg, 1927), 11 specimens (Fig. 5J–M); shell of medium size, slightly acute, uniform dark purple-violet or brown background, base flat, early whorls usually beaded, spiral cords more evident in early whorls, peripheral cord slightly more prominent, growth marks evident.

One PSH corresponded to a taxon inquirendum:

- *C. spongiarum* (*Trochus dubius* var. *spongiarum* Bucquoy, Dautzenberg & Dollfus, 1885), three specimens (Fig. 6S–T); shell of medium size, stout, brownish-reddish background with white or cream quadrangular vertically aligned blotches, base flat or slightly concave, early whorls smooth, spiral cords very faint (made more evident by the coloration), peripheral cord very prominent.

Six PSH corresponded to nominal taxa currently synonymized (as indicated in parentheses):

- *C. alexandrinum* (*Calliostoma alexandrinum* Pallary, 1912 = *C. zizyphinum*), three specimens (Fig. 5N); shell of small size, stout, whitish or cream background with sparse

reddish blotches, base flat, early whorls beaded, spiral cords faint and regularly spaced, peripheral cord prominent.

- *C. dubium* (*Trochus dubius* R. A. Philippi, 1844 = *C. conulus*), one specimen (Fig. 5P); shell of medium to large size, acute, cream background with reddish stripes, base flat, early whorls beaded, spiral cords faint, peripheral cord prominent.
- *C. kochi* (*Calliostoma dubium* var. *kochi* Pallary, 1902 = *C. laugieri*), eight specimens (Fig. 6i–j); shell of small size, stout, greenish, brown, or grey background with white blotches, base flat, early whorls beaded, spiral cords marked and regularly spaced, peripheral cord slightly more prominent.
- *C. neretinum* (*Calliostoma neretinum* Parenzan, 1970 = *C. gualterianum*), one specimen (not illustrated); Shell of small size, acute, bright purple background, base flat, early whorls beaded, spiral cords very faint and regularly spaced, peripheral cord very prominent, white with dark blotches.
- *C. planatum* (*Calliostoma planatum* Pallary, 1900 = *C. laugieri*), four specimens (Fig. 6g–h); shell of medium size, stout, last whorl elated, light greenish background with brown stripes and blotches, base flat, early whorls weakly beaded, spiral cords faint and regularly spaced, peripheral cord more prominent.
- *C. seriopunctatus* (*Trochus seriopunctatus* Blainville, 1830 = *C. laugieri*), one specimen (Fig. 6e); shell of small size, stout, reddish-brown background with white stripes and blotches, base flat, early whorls beaded, spiral cords faint or absent, peripheral cord prominent.

Five PSH loosely resembled known species:

- “stepped” morph, similar to *C. gualterianum*, but with stepped outline in last whorl (Fig. 6R), one specimen;
- “elegant” morph, similar to *C. conulus*, but of smaller size, with blotches and stripes, and elate last whorl (Fig. 6Y–d), 20 specimens;
- “uniform” morph, similar to a typical “*laugieri*”, but smooth and monochrome coloration (Fig. 6O), seven specimens;
- “big-cord” morph, similar to a typical “*laugieri*”, but with remarkably prominent peripheral cord (Fig. 6k–p), 22 specimens;
- “chessboard” morph, resembling *C. funiculatum* with a peculiar combination of colour and sculpture (Fig. 6U), one specimen.

The most supported ASAP partition ($p\text{-val} = 9.24\text{e-}02$; $p\text{-val rank} = 3$; $W = 8.25\text{e-}05$; $W \text{ rank} = 6$) split the whole dataset into 22 hypothetical species, with a threshold distance of 6.1%. According to this partition, the morphologically assessed Mediterranean and Eastern Atlantic samples constituted four hypothetical species, corresponding to *C. conulus*, *C. granulum*, *C. zizyphinum* (including PSH-*zizyphinum*, PSH-*lividum*, and PSH-*dubium*), and the rest of the specimens lumped together into a single unit, which will be hereafter referred to as the “*C. laugieri* complex”. This pattern was also retrieved from the second and third best partition, providing our Secondary Species Hypotheses (SSH). The seventh best partition ($p\text{-val} = 3.55\text{e-}01$; $p\text{-val rank} = 5$; $W = 1.13\text{e-}05$; $W \text{ rank} = 22$) found 44 species, splitting the “SSH-*zizyphinum*” and the “SSH-*laugieri* complex” into six and thirteen hypothetical species respectively, not matching the PSH, with a threshold distance of 1.01%. The ten best ASAP partitions are illustrated in Supplementary Material 5.

1
2
3 315 In the single gene ML and BI reconstructions, monophyly of most of the PSH was not confirmed,
4 316 including the currently accepted species *C. funiculatum*, *C. gualterianum*, *C. laugieri*, *C. lividum*, *C.*
5 317 *virescens*, and *C. zizyphinum*, the taxon inquirendum *C. spongiarum*, and the morphotypes within
6 318 the *C. laugieri* complex (Supplementary Material 6). The four SSH (identified by the three best
7 319 scored ASAP partitions) were instead well supported, with the exception of *C. zizyphinum* and the
8 320 *C. laugieri* complex in the 16S rRNA and ITS2 reconstructions, with the clade representing *C.*
9 321 *zizyphinum* nested inside the *C. laugieri* complex. The internal partitioning of the *C. laugieri*
10 322 complex (either morphological, geographic, or as proposed by the more splitting ASAP partitions)
11 323 was not supported.
12
13
14 324 The matrices of pairwise *cox1* distances (Supplementary Material 7) generated by Hooty included
15 325 those among the PSH (20 groups), the SSH (4 groups) and the groups of the seventh ASAP
16 326 partitions (21 groups). Most of the smallest and largest distances involved minibarcode sequences,
17 327 so they were excluded from the analysis (see Tables S7/4–6 for results including minibarcodes).
18 328 The SSH partition (Table S7-1) showed interspecific distances ranging from 8.84% to 23.89%. The
19 329 upper range of intraspecific distance was lower for *C. conulus* (2.16%) and *C. granulatum* (0.55%),
20 330 and higher for *C. zizyphinum* (5.19%), and the *C. laugieri* complex (6.59%). The PSH partition (Table
21 331 S7-2) had high maximum intraspecific values for PSH-*conulus* (21.20%) and PSH-*virescens*
22 332 (11.96%), followed by PSH-*laugieri* (6.28%) and PSH-“elegant” (6.37%). Minimum interspecific
23 333 distances were lower than 1%, between the morphotypes PSH-*alexandrinum*, PSH-*lividum*, and
24 334 PSH-*zizyphinum*, and between PSH-*funiculatum*, PSH-*gualterianum*, PSH-*kochi*, PSH-*laugieri*, PSH-
25 335 *spongiarum*, PSH-*virescens*, PSH-*planatum*, PSH-*seriopunctatus*, PSH-“stepped”, PSH-“elegant”,
26 336 PSH-“uniform”, and PSH-“big-cord”. The splitting ASAP partition (Table S7-3) had lower maximum
27 337 intraspecific values, the highest being one of the *C. laugieri* complex putative species (4.30%). Two
28 338 of the six hypothetical species within *C. zizyphinum* had slightly overlapping ranges (0–2.02% and
29 339 1.07–2.02%), as did five out of the thirteen hypothetical species of the *C. laugieri* complex. The
30 340 remaining showed a barcoding gap, but the minimum interspecific distances were low, varying
31 341 from 1.70% to 2.65%.
32
33
34 342 The *its2* sequence length within our dataset ranged from 471 to 494 nucleotides. Sequences of *C.*
35 343 *conulus* and *C. granulatum* were consistently shorter than the others. The common derived *its2*
36 344 secondary structure (Fig. 2) was organised in two conserved domains (D1–D2) and two variable
37 345 domains (D3–D4), with different loop regions for each of the SSH. The basal double strand RNA
38 346 portion of D1, consisting of the triplet 5'-CGC/GCG-3', was preceded by the single strand sequence
39 347 5'-UAAGCAAU-3' and followed by quadruplet 5'-CAUU-3' that delimited the separation between
40 348 the D1 and D2 domains. The basal double strand RNA portion of D2, consisting of the quadruplet
41 349 5'-GGGC/GCUC-3', was followed by the sequence stretch 5'-UAAGUGCAGAC-3', which contains the
42 350 consensus motif CAGAC box (Oliverio *et al.*, 2002). The longest double helix region was the D3
43 351 domain, located 3' downstream the middle portion of the *its2* rRNA. In all species examined there
44 352 was a very high conserved sequence motif located in the apical double helix-loop region of D3,
45 353 known in marine molluscs as the Apical STEM sequence (Salvi *et al.*, 2010; Salvi & Mariottini, 2012)
46 354 which included up to 15 base pairs: 5'-GACUGCGUGGACCGA-3' complementary to a 14 nucleotide
47 355 long 5'-UCGUUCAUGUGGUU-3'. The base-pairing between the two strands did not perfectly
48 356 match the entire sequences of the double helix, due to a C protruding at position 13 of the single
49 357 strand sequence at the 5' end of the stem-loop.

Among the Pacific species analysed, *Calliostoma sakashitai* (Sakurai, 1994) and *Tristichotrochus aculeatum* (G. B. Sowerby III, 1912) clustered in a single clade, as did *Akoya akoya* (Kuroda, 1942), *Otukaia ikukoe* Sakurai, 1994, and *Otukaia kiheiziebisu* (Otsuka, 1939). Each of these species was represented by only one specimen.

Phylogenetic reconstruction

The best fitted nuclear substitution models for each gene were as follows: *cox1* first codon, GTR+I+G; *cox1* second codon, F81; *cox1* third codon, TrNef+I+G; *16S rRNA*, HKY+I+G; *its2*, K80+G.

ML and BI reconstructions of the combined datasets (Fig. 3) both identified two main lineages for calliostomatids in the study area, one with *C. conulus* and the NW Atlantic *C. torrei* and the other with the remaining species plus *C. occidentale* from the N Atlantic. *Calliostoma laugieri*, *C. lividum*, and *C. zizyphinum* as morphologically delimited in our PSH were found paraphyletic. The *C. laugieri* complex showed a remarkable geographical structure of the Alboran, Tyrrhenian, Sicily channel, Adriatic, Aegean, and Levantine regions, with few exceptions. This pattern only partially matched the PSH and the splitting ASAP partition hypothesis.

For the samples from outside the study area, the phylogenetic analyses showed low support scores at the basal nodes. The genus *Calliostoma* as currently conceived was polyphyletic. The genera *Akoya* Habe, 1961, *Maurea* W.R.B. Oliver, 1926, *Otukaia* Ikebe, 1942, and *Tristichotrochus* Ikebe, 1942 were also polyphyletic in the analyses. The clade corresponding to *Tristichotrochus* also included *Calliostoma shinagawense* and *C. sakashitai*, both previously assigned to this genus. Additionally, four sequences of *T. unicus* (Dunker, 1860) from GenBank did not comprise a monophyletic group: we are not able to comment on their identity as we did not have access to the vouchers.

DISCUSSION

Species delimitation

Molecular data revealed that our dataset, including specimens ascribed morphologically to twenty morphotypes (of which eight corresponded to currently accepted species), actually comprised only four species corresponding to our SSH, that can be referred to the following nominal taxa: *C. conulus*, *C. granulatum*, *C. zizyphinum*, and *C. laugieri*.

The genetic delimitation of *Calliostoma conulus* (0–2.16% intraspecific *cox1* distance; samples from North Eastern Atlantic, Alboran Sea, Central Mediterranean, and Ionian Sea) and *C. granulatum* (0–0.55% intraspecific *cox1* distance; samples from North Eastern Atlantic, Alboran Sea, and Central Mediterranean) corresponded to the morphological identification. The species *C. zizyphinum* (0–5.19% intraspecific *cox1* distance; samples from North Eastern Atlantic, Azores, Alboran Sea, Central Mediterranean, and Adriatic Sea) included the morphs *alexandrinum*, *dubium* (currently synonymized with *C. conulus*), and *lividum* (currently considered a valid species). *Calliostoma lividum*, a taxon considered endemic to the Azores, is not genetically distinct from the other specimens included in *C. zizyphinum* (<1% genetic distance with samples from both the Mediterranean and the Atlantic), and the relevant specimens fall in a single clade in the combined phylogenetic reconstructions. *Calliostoma lividum* was originally described as a variety of *C. conulus*, and later revived as an independent taxon due to its limited distribution and peculiar phenotype: a uniform dark-brown/violet top-shell (Ávila *et al.*, 2011). Empty shells resembling this

morphotype have recently been found near Galicia at 150 m, although they are identified as *C. conulus* (Hoffman *et al.*, 2023). Despite the morphological distinctiveness for the Azorean specimens, none of the three molecular markers employed showed any significant difference between *C. lividum* and *C. zizyphinum*. *Calliostoma lividum* should therefore be considered as a mere variety of *C. zizyphinum*.

The *C. laugieri* complex (0–6.59% intraspecific *cox1* distance; samples from Alboran Sea, Central and Eastern Mediterranean) comprises all the remaining morphs (14 PSH), including 4 currently accepted species. The phylogenetic structure shows a geographic pattern, and the genetic distance between some specimens exceeds 6%. One interpretation of these results would be based on ASAP partitions (with low scores) that split this clade into thirteen or more species. However, there would be multiple issues with this multi-species hypothesis: in these partitions, the putative molecular species in the *C. laugieri*-complex did not match the morphology-based PSH; the interspecific distances frequently overlapped with the intraspecific ones; the geographic ranges of several hypothesised species overlapped; these partitions also split *C. zizyphinum* into incongruent multi-species hypotheses. The best scored ASAP partitions, suggest the alternative hypothesis that the *C. laugieri* complex represents only one species with a remarkable genetic and geographic structure, which we prefer as more evolutionarily plausible in the current framework. The number of endemic Mediterranean *Calliostoma* species is therefore largely overestimated, with specimens representing the nominal taxa *C. funiculatum*, *C. gualterianum*, *C. laugieri*, *C. spongiarum*, and *C. virescens* belonging to a single species. According to the principle of priority (ICZN, 1999: Art.23), the valid name for this species is *Calliostoma laugieri* (Payraudeau, 1826).

Although the *its2* alignment alone was not capable to effectively distinguish *C. laugieri* and *C. zizyphinum*, the structure obtained from its folding clearly differed among the four SSH (*C. conulus*, *C. granulatum*, *C. zizyphinum*, and *C. laugieri*). The combined analysis of the *its2* structure and sequence is crucial to establish positional homology in multiple alignments, and it also highlights conserved sequence-structure elements, like the CAGAC box and the Apical STEM sequence (Coleman, 2007; Garzia *et al.*, 2021; Oliverio *et al.*, 2002; Salvi *et al.*, 2010, 2014; Salvi & Mariottini, 2012, 2017). The latter motif in *Calliostoma* is consistent with other Vetigastropoda, but carries a unique mutation. The Apical STEM of the *Calliostoma* species sequenced in this study consists of a double strand helix of 14 complementary nucleotide pairs, with a single unpaired protruding C (Fig. 2). By contrast, the Apical STEM of all the other Vetigastropoda species studied so far, representing the genera *Oxystele* R. A. Philippi, 1847, *Phorcus* Risso, 1826, *Steromphala* Gray, 1847, *Tegula* Lesson, 1833, *Agathistoma* Olsson & Harbison, 1953, *Turbo* Linneus, 1758, *Haliothis* Linneus, 1758 (Coleman & Vacquier, 2002), and *Megathura* Pilsbry, 1890 (Supplementary Material 2-2), is one nucleotide longer, with the unpaired and protruding dinucleotide UY instead of a single C.

Phylogenetic reconstruction

The multilocus phylogenetic reconstruction including all currently sequenced Calliostomatinae revealed three main lineages, comprising species from (a) the Western Atlantic, (b) the Indo-West Pacific and (c) the Eastern Atlantic plus the Mediterranean Sea. However, the taxonomic coverage of the subfamily is still very low (6%), replicates are overall scarce (40% of the included species are represented by a single specimen), and the number of molecular markers is still reduced (a single marker available for 52% of the included species), resulting in low support values. There is also a

significant geographic gap, as the Eastern Atlantic species absent from the Mediterranean (Hoffman *et al.*, 2011, 2019; Nolf & Hubrecht, 2022a, 2022c) have not been sequenced yet. The two species that were retrieved as the most closely related to Mediterranean taxa were both originally described from the Western Atlantic: *Calliostoma torrei* Clench & Aguayo, 1940 and *Calliostoma occidentale* (Mighels & C.B. Adams, 1842).

Calliostoma torrei was described on a single large specimen found in deep waters off Cuba (Clench & Turner, 1960). More than 60 years later the same species was sampled in Florida and both specimens were barcoded (Harasewych & Sedberry, 2006). Remarkably, *C. torrei* did not cluster with the other Western Atlantic calliostomatids, but rather with *C. conulus*. It is noteworthy that — contrary to recent predictions (Gofas & Hoffman, 2020) — the type taxon of the genus was more closely related to this species than to the other Mediterranean and NE Atlantic calliostomatids we sampled. *Calliostoma occidentale* (originally described from Casco Bay, Maine, USA) is a species with an apparent amphiatlantic distribution (Clench & Turner, 1960), although only a single specimen from the northeastern Atlantic (off Sweden) has currently been sequenced. The remaining Mediterranean calliostomatids were retrieved as more closely related to this specimen than to *C. conulus*. A comprehensive interpretation of these interesting results would require extending the molecular coverage to the remaining N Atlantic species.

Species identification issues in Calliostoma

There are currently nine accepted species of *Calliostoma* for the Mediterranean Sea (WoRMS, accessed on 25th March 2024), five of which are considered as endemic to the basin. To account for the broad morphological variation observed across the area, a very large number of nominal taxa have been introduced for Mediterranean calliostomatids, and subsequently downranked as synonyms of a smaller number of accepted species. For example, for the north Adriatic Sea, Coen (1933) reported five endemic species of *Calliostoma*, of which only one, *C. virescens*, is currently considered as valid (and we now subsume it within the synonymy of *C. laugieri*). Parenzan (1970) identified a total of twelve valid Mediterranean species and at least ten other dubious taxa: they are currently reduced to five accepted species, of which we recognise only four as valid.

In this work, most of the species hypothesised using morphological characters were not confirmed after molecular analyses. Determining which morphological traits are diagnostic for species (or genera) in this group has always been challenging (Landau *et al.*, 2017; Marshall, 1995a, b; Quinn, 1992), making the process of identification quite troublesome. An example of misleading traits in *Calliostoma* is the beaded sculpture of the early whorls of the teleoconch, a feature that when misinterpreted (especially in combination with a yellow/orange coloration) leads to the erroneous diagnosis of *C. conulus* for specimens of *C. laugieri* (especially in the morphotype “elegant”) and *C. zizyphinum*. Another example is the typical greenish coloration striped with brown of *C. virescens* and the *kochi* morphotype found in our dataset in both *C. laugieri* (Alboran, Adriatic) and *C. zizyphinum* (Portugal). The issue is further complicated when dealing with juveniles, which lack many other morphological characters.

Colour, shell sculpture, and size turned out to be extremely variable among specimens of both *C. laugieri* and *C. zizyphinum* in our dataset. Species with this degree of variability can generate further issues when a new, undescribed phenotypic variant is found, often leading to the hasty conclusion of the discovery of a new species. The underestimation of the actual range of variation

of these species is evidently the reason for the large number of established names in Mediterranean *Calliostoma* (Ghisotti & Melone, 1971; Russo, 2023) as well as in other taxa with highly variable species (Chiappa *et al.*, 2022; Russini *et al.*, 2020). Anatomical characters could be screened to find truly diagnostic differences between gastropod species with unreliable or confusing conchological features, but few species have received detailed anatomical diagnoses in *Calliostoma* (Dornellas, 2012; Dornellas & Simone, 2013; Cavallari & Simone, 2018).

Due to the difficulties in defining reliably diagnostic morphological characters in this group (and also considering that our morphological identification of several specimens had to be reconsidered after genetic characterization), we recommend that caution be exercised in describing new species in calliostomatines based solely on morphological features, at least until the actual significance of shell features is tested by independent molecular data (Landau *et al.*, 2017). In the Mediterranean area in particular, one should consider all the morphological variability observed in both *C. zizyphinum* and *C. laugierii* in this study before raising a different morphotype to a new species. In this regard, a comprehensive molecular survey of the Eastern Atlantic calliostomatids is certainly desirable to detect the presence of polymorphic species that may have led to the description of invalid taxa, or to grant a further confirmation of new species recently described on conchological characters (Nolf & Hubrecht, 2022b, 2022c, 2022a; Vilvens & Swinnen, 2017; Hoffman *et al.*, 2023). When collecting live samples, preserving a small amount of tissue in 95–100% ethanol is a low-effort procedure that allows confirmation of a new discovery, avoiding future taxonomic issues.

Other Calliostoma species in the Mediterranean

After this revision, the number of valid species of *Calliostoma* in the Mediterranean is lowered to six, including *C. gubbiolii* Nofroni, 1984 and *C. agrigentinum* Coen, 1936, which have not been studied molecularly yet. Additionally, there are two dubious cases, with the nominal taxa *C. inopinatum* Dautzenberg, 1911 and *C. lusitanicum* F. Nordsieck & Garcia-Talavera, 1979 waiting for validation (WoRMS, accessed on 25th March 2024). It would be highly desirable to obtain genetic data for these taxa in order to confirm their status and complete the checklist of the genus *Calliostoma* in the Mediterranean. They are briefly commented on here (see also Supplementary Material 3):

Calliostoma gubbiolii is quite likely a valid species with an Atlantic distribution extending to the Alboran Sea. Morphologically, it closely resembles *C. granulatum*, but can be recognised by its smaller size (14 mm v. 30 mm), the more outstandingly prominent peripheral cord, and the flat base with a wavy outline (markedly convex with regularly circular outline in *C. granulatum*) (Gofas *et al.*, 2011; Nolf & Hubrecht, 2022a).

Calliostoma agrigentinum is a taxon based on a single shell found in Agrigento (Sicily). Subsequently considered to fall within the variability of *C. zizyphinum* (Sabelli *et al.*, 1990), this entity was recently reevaluated because of some peculiar aspects of its shell morphology (Micali, 2013). However, the incredible variability observed in the present work for *C. laugierii* and *C. zizyphinum* in the Mediterranean casts some doubts on the validity of this species.

Calliostoma inopinatum was based on some specimens found in the Gulf of Gabès (Tunisia), with a shell morphology overall similar to specimens traditionally called *C. gualterianum* and a peculiar dark stripe at the base of each whorl. The other distinctive traits reported in its original

description, including a beaded apex, a slightly more elongated shell, and an inclination of the base (Dautzenberg, 1911), could fall well in the variation observed for *C. laugieri* in the present work.

Calliostoma lusitanicum is an Atlantic species from Macaronesia with medium-sized shells, dark granulated apex, and maculated basal keel. Avila et al. (2011) illustrated the holotype of this species, similar to specimens traditionally called *C. dubium* (R. A. Philippi, 1844) and *C. semisulcatum* Coen, 1937. Recent findings of specimens with this phenotype from the Gulf of Gabès (Tunisia) raised the possibility that this species might occur in the Mediterranean Sea (Nappo et al., 2021).

Supraspecific classification

The subfamily Calliostomatinae currently comprises 18 accepted Recent genera and approximately 400 species, but almost three quarters of them are included in the genus *Calliostoma*, making it the most species rich genus of the order Trochida (WoRMS, Accessed on 25th March 2024). This is the result of a conservative approach, given the difficulty in defining genera using morphology (Clench & Turner, 1960; Marshall, 1995a; Quinn, 1992). The present results suggest that *Calliostoma* might be restricted to include only a group of Mediterranean and Atlantic Calliostomatinae related to *C. conulus* (as in Marshall, 1995a), whereas it is currently employed to encompass a large number of — probably unrelated — entities. Our very preliminary analysis suggests that a molecular survey would contribute significantly to a reassessment of calliostomatine systematics. This hypothesis should be tested with a revision based on a wider taxonomic and geographic coverage. For instance, *C. sakashitai* and *C. shinagawaense* appeared to be more closely related to species of *Tristichotrochus* than to *Calliostoma conulus*.

A trend of diversification

The *Calliostoma* species examined in this work showed different degrees of variation, which was remarkably higher in *C. laugieri* and *C. zizyphinum*. Especially for the latter, small genetic distances were estimated between strikingly different phenotypes, but a geographic structure seems evident. The factors driving these patterns should be the focus of evolutionary, population genetic and ecological studies. A possible explanation is adaptation to different environments. Abiotic factors like temperature, acidity and hydrodynamism are known to have an impact in the calcification process of gastropod shells (Melatunan et al., 2013). The heavier, darker, stouter shells of *C. zizyphinum* from the Atlantic could be the result of an adaptation to oceanic environmental conditions. Similarly, the multiple morphotypes of *C. laugieri* could be linked to local climatic regimes, e.g. the two shell types retrieved from Cyprus (Figs 6n-o) were collected from areas with 3 °C difference in temperature. The rather low genetic intraspecific distances recovered among specimens from distant sampling localities showed that populations in the Mediterranean and Atlantic are extremely well connected for *C. conulus*, *C. granulatum* and *C. zizyphinum*. Conversely, *C. laugieri* displayed a geographically structured genetic pattern. The relatively few studies on the reproductive strategy of calliostomatids suggested very limited dispersal capabilities, owing to the short period of time the eggs are suspended in the water (Ferro & Cretella, 1993; Lebour, 1936, 1937; Ramón, 1990) or to short living lecithotrophic larvae observed in a few Pacific species (Holyoak, 1988). It is not clear whether differences in larval

developmental timing are reflected in protoconch morphology and size in these vetigastropods, and no clear difference was observed in the protoconchs among the species investigated in this study. In calliostomatids, a gelatinous ribbon enveloping the eggs is used in the spawning process (Ferro & Cretella, 1993; Lebour, 1936, 1937), and there is no current knowledge on the properties of these secretions and their role in the dispersal potential of the eggs. The reasons behind the apparently lower dispersal capability in *C. laugierii* compared to the other studied species might prove of great importance, as they could be linked to microevolutionary dynamics within this clade. A population genetic approach based on a larger sample and other markers (e.g. nuclear, genome-scale) may help testing the single-species hypothesis, and explaining the evolutionary and ecological factors subtending the observed pattern.

Calliostomatids are very peculiar among Vetigastropoda for being able to feed on cnidarians and sponges. Diet is known to influence shell colour (Williams, 2017), and the evolution of different strategies (generalist vs selective) in combination to prey availability, may explain the wider morphological range of some species. In particular, the radula of *C. granulatatum* is extremely different from the others, suggesting the diet of this species could include different prey species, and a trophic adaptation may have accompanied the evolution of this taxon.

SYSTEMATIC DESCRIPTIONS

Subclass Vetigastropoda Salvini-Plawen, 1980

Order Trochida Rafinesque, 1815

Superfamily Trochoidea Rafinesque, 1815

Family Calliostomatidae Thiele, 1924 (1847)

***Calliostoma* Swainson, 1840**

Calliostoma Swainson, 1840: 351

Type species: *Trochus conulus* Linnaeus, 1758 by subsequent designation (Herrmannsen, 1846)

Description

Shell: trochiform, from small (4 mm: *Calliostoma valkuri* Cavallari, Salvador, Dornellas & Simone, 2019) to large (approx. 60 mm for *Calliostoma formosense* Smith, 1907: Vilvens, Nolf & Verstraeten, 2004) size for the family, imperforate; protoconch dome-shaped, paucispiral (0.75–1.25 whorls), sculptured with a honeycomb pattern; teleoconch of 3.5 (*C. valkuri*) to approx. ten whorls (*C. conulus*), smooth or sculptured by tubercles, striae, or spiral cords; base usually convex, occasionally flat or slightly concave; coloration variable, background from white to reddish, purplish, violet, yellowish, monochrome or with lines, flammulae, blotches or spots.

Radula: rhipidoglossate, formula: $\infty+1-2+5-7+1+5-7+1-2+\infty$; central tooth membranaceous, 60–150 μm wide, triangular shaped, serrated, followed by five thinner serrated teeth, threadlike, slightly wider than the marginal teeth. Innermost marginal tooth — sometimes diagnostic at the species level — strong, with thick base and carrying 3–7 triangular shaped teeth, progressively smaller near the distal end. Second innermost marginal tooth thin, hooked, with a comb-like indentation of 5–7 teeth. Marginal teeth threadlike, finely serrated, of variable number.

Characters variable within the same specimen in different portions of the radula.

Operculum: not very thick, usually subcircular, multispiral, smooth externally, yellowish or brownish.

***Calliostoma conulus* (Linnaeus, 1758) (Fig. 4A–J)**

Trochus conulus Linnaeus, 1758: 759

Trochus lucidus Risso, 1826: 126

Trochus violaceus Risso, 1826, 127, pl. 8 fig. 111

?*Trochus dubius* R. A. Philippi, 1844: 149, pl. 25 fig. 7

Trochus flavidus Dunker, 1857: 354

Zizyphinus candidus Brusina, 1865: 25

Zizyphinus basalis Monterosato, 1889: 81

Calliostoma tumidulum Coen, 1933: 155

Calliostoma bullatum Coen, 1933: 156, pl. 2 fig. 12

Type material: Ten syntypes (Linnean Society, A–F 0020114).

Type locality: “*M. Mediterraneo*” (Linnaeus, 1758: 759).

Distribution: Mediterranean and Eastern North Atlantic

Diagnosis

Shell: acute, moderately solid, of medium size for the genus, 20–25 mm high; teleoconch of 8–9 whorls; sculpture variable, of faint or smooth discontinuous spiral cords or regular stronger spiral cords with regularly spaced interspaces, with prominent peripheral cord; first 3–5 whorls always with spirally arranged beaded tubercles. Base from relatively narrow to very wide, sculptured by 10–12 spiral cords, regularly spaced. Aperture subquadrangular, with callous columella. Colour usually orange-red or cream background, sometimes with reddish or brownish flammulae, usually with white blotches on the peripheral cord.

Radula: innermost marginal tooth slightly hooked, wide, with 3–5 rounded teeth.

Remarks

The pearl-like tubercles extend over a higher number of whorls compared to other species, and their shape (more prominent and slightly spinulose) is also different (similar to those of *C. torrei*). Purple (Risso, 1826), violet (Giannuzzi-Savelli, 1994; Monterosato, 1889) almost monochrome reddish (Manousis *et al.*, 2021) and white (Brusina, 1865; Giannuzzi-Savelli, 1994) phenotypes have been reported. The type series (Linnean Society, A–F 0020114 <https://linnean-online.org/17277>) includes at least two specimens belonging to *C. zizyphinum*. *Trochus dubius* R. A. Philippi, 1844 is commonly regarded as a synonym of *C. conulus*. No type material is known of *T. dubius* (Kabat & Coan, 2017); however, specimens originally identified by R. A. Philippi (potential syntypes in the Monterosato collection, Fig. 7M) congruent with the original figure, are instead typical *C. zizyphinum*.

***Calliostoma granulatum* (Born, 1778) (Fig. 4K–S)**

Trochus granulatus Born, 1778: 343

Trochus papillosus da Costa, 1778: 38, pl. 3, fig. 5–6

Trochus fragilis Pulteney, 1799: 44

Trochus tenuis Montagu, 1803: 275, pl. 10, fig. 3

Type material: Holotype (Naturhistorisches Museum Wien, Austria; NHMW-MO-14000).

Type locality: unknown

Distribution: Mediterranean and Eastern Atlantic

Diagnosis

Shell: stout, moderately solid, of medium size for the genus, 20–30 mm high; teleoconch of 7–8 inflated whorls; sculpture rather constant, of 8–10 regular, heavily granulated spiral cords, with irregular interspaces, two peripheral granular and closely aligned spiral cords on the last whorl. Base wide, strongly convex, sculptured by 13–15 unequal, smooth, flat spiral cords, irregularly spaced. Aperture rounded, slightly compressed laterally, internally slightly nacreous with curved and slightly callous columella. Colour background usually dark cream, occasionally with vertical wavy reddish-brown flammulae and blotches; spiral cords often lighter (whitish on the base) with small brown spots; base with a dark cream to brown background with alternating reddish brownish and whitish spots.

Radula: central tooth ~150 µm wide, triangular, finely serrated, followed by five triangular lateral teeth ~50 µm wide, serrated. Innermost marginal tooth partially hooked, pointed, with 5–7 small triangular teeth. Second innermost marginal tooth thinner, hooked, with comb-like indentation (8–11 teeth).

Remarks

The morphology of this species is rather constant across the Mediterranean Sea, but the species displays more morphological variation along the African coasts (Nolf & Hubrecht, 2022a). The variety *lactea* (Jeffreys, 1862) and albino specimens are reported (Giannuzzi-Savelli, 1994; Rolán Mosquera, 1983). The radula of this species differs remarkably from other species within the same range, suggesting potential trophic differences.

***Calliostoma laugier* (Payraudeau, 1826) (Fig. 6)**

Trochus laugier Payraudeau, 1826: 125–126, pl. 6 fig. 2–3

Trochus maculatus Risso, 1826: 128

Trochus hyacinthinus Blainville, 1830: 259, pl. 10 fig. 2

Trochus seriopunctatus Blainville, 1830: 263, pl. 10 fig. 1

Trochus flammiger Dunker, 1857: 355

Trochus pallidulus Dunker, 1857: 355

Trochus gualterianus R. A. Philippi, 1848: 69, pl. 11 fig. 15 new synonym

Trochus dubius var. *spongiorum* Bucquoy, Dautzenberg & Dollfus, 1885: 353, pl. 42 figs 8–9

Calliostoma planatum Pallary, 1900: 353

Calliostoma kochi Pallary, 1902: 25, pl. 1 fig. 23–24

Calliostoma virescens Coen, 1933: 156, pl. 1 fig. 9 new synonym

Calliostoma neretinum Parenzan, 1970: 40, pl. 4 fig. 50 new synonym

Calliostoma floreanae Parenzan, 1970: 41, pl. 4 fig. 51

Calliostoma funiculatum Ardovalini, 2011: 19–20 new synonym

Type material: unknown

Type locality: Corsica (“*sur toutes les plages*”. Payraudeau, 1826: 125)

Distribution: Mediterranean and Eastern Atlantic

Diagnosis

Shell: from stout to acute, rather solid in adult specimens, of small to medium size for the genus, 9–15 mm high; teleoconch of 6.5–9 whorls, the first 2–3 with three granulose cords; surface of the

teleoconch rather smooth or with fine irregular spiral striae, with a barely evident peripheral cord, or three wide and flat spiral cords with narrow interspaces, or with four elevated and tessellated cords with wide interspaces (additional variation in remarks).

Base convex, sculptured by 4–5 spiral cords barely visible in smooth specimens, 7–8 wide and flat cords with engraved interspaces in heavily sculptured specimens. Aperture rounded, compressed laterally outwards, internally nacreous bluish purple, columella white and thickened.

Colour background usually dark brownish-grey, with reddish or lighter vertical flammulae, or tessellated.

Radula: innermost marginal tooth partially hooked, with four triangular teeth, the most distant one being very small.

Remarks

Colour variation is particularly evident, with background colour ranging from cream or very light brown to dark green or dark brown, with reddish to brownish flammulae of various shapes and sizes, as well as completely monochrome specimens, sometimes albino (Giannuzzi-Savelli, 1994) or completely violet (Giannuzzi-Savelli, 1994; Manousis *et al.*, 2021; Terreni, 1981). The outline is also variable, ranging from stout to rather acute.

The morphological variability of this species is extremely complex, with some morphotypes that can be confused with other species and a variation frequently (yet not consistently) distributed on a geographical scale: for instance, the variety *funiculatum* is usually found in the Strait of Sicily and in the Alboran Sea (Vilvens & Swinnen, 2017); the greenish coloration with brown flammulae of the varieties *planatum*, *kochi* and the *virescens* morphotype appears to be restricted to the area of Alboran and in the northern Adriatic, respectively; dark-green shells are found mostly in the Eastern Mediterranean. In Cyprus two strongly diverging morphotypes occur between 5 and 30 m depth at two sites ca 100 km apart (1–2% *cox1* distance): a dark green morphotype occurs along the south-western shores, whereas a morphotype orange to brown in colour with white spots occurs along the eastern shores.

Shape, colour and size can sometimes be reminiscent of *C. conulus*, but the granulose spiral cords never exceed three whorls (3–5 whorls in *C. conulus*), and the tubercles are more rounded and never spinulose. Although usually the shells of *C. zizyphinum* are stouter (and the colour of the shell is diagnostic in most cases), some peculiar morphotypes of *C. laugieri* and *C. zizyphinum* can be extremely similar (see Figures 4Q and 5i), so that molecular or radular data are needed for confirmation.

***Calliostoma zizyphinum* (Linnaeus, 1758) (Fig. 5)**

Trochus zizyphinum Linnaeus, 1758: 759

Trochus laevigatus J. Sowerby, 1817: 179, pl. 181 fig. 1

Trochus discrepans T. Brown, 1818: 519–209, pl. 24 fig. 4

Trochus conuloides Lamarck, 1822: 24

Trochus lineolatus Risso, 1826: 128

Trochus albidus W. Wood, 1828: 16, pl. 5 fig. 14

Trochus agrestis R. A. Philippi, 1843: 33, pl. 1 fig. 6

Conulus typus Nardo, 1847: 70

Trochus chemnitzii R. A. Philippi, 1848: 63, pl. 13 fig. 7

Trochus flavus Anton, 1849 [in R. A. Philippi, 1849: 105]

1
23 738 *Trochus lyonsii* Leach, 1850 [in Forbes & Hanley, 1849-1850: 491]4 739 *Zizyphinus vulgaris* J. E. Gray, 1850: 895 740 *Trochus cranchi* Leach, 1852: 1716 741 *Trochus irregularis* Leach, 1852: 1737 742 *Zizyphinus linnaei* Monterosato, 1884: 448 743 *Zizyphinus dalmaticus* Monterosato, 1889: 789 744 *Zizyphinus demissus* Monterosato, 1889: 7810 745 *Zizyphinus lissus* Monterosato, 1889: 7811 746 *Zizyphinus novegradensis* Monterosato, 1889: 7812 747 *Zizyphinus altus* Locard, 1904: 3513 748 *Calliostoma alexandrinum* Pallary, 1912: 143, pl. 16 fig. 11-1214 749 *Calliostoma conulus* var. *livida* Dautzenberg, 1927: 191 new synonymy15 750 **Type material:** five syntypes (Linnean Society, P-Z 0010917)16 751 **Type locality:** “*M. Mediterraneo et Europaeo*”. (Linnaeus, 1758: 759).17 752 **Distribution:** Mediterranean, Eastern Atlantic, Azores18 753 **Diagnosis**

19 754 *Shell:* from very stout to acute, solid and thick, of medium to large size for the genus, 20–43 mm
 20 755 high; teleoconch of 7.5–9 whorls, the first 3–5 with 3–4 granulated cords; rest of teleoconch from
 21 756 smooth or finely striated, to strongly sculptured by 3–4 thick spiral cords with well-engraved
 22 757 interspaces, 1–3 peripheral cords distinctly prominent. Base wide and convex with 7–9 spiral cords
 23 758 with narrow and well-engraved interspaces; prosocline growth scars evident. Aperture
 24 759 subquadrangular, compressed laterally outwards, quadrangular in juveniles, internally nacreous
 25 760 bluish white, with whitish and thickened columella. Colour background usually brownish grey or
 26 761 reddish brownish, monochrome or with flammulae or reddish-brownish prosocline striae, or
 27 762 reddish-brownish spots on the cords.

28 763 *Radula:* innermost marginal tooth thickened dorsally and hooked in the distal portion, with up to
 29 764 eight very small triangular teeth.

30 765 **Remarks**

31 766 Some colour varieties are known, such as “*violacea*” and “*albina*” (Monterosato, 1889; Rolán
 32 767 Mosquera, 1983; Trigo *et al.*, 2018) as well as the monochrome creamy white “*lyonsii*” (Giannuzzi-
 33 768 Savelli, 1994), or *lividum* from Azores, almost monochrome with fine spiral striae and wavy and
 34 769 prosocline growth lines.

35 770 While the morphological variability of *C. laugierii* includes a multitude of combinations of
 36 771 characters, *C. zizyphinum* has a few distinct phenotypes. Almost any *C. zizyphinum* specimen falls
 37 772 within one of the following:

- 38 773 • The predominant and typical phenotype of the species, especially in the Atlantic
 39 774 continental waters and the westernmost Mediterranean, with a very stout shell and
 40 775 prominent peripheral cord. Colour background is usually light cream, with red-violet
 41 776 blotches on the peripheral cord, turning darker at lower latitudes (Fig. 5A–I).
- 42 777 • A smaller and less stout shell of light colour, with beaded early teleoconch whorls (Fig. 5N),
 43 778 sometimes resembling *C. conulus* (Fig. 5O). The peripheral cord can be extremely
 44 779 prominent, as in the *dubium* morphotype (Fig. 5P).
- 45 780 • The *lividum* morph, only reported from Macaronesia, less stout and of brownish-violet
 46 781 background colour, without the typical blotched cord of the species (Fig. 5J–M).

- Stout shell, greenish background with brown stripes and strongly funiculated whorls (Fig. 5Q). Shells resemble those of *C. laugier* from Alboran and the Adriatic Sea. It is only reported from Portugal.

ACKNOWLEDGEMENTS

We would like to thank: Cristina Lemorini for her help with the SEM microscope (Laboratory of Technological and Functional Analyses of Prehistoric Artefacts, Department of Classics, Sapienza University of Rome); Pierfilippo Cerretti e Maurizio Mei for providing the photographic equipment (Zoological Museum, Sapienza University of Rome); Giampiero Lanzotti and Rita Graziano for the coating of the radulae for SEM (Stazione Zoologica Anton Dohrn, Italy); Serge Gofas, Juan Moles, Garcia Raso, Walter Renda, Roberto Ardevini, Daniela Pica, Franck Finelli, Renato Chemello, Enrico Delfiero, Rino Bertolino, Ivan Merulo, Paolo Russo, Eva Turicchia, Michel le Qument, Gianni Spada, Bruno Sabelli, Francesca Evangelisti and the late Mauro Pizzini for providing valuable samples. MNHN specimens were collected during scientific expeditions under the auspices of the Our Planet Reviewed program (CORCABENTHOS 1–3). This work was in part supported by ‘Service de Systématique Moléculaire’ (UAR 2700 2AD, MNHN, CNRS).

Massimo Appolloni (Museo Civico di Zoologia, Rome, Italy) helped with the search in the Monterosato collection; We thank Luigi Romani for providing valuable bibliographic material and Brian Cunningham for his opinion on relevant morphologies. Stefano Piraino, Anna Maria Miglietta and Giulia Furfaro (Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Porto Cesareo, Lecce, Italy) sent photos of the types of *Calliostoma neretinum* and *Calliostoma floreanae* (Museo di Biologia Marina “Pietro Parenzan”, Porto Cesareo, Università del Salento, Lecce, Italy); Oz Rittner (The Steinhardt Museum of Natural History, Tel Aviv, Israel) sent photos of the type of *Calliostoma agrigentinum* from the Coen collection. Anita Eschner (Naturhistorisches Museum Wien, Austria) sent photos of the type of *Calliostoma granulatum*. We thank Andrea Deneau (The Linnean Society of London, UK) for permission to publish images of the types of *Trochus conulus* and *Trochus zizyphinum*.

We are deeply grateful to Suzanne Williams, Serge Gofas, and one anonymous reviewer for their thorough revision of this work and their valuable comments.

FUNDING STATEMENT

Funding: This work was partly supported by the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 (Project code CN_00000033, “National Biodiversity Future Center - NBFC”, CUP B83C22002950007, Sapienza University of Rome).

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

Giacomo Chiappa: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing - original draft; Writing - review & editing. **Giulia Fassio:** Conceptualization; Data curation; Methodology; Supervision; Validation; Writing – review & editing. **Carlo Smriglio:** Data curation; Investigation; Supervision; Writing – review & editing. **Paolo Mariottini:** Data curation; Formal analysis; Investigation; Methodology; Writing – review & editing. **Paolo Albano:** Data curation; Investigation; Writing – review & editing. **Maria Vittoria Modica:** Supervision; Validation; Writing – review & editing. **Dario Zuccon:** Data curation; Writing – review & editing. **Nicolas Puillandre:** Data curation; Methodology; Resources; Validation; Writing – review & editing. **Marco Oliverio:** Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing.

DATA AVAILABILITY

All newly produced data is publicly available in GenBank with the following NCBI Accession Numbers: *cox1*, OR633461–OR633676, PP528820–PP528828; *16S rRNA*, OR632437–632560, PP528829–PP528853; *its2*, OR678225–OR678266. Additional data downloaded from GenBank listed in Supplementary material 2.

REFERENCES

AKTIPIS, S.W. & GIRIBET, G., 2012. Testing relationships among the vetigastropod taxa: a molecular approach. *Journal of Molluscan Studies*, **78**: 12–27.

ALJANABI, S.M. & MARTINEZ, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**: 4692–4693.

APPOLLONI, M., SMRIGLIO, C., AMATI, B., LUGLIÈ, L., NOFRONI, I., TRINGALI, L.P., MARIOTTINI, P., OLIVERIO, M., 2018. Catalogue of the primary types of marine molluscan taxa described by Tommaso Allery Di Maria, Marquis of Monterosato, deposited in the Museo Civico di Zoologia, Roma. *Zootaxa*, **4477(1)**: 1–138

ARDOVINI, R., 2011. *Calliostoma funiculatum* n. sp. (Gastropoda, Callisotomatidae) Sicilia occidentale, Isole Egadi. *Malacologia Mostra Mondiale*, **71**: 19–20.

ÁVILA, S.P., BORGES, J.P., DE FRIAS MARTINS, A.M., 2011. The littoral Trochoidea (Mollusca: Gastropoda) of the Azores. *Journal of Conchology*, **40(4)**: 408–427.

BARCO, A., HOUART, R., BONOMOLO, G., CROCETTA, F., OLIVERIO, M., 2013. Molecular data reveal cryptic lineages within the northeastern Atlantic and Mediterranean small mussel drills of the *Ocenebrina edwardsii* complex (Mollusca: Gastropoda: Muricidae). *Zoological Journal of the Linnean Society*, **169**: 389–407.

- BLAINVILLE, H.M.D. DE, 1830 [1828–1830]. *Malacozoaires ou Animaux Mollusques*. [in] Faune Française, livr. 28 (3 July 1830) : 241–320. Levrault, Paris. 320 pp.
- BORGES, L.M.S., HOLLATZ, C., LOBO, J., CUNHA, A.M., VILELA, A.P., CALADO, G., COELHO, R., COSTA, A.C., FERREIRA, M.S.G., COSTA, M.H., COSTA, F.O., 2016. With a little help from DNA barcoding: investigating the diversity of Gastropoda from the Portuguese coast. *Scientific Reports*, **6**: 20226.
- BORN, I. VON, 1778. Index rerum naturalium Musei Cæsarei Vindobonensis. Pars I.ma. *Testacea. Verzeichniß der natürlichen Seltenheiten des k. k. Naturalien Cabinets zu Wien*. Erster Theil, Schalthiere. Vienna [Vindobonae].
- BOUCHET, P., FRÝDA, J., HAUSDORF, B., PONDER, W., VALDÉS, A., WARÉN, A., 2005. Classification and nomenclator of gastropod families. *Malacologia*, **47(1-2)**: 1–397.
- BROWN, T., 1818. Account of the Irish testacea. *Memoirs of the Wernerian Natural History Society* **2**: 501–536.
- BRUSINA, S., 1865. Conchiglie dalmate inedite. *Verhandlungen der Kaiserlich-königlichen Zoologisch-botanisch Gesellschaft in Wien*, **15**: 3–42.
- CALLEA, A., 2012. Genere *Calliostoma* Swainson, 1840: il sottogenere *Maurea* Oliver, 1926 (Vetigastropoda: Calliostomatidae). *Notiziario Società Italiana di Malacologia*, **30**: 17–29.
- CANTRAINE, F.J., 1835. Diagnoses ou descriptions succinctes de quelques espèces nouvelles de mollusques. *Bulletins de l'Académie Royale des Sciences et Belles-Lettres de Bruxelles*, **2**: 380–401.
- CASTELIN, M., VAN STEENKISTE, N., PANTE, E., HARBO, R., LOWE, G., GILMORE, S.R., THERRIAULT, T.W., ABBOTT, C.L., 2016. A new integrative framework for large-scale assessments of biodiversity and community dynamics, using littoral gastropods and crabs of British Columbia, Canada. *Molecular Ecology Resources*, **16**: 1322–1339.
- CAVALLARI, D.C. & SIMONE, L.R.L., 2018. A new species of *Calliostoma* (Vetigastropoda: Calliostomatidae) from Canopus Bank, off northeastern Brazil. *Zootaxa*, **4457(1)**: 156–166
- CAVALLARI, D.C., SALVADOR, R.B., DORNELLAS, A.P.S., SIMONE, L.R.L., 2019. Calliostomatidae, Colloniidae, Margaritidae, and Solariellidae (Gastropoda: Trochoidea) collected by the Marion Dufresne (MD55) expedition in southeastern Brazil, with description of a new species of *Calliostoma*. *Zootaxa*, **4609(3)**: 401–428.
- CHAMPLIT, S., BERTHELOT, C., PRUVOST, M., BENNETT, E.A., GRANGE, T., GEIGL, E.-M., 2010. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS One*, **5**: e13042.
- CHEN, C., WATANABE, H.K., MIYAZAKI, J., & KAWAGUCCI, S., 2017. Unanticipated discovery of two rare gastropod molluscs from recently located hydrothermally influenced areas in the Okinawa Trough. *PeerJ*, **5**: e4121.
- CHIAPPA, G., FASSIO, G., CORSO, A., CROSETTA, F., MODICA, M.V., OLIVERIO, M., 2022. How many abalone species live in the Mediterranean Sea? *Diversity*, **14**: 1107.
- CLENCH, W.J. & TURNER, R.D., 1960. The genus *Calliostoma* in the western Atlantic. *Johnsonia*, **4**: 1–80.
- COAN E.V. & KABAT A.R., 2017. The malacological contributions of Rudolph Amandus Philippi (1808–1904). *Malacologia*, **60(1-2)**: 31–322.

- 1
2
3 896 COEN, G., 1933. Saggio di una Sylloge Molluscorum Adriaticorum. *Memorie del Regio Comitato*
4 897 *Talassografico Italiano*, **192**: 1–186.
- 6 898 COLEMAN, A.W., 2007. Pan-eukaryote ITS2 homologies revealed by RNA secondary structure. *Nucleic Acids*
7 899 *Research*, **35**: 3322–3329.
- 9 900 COLEMAN, A.W. & VACQUIER, V.D., 2002. Exploring the phylogenetic utility of ITS sequences for animals: A
10 901 test case for abalone (*Haliotis*). *Journal of Molecular Evolution*, **54**: 246–257.
- 12 902 DA COSTA, E. M. (1778). *Historia naturalis testaceorum Britanniae, or, the British conchology; containing the*
13 903 *descriptions and other particulars of natural history of the shells of Great Britain and Ireland:*
14 904 *illustrated with figures*. Millan, White, Emsley & Robson, London.
- 16 905 DARRIBA, D., TABOADA, G.L., DOALLO, R., POSADA, D., 2012. jModelTest 2: more models, new heuristics
17 906 and parallel computing. *Nature Methods*, **9**: 772–772.
- 19 907 DAUTZENBERG, P., 1911. Contributions à la faune malacologique méditerranéenne. *Journal de*
20 908 *Conchyliologie*, **58**: 205–211.
- 22 909 DAUTZENBERG, P., 1927. Mollusques provenant des campagnes scientifiques du Prince Albert Ier de
23 910 Monaco dans l'Océan Atlantique et dans le Golfe de Gascogne. *Résultats des Campagnes*
24 911 *Scientifiques Accomplies sur son Yacht par Albert Ier Prince Souverain de Monaco*, **72**: 401 pp.
- 26 912 DAYRAT, B., 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society*, **85**: 407–417.
- 28 913 DORNELLAS, A.P.S., 2012. Description of a new species of *Calliostoma* (Gastropoda, Calliostomatidae) from
29 914 Southeastern Brazil. *Zookeys*, **224**: 89–106.
- 31 915 DORNELLAS, A.P.S. & SIMONE, L.R.L., 2013. Comparative morphology and redescription of three species of
32 916 *Calliostoma* (Gastropoda, Trochoidea) from the Brazilian coast. *Malacologia*, **56(1–2)**: 267–293
- 34 917 DUDA, T.F., BOLIN, M.B., MEYER, C.P., KOHN, A.J., 2008. Hidden diversity in a hyperdiverse gastropod
35 918 genus: Discovery of previously unidentified members of a *Conus* species complex. *Molecular*
36 919 *Phylogenetics and Evolution*, **49**: 867–876.
- 38 920 DUNKER, W., 1857. Mollusca nova collectionis Cumingianae. *Proceedings of the Zoological Society of*
39 921 *London*, **24**: 354–358.
- 41 922 ESPIRITU, D.J.D., WATKINS, M., DIA-MONJE, V., CARTIER, G.E., CRUZ, L.J., OLIVERA, B.M., 2001. Venomous
42 923 cone snails: molecular phylogeny and the generation of toxin diversity. *Toxicon*, **39**: 1899–1916.
- 44 924 FASSIO, G., MODICA, M.V., ALVARO, M.C., BUGÉ, B., SALVI, D., OLIVERIO, M., SCHIAPARELLI, S., 2019. An
45 925 Antarctic flock under the Thorson's rule: Diversity and larval development of Antarctic Velutinidae
46 926 (Mollusca: Gastropoda). *Molecular Phylogenetics and Evolution*, **132**: 1–13.
- 48 927 FASSIO, G., RUSSINI, V., BUGÉ, B., SCHIAPARELLI, S., MODICA, M.V., BOUCHET, P., OLIVERIO, M., 2020. High
49 928 cryptic diversity in the kleptoparasitic genus *Hyalorisia* Dall, 1889 (Littorinimorpha: Capulidae) with
50 929 the description of nine new species from the Indo-West Pacific. *Journal of Molluscan Studies*, **86**:
51 930 401–421.
- 53 931 FASSIO, G., BOUCHET, P., OLIVERIO, M., STRONG, E.E., 2022a. Re-evaluating the case for poecilogony in the
54 932 gastropod *Planaxis sulcatus* (Cerithioidea, Planaxidae). *BMC Ecology and Evolution*, **22**: 13.
- 56 933 FASSIO, G., RUSSO, P., BONOMOLO, G., FEDOSOV, A.E., MODICA, M.V., NOCELLA, E., OLIVERIO, M., 2022b.
57 934 A molecular framework for the systematics of the Mediterranean spindle-shells (Gastropoda,
58 935 Neogastropoda, Fascioliariidae, Fusininae). *Mediterranean Marine Science*, **23**: 623–636.

- FASSIO, G., STEFANI, M., RUSSINI, V., BUGÉ, B., BOUCHET, P., TRENEMAN, N., MALAQUIAS, M.A.E., SCHIAPARELLI, S., MODICA, M.V., OLIVERIO, M., 2022c. Neither slugs nor snails: a molecular reappraisal of the gastropod family Velutinidae. *Zoological Journal of the Linnean Society*, **199** (1): 321–325.
- FERRO, R. & CRETELLA, M., 1993. Osservazioni sulla biologia di *Calliostoma granulatum* (Born) (Gastropoda: Trochidae). *Bollettino Malacologico*, **29**(1–4): 49–56
- FOLMER, O., HOEH, W.R., BLACK, M.B., VRIJENHOEK, R.C., 1994. Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnologies*, **3**: 294–299.
- FORBES, E. & HANLEY, S. C. 1850 [1848-1853]. *A history of British Mollusca and their shells*. Vol. 2: 481–557 [1850]. Van Voorst, London.
- FURFARO, G., SALVI, D., MANCINI, E., MARIOTTINI, P., 2018. A multilocus view on Mediterranean aeolid nudibranchs (Mollusca): Systematics and cryptic diversity of Flabellinidae and Piseinotecidae. *Molecular Phylogenetics and Evolution*, **118**: 13–22.
- FURFARO, G., SCHREIER, C., TRAINITO, E., PONTES, M., MADRENAS, E., GIRARD, P., MARIOTTINI, P., 2022. The sea slug *Doriopsilla areolata* Bergh, 1880 (Mollusca, Gastropoda) in the Mediterranean Sea: Another case of cryptic diversity. *Diversity*, **14**(4): 297.
- GALINDO, L.A., PUILLANDRE, N., STRONG, E.E., BOUCHET, P., 2014. Using microwaves to prepare gastropods for DNA barcoding. *Molecular Ecology Resources*, **14**: 700–705.
- GARZIA, M., MARIOTTINI, P., SALVI, D., FURFARO, G., 2021. Variation and diagnostic power of the Internal Transcribed Spacer 2 in Mediterranean and Atlantic aeolid nudibranchs (Mollusca, Gastropoda). *Frontiers in Marine Science*, **8**: 693093.
- GELLER, J., MEYER, C., PARKER, M., HAWK, H., 2013. Redesign of PCR primers for mitochondrial Cytochrome c Oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, **13**: 851–861.
- GHISOTTI, F. & MELONE, G.C., 1971. Catalogo illustrato delle conchiglie marine del Mediterraneo. 3. Trochidae. *Conchiglie*, supplement to **7**(1–2): 47–145.
- GIANNUZZI-SAVELLI, R., 1994. *Atlante delle conchiglie marine del Mediterraneo: Archaeogastropoda*. Edizioni “La Conchiglia.”, Roma.
- GOFAS, S., MORENO, D., SALAS, C., 2011. *Moluscos Marinos de Andalucía*. Universidad de Málaga. Servicio de Publicaciones e Intercambio Científico, Málaga.
- GOFAS, S. & HOFFMAN, L., 2020. Deep-water Calliostomatidae (Vetigastropoda, Gastropoda) from the South Azorean Seamount Chain. *Iberus*, **38**(2): 195–211.
- GRAY, J.E., 1850. [text]. In: Gray, M. E., *Figures of molluscos animals, selected from various authors*. Vol. 4, iv + 219 pp. Longman, Brown, Green and Longmans, London.
- HARASEWYCH, M.G. & SEDBERRY, G.R., 2006. Rediscovery, range extension, and redescription of *Calliostoma torrei* Clench and Aguayo, 1940 (Gastropoda: Vetigastropoda: Trochidae). *Nautilus*, **120**(2): 39–44.
- HAYASHI, S., 2005. The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives. *Molluscan Research*, **25**: 85–98.

- 1
2
3 977 HEBERT, P.D.N., CYWINSKA, A., BALL, S.L., DEWAARD, J.R., 2003. Biological identifications through DNA
4 978 barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**: 313–321.
5
6 979 HERRMANNSEN, A.N., 1846 [1846-1852]. *Indicis Generum Malacozoorum primordia*. Vol. 1. Fischer, Cassel.
7
8 980 HICKMAN, C.S. & MCLEAN, J.H., 1990. Systematic revision and suprageneric classification of trochacean
9 981 gastropods. *Science Series of Natural History Museum of Los Angeles County*, **35**: 1–169.
10
11 982 HICKMAN, C.S. & TAYLOR, J., 1996. Phylogeny and patterns of evolutionary radiation in trochoidean
12 983 gastropods. In: *Origin and Evolutionary Radiation of the Mollusca* (Taylor, J.D. ed), pp. 177–198.
13 984 Oxford University Press, Oxford.
14
15 985 HOFFMAN, L., HEUGTEN, B., LAVALEYE, M., 2011. Gastropoda (Mollusca) from the Rockall and Hatton
16 986 Banks, northeastern Atlantic Ocean. Part 2. *Miscellanea Malacologica*, **4**: 85–118.
17
18 987 HOFFMAN, L., BEUCK, L., VAN HEUGTEN, B., LAVALEYE, M., FREIWALD, A., 2019. Last snails standing since
19 988 the Early Pleistocene, a tale of Calliostomatidae (Gastropoda) living in deep-water coral habitats in
20 989 the north-eastern Atlantic. *Zootaxa*, **4613**: 93–110.
21
22
23 990 HOFFMAN, L., URGORRI, V., OLIVER, J.D., TEMPLADO, J., 2023. Data on species of the genus *Calliostoma*
24 991 (Vetigastropoda: Trochoidea: Calliostomatidae) on the shelf and bathyal bottoms off Galicia (NW
25 992 Iberian Peninsula). *Iberus*, **42(1)**: 55–64
26
27 993 HOFFMAN, L., VAN HEUGTEN, B., GOUD, J., 2023. Description of a new species of *Calliostoma* (Gastropoda,
28 994 Vetigastropoda: Calliostomatidae) from the Cape Verde Islands. *Basteria*, **87(2)**: 156
29
30 995 HOLYOAK, A.R., 1988. Spawning and larval development of the trochid gastropod *Calliostoma ligatum*
31 996 (Gould, 1849). *Veliger*, **30**: 369–371.
32
33 997 ICZN [INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE], 1999. *International code on*
34 998 *zoological nomenclature*, 4th edn. The International Trust for Zoological Nomenclature, London.
35
36 999 IKEBE, N., 1942. Trochid Mollusca *Calliostoma* of Japan, fossil and recent. *Japanese Journal of Geology and*
37 1000 *Geography*, **18**: 249–282.
38
39 1001 JEFFREYS, J.G., 1865 [1862-1869]. *British conchology*. Vol. 3. van Voors, London.
40
41 1002 KABAT, A.R. & COAN, E.V., 2017. The life and work of Rudolph Amandus Philippi. *Malacologia*, **60(1–2)**: 1–
42 1003 30
43
44 1004 KIMURA, M., 1980. A simple method for estimating evolutionary rates of base substitutions through
45 1005 comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**: 111–120.
46
47 1006 KRUG, P.J., VENDETTI, J.E., RODRIGUEZ, A.K., RETANA, J.N., HIRANO, Y.M., TROWBRIDGE, C.D., 2013.
48 1007 Integrative species delimitation in photosynthetic sea slugs reveals twenty candidate species in three
49 1008 nominal taxa studied for drug discovery, plastid symbiosis or biological control. *Molecular*
50 1009 *Phylogenetics and Evolution*, **69**: 1101–1119.
51
52
53 1010 LAMARCK, J.-B., 1822. *Histoire naturelle des animaux sans vertèbres*. Tome septième. Published by the
54 1011 Author, Paris.
55
56 1012 LANDAU, B.M., VAN DINGENEN, F., CEULEMANS, L., 2017. The upper Miocene gastropods of northwestern
57 1013 France, 1. Patellogastropoda and Vetigastropoda. *Cainozoic research*, **17**: 75–166.
58
59 1014 LAYTON, K.K.S., MARTEL, A.L., HEBERT, P.D.N., 2014. Patterns of DNA barcode variation in Canadian marine
60 1015 molluscs. *PLoS One*, **9**: e95003.

- LEACH, W.E., 1852. *Molluscorum Britanniae Synopsis. A synopsis of the Mollusca of Great Britain arranged according to their natural affinities and anatomical structure*. Van Voorst, London (edited posthumously by J. E. Gray).
- LEBOUR, M.V., 1936. Notes on the eggs and larvae of some Plymouth prosobranchs. *Journal of the Marine Biological Association of the United Kingdom*, **20**: 547–565.
- LEBOUR, M.V., 1937. The eggs and larvae of the British prosobranchs with special reference to those living in the plankton. *Journal of the Marine Biological Association of the United Kingdom*, **22**: 105–166.
- LEE, M.S.Y., 2004. The molecularisation of taxonomy. *Invertebrate Systematics*, **18**: 1–6.
- LERAY, M., YANG, J.Y., MEYER, C.P., MILLS, S.C., AGUDELO, N., RANWEZ, V., BOEHM, J.T., MACHIDA, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, **10**: 34.
- LINNAEUS, C., 1758. *Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Editio decima, reformata [10th revised edition], vol. 1: 824 pp. Laurentius Salvius, Stockhol [Holmiae].
- LOCARD, A., 1904. Coquilles des Mers d'Europe. Turbinidæ. *Annales de la Société d'Agriculture, Sciences et Industrie de Lyon*, **8**: 31–89.
- MANOUSIS, T., GALINO-MITSOUNDI, S., KONTADAKIS, C., MPAZIOS, G., POLYZOULIS, G., 2021. *Hellenic Conches*. ConchBooks, Harxheim.
- MARSHALL, B.A., 1995a. Calliostomatidae (Gastropoda: Trochoidea) from New Caledonia, the Loyalty Islands, and the northern Lord Howe Rise. In: Résultats des Campagnes MUSORSTOM 14. *Mémoires du Muséum national d'Histoire naturelle, Série A, Zoologie* (Bouchet, P. ed.), **167**: 381–458.
- MARSHALL, B.A., 1995b. A revision of the Recent *Calliostoma* species of New Zealand (Mollusca: Gastropoda: Trochoidea). *Nautilus*, **108**: 83–127.
- MATHEWS, D.H., SABINA, J., ZUKER, M., TURNER, D.H., 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology*, **288**: 911–940.
- MEIER, R., ZHANG, G., ALI, F., 2008. The use of mean instead of smallest interspecific distances exaggerates the size of the “barcoding gap” and leads to misidentification. *Systematic Biology*, **57**: 809–813.
- MELATUNAN, S., CALOSI, P., RUNDLE, S.D., WIDDICOMBE, S., MOODY, A.J., 2013. Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. *Marine Ecology Progress Series*, **472**: 155–168.
- MERINO VALENCIA, N.M., SOTIL, G., INDACOCHEA, A., 2020. *DNA barcoding of five benthic invertebrate taxa from the Mackellar Cove of the Antarctic Peninsula*. Bachelor Thesis, Universidad Científica del Sur, Facultad de Ciencias Veterinarias y Biológicas, Lima, Perú.
- MICALI, P., 2013. Riscoperta di *Calliostoma agrigentinum* Coen, 1936, con note su alcuni sinonimi di *C. zizyphinum* (Mollusca: Gastropoda: Calliostomatidae). *Malacologia Mostra Mondiale*, **81**: 25–27.
- MONTAGU, G., 1803. *Testacea Britannica or natural history of British shells, marine, land, and fresh-water, including the most minute: Systematically arranged and embellished with figures*. J. White, London.

- 1
2
3 1055 MONTEROSATO, T.A., 1884. *Nomenclatura generica e specifica di alcune conchiglie mediterranee*. Virzi,
4 1056 printed for the author, Palermo.
- 6 1057 MONTEROSATO, T.A., 1889. Molluschi del Porto di Palermo. Specie e varietà. *Bullettino della Società*
7 1058 *Malacologica Italiana*, **14**(4–6): 75–81.
- 9 1059 MUGNAI, F., COSTANTINI, F., CHENUIL, A., LEDUC, M., ORTEGA, J.M.G., & MEGLÉCZ, E. 2023. Be positive:
10 1060 customized reference databases and new, local barcodes balance false taxonomic assignments in
11 1061 metabarcoding studies. *PeerJ*, **11**, e14616.
- 13 1062 NAPPO, A., PELLEGRINI, D., PAGLI, A., 2021. First record of *Calliostoma lusitanicum* F. Nordsieck et García-
14 1063 Talavera, 1979 (Gastropoda Calliostomatiidae) in the Mediterranean Sea and remarks on *C.*
15 1064 *alexandrinum* Pallary, 1912. *Biodiversity Journal*, **12**: 225–228.
- 17 1065 NARDO, G.D., 1847. *Sinonimia Moderna delle Specie Registrate nell’Opera Intitolata: Descrizione*
18 1066 *de’ Crostacei, de’ Testacei e de’ Pesci che Abitano le Lagune e Golfo Veneto Rappresentati in Figure, a*
19 1067 *Chiario-scuro ed a Colori dall’Abate Stefano Chiereghini Ven. Clodiense*. Antonelli, Venezia.
- 21 1068 NOLF, F. & HUBRECHT, S., 2022a. A new look at East Atlantic calliostomatids, resulting in the introduction of
22 1069 two new species. *Neptunea*, **16**: 1–34.
- 24 1070 NOLF, F. & HUBRECHT, S., 2022b. A tiny new *Calliostoma* species from Senegal. *Neptunea*, **16**: 17–24.
- 26 1071 NOLF, F. & HUBRECHT, S., 2022c. A case of stolen identity: *Calliostoma lithocolletum*, and the description of
27 1072 a new species from the Canary Islands. *Neptunea*, **16**: 1–16.
- 29 1073 OLIVERIO, M. & MARIOTTINI, P., 2001. Contrasting morphological and molecular variation in *Coralliophila*
30 1074 *meyendorffii* (Muricidae, Coralliophilinae). *Journal of Molluscan Studies*, **67**: 243–246.
- 32 1075 OLIVERIO, M., CERVELLI, M., MARIOTTINI, P., 2002. ITS2 rRNA evolution and its congruence with the
33 1076 phylogeny of muricid neogastropods (Caenogastropoda, Muricoidea). *Molecular Phylogenetics and*
34 1077 *Evolution*, **25**: 63–69.
- 36 1078 PALLARY, P., 1900. Coquilles marines du littoral du département d’Oran. *Journal de Conchyliologie*, **48**: 211–
37 1079 422.
- 39 1080 PALLARY, P., 1902. Liste des mollusques testacés de la baie de Tanger. *Journal de Conchyliologie*, **50**: 1–39.
- 41 1081 PALLARY, P., 1912. Catalogue des mollusques du littoral méditerranéen de l’Egypte. *Mémoires de l’Institut*
42 1082 *d’Egypte*, **7**(3): 69–207.
- 44 1083 PALUMBI, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: *Molecular systematics*, 2nd edition
45 1084 (D.M. Hillis, C. Moritz, B. K. Mable, eds), pp. 205–247. Sinauer, Sunderland.
- 47 1085 PARENZAN, P., 1970. *Carta d’identità delle conchiglie del Mediterraneo. Gasteropodi*. Bios Taras Ed,
48 1086 Taranto.
- 50 1087 PAYRAUDEAU, B.C., 1826. *Catalogue descriptif et méthodique des annélidés et des mollusques de l’île de*
51 1088 *Corse; avec huit planches représentant quatre-vingt-huit espèces, dont soixante-huit nouvelles*.
52 1089 Imprimerie de J. Tastu, Paris.
- 54 1090 PHILIPPI, R.A., 1843 [1842–1850]. *Abbildungen und Beschreibungen neuer oder wenig gekannter Conchylien*
55 1091 *unter Mithülfe mehrerer deutscher Conchyliologen*. Vol. 1: 21–76 [1843]. Fischer, Cassel.
- 57 1092 PHILIPPI, R.A., 1844. *Enumeratio molluscorum Siciliae cum viventium tum in tellure tertiaria fossilium, quae*
58 1093 *in itinere suo observavit*. Vol. 2. Eduard Anton, Halle [Halis Saxorum].

- PHILIPPI, R.A., 1848 [1846–1855]. Die Kreiselschnecken oder Trochoideen (Gattungen *Turbo*, *Trochus*, *Solarium*, *Rotella*, *Delphinula*, *Phasianella*). In *Abbildungen nach der Natur mit Beschreibungen*. In: *Systematisches Conchylien Cabinet von Martini und Chemnitz*. Zweiten Bandes, Dritte Abtheilung. (Küster, H. C. ed.), **2(3)**: 33–72 [1–372, pl. 1–49]. Bauer & Raspe, Nürnberg.
- PHILIPPI, R.A., 1849. Centuria altera testaceorum novorum. *Zeitschrift für Malakozoologie*, **5(7)**: 99–112.
- PUILLANDRE, N., BAYLAC, M., BOISSELIER, M.-C., CRUAUD, C., SAMADI, S., 2009. An integrative approach to species delimitation in *Benthomangelia* (Mollusca: Conoidea). *Biological Journal of the Linnean Society*, **96**: 696–708.
- PUILLANDRE, N., MODICA, M.V., ZHANG, Y., SIROVICH, L., BOISSELIER, M., CRUAUD, C., HOLFORD, M., SAMADI, S., 2012. Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology*, **21**: 2671–2691.
- PUILLANDRE, N., STÖCKLIN, R., FAVREAU, P., BIANCHI, E., PERRET, F., RIVASSEAU, A., LIMPALAËR, L., MONNIER, E., BOUCHET, P., 2014. When everything converges: Integrative taxonomy with shell, DNA and venomic data reveals *Conus conco*, a new species of cone snails (Gastropoda: Conoidea). *Molecular Phylogenetics and Evolution*, **80**: 186–192.
- PUILLANDRE, N., BROUILLET, S., ACHAZ, G., 2021. ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, **21**: 609–620.
- PULTENEY, R., 1799. *Catalogue of the birds, shells, and some of the more rare plants of Dorsetshire*. Nichols, London.
- QUINN, J.F., 1992. New species of *Calliostoma* Swainson, 1840 (Gastropoda: Trochidae), and notes on some poorly known species from the Western Atlantic Ocean. *The Nautilus*, **106**: 77–114.
- RAMBAUT, A., DRUMMOND, A.J., XIE, D., BAELE, G., SUCHARD, M.A., 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. *Systematic Biology*, **67**: 901–904.
- RAMÓN, M., 1990. Spawning and development of *Calliostoma granulatum* in the Mediterranean Sea. *Journal of the Marine Biological Association of the United Kingdom*, **70**: 321–328.
- RISSE, A., 1826. *Histoire naturelle des principales productions de l'Europe méridionale et particulièrement de celles des environs de Nice et des Alpes Maritimes*. Vol. 4. Mollusques. Annélides. F.-G. Levrault. Paris.
- ROLÁN MOSQUERA, E., 1983. *Moluscos de la Ria de Vigo: Gasteropodos*. Velograf, Santiago de Compostela, Spain.
- RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D.L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M.A., HUELSENBECK, J.P., 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**: 539–542.
- RUSSINI, V., FASSIO, G., MODICA, M.V., DEMAINTENON, M.J., OLIVERIO, M., 2017. An assessment of the genus *Columbella* Lamarck, 1799 (Gastropoda: Columbellidae) from eastern Atlantic. *Zoosystema*, **39**: 197–212.
- RUSSINI, V., GIANNUZZI-SAVELLI, R., PUSATERI, F., PRKIĆ, J., FASSIO, G., MODICA, M.V., OLIVERIO, M., 2020. Candidate cases of poecilogony in Neogastropoda: Implications for the systematics of the genus *Raphitoma* Bellardi, 1847. *Invertebrate Systematics*, **34**: 293–318.
- RUSSO, P., 2023. Storia tassonomica di *Trochus Laugierii* Payraudeau, 1826 (Gastropoda: Calliostomatiidae) e del complesso di specie affini. *Alleryana*, **41**: 1–17.

- 1
2
3 1135 SABELLI, B., GIANNUZZI-SAVELLI, R., BEDULLI, D., 1990. *Catalogo annotato dei molluschi marini del*
4 1136 *Mediterraneo (Annotated checklist of Mediterranean marine mollusks)*. Libreria naturalistica
5 1137 bolognese, Bologna.
- 7 1138 SALVI, D., BELLAVIA, G., CERVELLI, M., MARIOTTINI, P., 2010. The analysis of rRNA sequence-structure in
8 1139 phylogenetics: An application to the family Pectinidae (Mollusca: Bivalvia). *Molecular Phylogenetics*
10 1140 *and Evolution*, **56**: 1059–1067.
- 11 1141 SALVI, D. & MARIOTTINI, P., 2012. Molecular phylogenetics in 2D: ITS2 rRNA evolution and sequence-
12 1142 structure barcode from Veneridae to Bivalvia. *Molecular Phylogenetics and Evolution*, **65**: 792–798.
- 14 1143 SALVI, D., MACALI, A., MARIOTTINI, P., 2014. Molecular phylogenetics and systematics of the bivalve family
15 1144 Ostreidae based on rRNA sequence-structure models and multilocus species tree. *PLoS One*, **9**:
17 1145 e108696.
- 18 1146 SALVI, D. & MARIOTTINI, P., 2017. Molecular taxonomy in 2D: a novel ITS2 rRNA sequence-structure
20 1147 approach guides the description of the oysters' subfamily Saccostreinae and the genus *Magallana*
21 1148 (Bivalvia: Ostreidae). *Zoological Journal of the Linnean Society*, **179**: 263–276.
- 23 1149 SEIBEL, P.N., MÜLLER, T., DANDEKAR, T., WOLF, M., 2008. Synchronous visual analysis and editing of RNA
24 1150 sequence and secondary structure alignments using 4SALE. *BMC Research Notes*, **1**: 1–7.
- 26 1151 SOWERBY, J. 1817 [1812-1845, continued by J.D.C. Sowerby]. *The mineral conchology of Great Britain; or*
27 1152 *coloured figures and descriptions of those remains of testaceous animals or shells, which have been*
29 1153 *preserved at various times and depths in the earth*. London (Sowerby), 2 (for authorship, collation
30 1154 and dates of parts 1-7 see *Bulletin of Zoological Nomenclature*, 1987, **44**: 64–67).
- 31 1155 SWOFFORD, D.L., 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), Version 4.
33 1156 Sinauer Associates, Sunderland, Massachusetts.
- 35 1157 TERRENI, G., 1981. *Molluschi conchiferi del mare antistante la costa Toscana (Gastropoda, Scaphopoda,*
36 1158 *Amphineura, Bivalva, Cephalopoda)*. Tip. Benvenuti & Cavaciocchi, Livorno, Italy.
- 38 1159 TRIFINOPOULOS, J., NGUYEN, L.-T., VON HAESELER, A., MINH, B.Q., 2016. W-IQ-TREE: a fast online
39 1160 phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research*, **44**: W232–W235.
- 41 1161 TRIGO, J.E., ROLÁN, E., AGRAS, G.J.D., GARCÍA-ÁLVAREZ, Ó., SIERRA, Á.G., DA ROCHA, J.M., DIESTE, J.P.,
42 1162 TRONCOSO, J.S., URGORRI, V., 2018. *Guía de los moluscos marinos de Galicia*. Servizo de Publicacións
43 1163 da Universidade de Vigo, Vigo.
- 45 1164 URIBE, J.E., WILLIAMS, S.T., TEMPLADO, J., ABALDE, S., ZARDOYA, R., 2017. Denser mitogenomic sampling
46 1165 improves resolution of the phylogeny of the superfamily Trochoidea (Gastropoda: Vetigastropoda).
47 1166 *Journal of Molluscan Studies*, **83**: 111–118.
- 49 1167 VILVENS, C., NOLF, F., VERSTRAETEN, J., 2004. Description of *Calliostoma madagascarensis* n. sp.
51 1168 (Gastropoda: trochidae: Calliostomatinae) from Madagascar. *Novapex*, **5(2-3)**: 49–55.
- 52 1169 VILVENS, C. & SWINNEN, F., 2017. A new record and a new species of *Calliostoma* (Gastropoda: Trochoidea:
54 1170 Calliostomatidae) from southwestern Mediterranean Sea and adjacent Atlantic area. *Gloria Maris*,
55 1171 **56**: 20–23.
- 57 1172 WARÉN, A., 1990. Ontogenetic changes in the trochoidean (Archaeogastropoda) radula, with some
58 1173 phylogenetic interpretations. *Zoologica Scripta*, **19**: 179–187.
- 59 1174 WILLIAMS, S.T., 2017. Molluscan shell colour. *Biological Reviews*, **92**: 1039–1058.

- 1
2
3 1175 WILLIAMS, S.T., KARUBE, S., OZAWA, T., 2008. Molecular systematics of Vetigastropoda: Trochidae,
4 1176 Turbinidae and Trochoidea redefined. *Zoologica Scripta*, **37**: 483–506.
5
6 1177 WILLIAMS, S.T., DONALD, K.M., SPENCER, H.G., NAKANO, T., 2010. Molecular systematics of the marine
7 1178 gastropod families Trochidae and Calliostomatidae (Mollusca: Superfamily Trochoidea). *Molecular*
8 1179 *Phylogenetics and Evolution*, **54**: 783–809.
9
10 1180 WOOD, W., 1828. *Supplement to the Index Testaceologicus; or A catalogue of shells, British and foreign.*
11 1181 Richard Taylor, London.
12
13 1182 YEATES, D.K., SEAGO, A., NELSON, L., CAMERON, S.L., JOSEPH, L.E.O., TRUEMAN, J.W.H., 2011. Integrative
14 1183 taxonomy, or iterative taxonomy? *Systematic Entomology*, **36**: 209–217.
15
16 1184 ZUKER, M. & JACOBSON, A.N.N.B., 1998. Using reliability information to annotate RNA secondary
17 1185 structures. *RNA*, **4**: 669–679.
18
19 1186 ZUKER, M., 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids*
20 1187 *Research*, **31**: 3406–3415.
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2 1188
3 1189
4 1190
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

Table 1. Samples sequenced in the present work. Species names correspond to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Fig. 3); “*r*” indicates samples whose radula was dissected and photographed; morphotype names represent preliminary species hypotheses (PSH), see text for details; locality numbers are displayed in Figure 1. The asterisks in NCBI accession numbers indicate minibarcode sequences.

Species	ID Code	Morphotype	Locality	lat	long	depth	cox1	16S rDNA	its2
<i>C. conulus</i>	BAU 2730	<i>conulus</i>	18. Sicily, Capo D'Orlando	38.1650	14.7508	40 m	OR633461*	OR632437	
<i>r</i>	BAU 3704	<i>conulus</i>	14. Sardinia, Bosa	40.3067	8.4622	110 m		OR632438	
<i>r</i>	BAU 3705	<i>conulus</i>	12. Italy, Giannutri Island	42.2542	11.1083	35 m	OR633462	OR632439	OR678225
	BAU 3707	<i>conulus</i>	14. Sardinia, Bosa	40.3067	8.4622	110 m	OR633463	OR632440	OR678226
<i>r</i>	BAU 3713	<i>conulus</i>	22. Italy, Palizzi	37.9192	16.0167	42 m		OR632441	
	BAU 3714	<i>conulus</i>	12. Italy, Giannutri Island	42.2542	11.1083	5 m		OR632442	
	BAU 3777	<i>conulus</i>	12. Italy, Giglio Island	42.3556	10.9267	15 m		OR632443	
	BAU 3778	<i>conulus</i>	10. Corsica, Scandola Reserve, Palazzino	42.3800	8.5500	42 m	OR633464	OR632444	OR678227
<i>r</i>	BAU 3841	<i>conulus</i>	3. Spain, Malpica	43.3400	-8.8400	18 m	OR633465	OR632445	OR678228
	BAU 3851.1	<i>conulus</i>	23. Italy, Taranto, Mar Piccolo	40.4742	17.3094	2 m	OR633466	OR632446	OR678229
	BAU 3851.2	<i>conulus</i>	23. Italy, Taranto, Mar Piccolo	40.4742	17.3094	2 m	OR633467	OR632447	OR678230
	BAU 4161	<i>conulus</i>	22. Italy, Scilla	38.2544	15.7189	35 m	OR633468*	OR632448	
	MNHN-IM-2019-4548	<i>conulus</i>	9. Corsica	42.7283	9.4650	32 m	OR633469		
	MNHN-IM-2019-4734	<i>conulus</i>	9. Corsica, Cap Corse	42.7350	9.4683	50 m	OR633470		
	MNHN-IM-2019-4735	<i>conulus</i>	9. Corsica, Cap Corse	42.8933	9.5050	40 m	OR633471		
	MNHN-IM-2019-5657	<i>conulus</i>	9. Corsica, Nonza shoal	42.7867	9.3300	37 m	OR633472		
	MNHN-IM-2019-5726	<i>conulus</i>	9. Corsica, Pietranera	42.7283	9.4650	33 m	OR633473		
	MNHN-IM-2019-5728	<i>conulus</i>	9. Corsica, Pietranera	42.7283	9.4650	33 m	OR633474		
	MNHN-IM-2019-5891	<i>conulus</i>	9. Corsica, Centuri	42.9917	9.2917	36 m	OR633475		
	MNHN-IM-2019-6374	<i>conulus</i>	9. Corsica, Cap Corse	43.0433	9.3317	60 m	OR633476		
	MNHN-IM-2019-6375	<i>conulus</i>	9. Corsica, Cap Corse	43.0433	9.3317	60 m	OR633477		
	MNHN-IM-2019-12778	<i>conulus</i>	11. Corsica	41.5283	9.4017	32–42 m	OR633478		
	MNHN-IM-2019-12818	<i>conulus</i>	11. Corsica, Les Griffes du Toro	41.5100	9.3983	50 m	OR633479		
	MNHN-IM-2019-13704	<i>conulus</i>	11. Corsica, Les Moines	41.4467	8.9000	25–34 m	OR633480		
	MNHN-IM-2019-16536	<i>conulus</i>	10. Corsica, Gargalo Island	42.3667	8.5417	28–30 m	OR633481		
	MNHN-IM-2019-17180	<i>conulus</i>	10. Corsica, Gargalo Island	42.3717	8.5400	25–39 m	OR633482		
	MNHN-IM-2019-17181	<i>conulus</i>	10. Corsica, Gargalo Island	42.3717	8.5400	25–39 m	OR633483		
	MNHN-IM-2019-18123	<i>conulus</i>	10. Corsica, Solana Bay	42.3567	8.5533	3–15 m	OR633484		
	MNHN-IM-2019-18124	<i>conulus</i>	10. Corsica, Solana Bay	42.3567	8.5533	3–15 m	OR633485		

C. granulatum	r	MNHN-IM-2019-18131	conulus	10. Corsica, Garganellu island	42.3650	8.5383	3–51 m	OR633486		
		BAU 3718.1	granulatum	13. Italy, off Fiumicino	41.7811	12.2242	160 m	OR633487*	OR632449	
		BAU 3718.2	granulatum	13. Italy, off Fiumicino	41.7811	12.2242	160 m	OR633488	OR678231	
		BAU 3718.3	granulatum	13. Italy, off Fiumicino	41.7811	12.2242	160 m	OR633489*		
		BAU 4499	granulatum	17. Italy, Curinga	38.8347	16.2156	60–72 m	OR633490	OR632450	
		BAU 4517.1	granulatum	7. Spain, Vilanova i la Geltrù	41.2131	1.7338	20 m	OR633491	OR632451	
		BAU 4517.2	granulatum	7. Spain, Vilanova i la Geltrù	41.2131	1.7338	20 m	OR633492	OR632452	
		BAU 4528	granulatum	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633493		
		BAU 4627.1	granulatum	2. France, Roscoff	48.7261	-3.9908	80 m	OR633494	OR632453	
			MNCN-15.05/200522	granulatum	5. Spain, Malaga	36.6032	-4.3465	154–156 m	OR633495	OR632454
C. zizyphinum		MNHN-IM-2019-4176	granulatum	9. Corsica, Cap Corse	42.9400	9.5233	71 m	OR633496		
		MNHN-IM-2019-12422	granulatum	11. Corsica, Punta di u Capicciolu	41.4050	9.2817	71–71 m	OR633497		
				11. Corsica, between Bonifacio and Porto-Vecchio	41.4217	9.5550	101–102 m	OR633498		
		MNHN-IM-2019-13088	granulatum							
		MNHN-IM-2019-13329	granulatum	11. Corsica, Bonifacio	41.3633	9.1600	73–73 m	OR633499		
		MNHN-IM-2019-17969	granulatum	10. Corsica, Mursetta	42.4883	8.6417	90–91 m	OR633500		
		BAU 703.1	lividum	29. Azores, San Miguel Island, Nordeste	37.8292	-25.1394	5 m	OR633501	OR632455	
		BAU 703.2	lividum	29. Azores, San Miguel Island, Nordeste	37.8292	-25.1394	5 m	OR633502*		
		BAU 703.3	lividum	29. Azores, San Miguel Island, Nordeste	37.8292	-25.1394	5 m	OR633503*	OR632456	
		BAU 722.1	lividum	29. Azores, San Miguel Island, Caloura	37.7125	-25.4961	3 m	OR633504	OR632457	
		BAU 722.2	lividum	29. Azores, San Miguel Island, Caloura	37.7125	-25.4961	3 m	OR633505	OR632458	
		r	BAU 722.3	lividum	29. Azores, San Miguel Island, Caloura	37.7125	-25.4961	3 m	OR633506	OR632459
		BAU 737.1	lividum	30. Azores, Flores Island, Santa Cruz	39.4514	-31.1269	2 m	OR633507	OR632460	
		BAU 749	lividum	29. Azores, San Miguel Island, Caloura	37.7125	-25.4961	3 m	OR633508*	OR632461	
				29. Azores, San Miguel Island, Vila Franca do Campo Island	37.7061	-25.4431	3 m	OR633509	OR632462	
		r	BAU 765.1	lividum						
				29. Azores, San Miguel Island, Vila Franca do Campo Island	37.7061	-25.4431	3 m	OR633510*	OR632463	
			BAU 2042.1	zizyphinum	2. France, Roscoff	48.7261	-3.9908	0–1 m		OR632464
		r	BAU 2042.2	zizyphinum	2. France, Roscoff	48.7261	-3.9908	0–1 m	OR633511	OR632465
			BAU 2042.3	zizyphinum	2. France, Roscoff	48.7261	-3.9908	0–1 m	OR633512*	
	r	BAU 2042.4	zizyphinum	2. France, Roscoff	48.7261	-3.9908	0–1 m	OR633513*	OR632466	
		BAU 2864.1	alexandrinum	21. Sicily, Portopalo di Capo Passero	36.6808	15.1383	60 m	OR633514	OR632467	
		BAU 2864.2	alexandrinum	21. Sicily, Portopalo di Capo Passero	36.6808	15.1383	60 m	OR633515	OR632468	
									OR678235	

1									
2		BAU 3623	<i>lividum</i>	28. Azores, Terceira Island, Biscoitos	38.8017	-27.2594	4 m	OR633516	OR632469
3	r	BAU 3712	<i>alexandrinum</i>	14. Sardinia, Bosa	40.3067	8.4622	2 m	OR633517	OR632470
4				13. Italy, Lazio, Civitavecchia, Asia					
5		BAU 3715	<i>dubium</i>	shipwreck	42.0569	11.7831	35 m	OR633518	OR632471
6		BAU 3839.1	<i>zizyphinum</i>	3. Spain, Ria de Arousa	42.5249	-8.8667	5 m	OR633519	OR632472
7	r	BAU 3839.2	<i>zizyphinum</i>	3. Spain, Ria de Arousa	42.5249	-8.8667	5 m	OR633520	
8		BAU 3840.2	<i>zizyphinum</i>	3. Spain, Ria de Arousa, Cambados	42.5267	-8.8274	5 m	OR633521	OR678236
9		BAU 4480.1	<i>virescens</i>	4. Portugal, Aveiro	40.6433	-8.6742	tide pools	OR633522	OR632473
10		BAU 4480.2	<i>virescens</i>	4. Portugal, Aveiro	40.6433	-8.6742	tide pools	OR633523	
11		BAU 4480.3	<i>virescens</i>	4. Portugal, Aveiro	40.6433	-8.6742	tide pools	OR633524	
12		BAU 4519	<i>zizyphinum</i>	1. United Kingdom, Plymouth	50.3636	-4.1413	intertidal	OR633525	
13		BAU 4520.1	<i>zizyphinum</i>	1. United Kingdom, Plymouth	50.3636	-4.1413	intertidal	OR633526	
14		BAU 4520.2	<i>zizyphinum</i>	1. United Kingdom, Plymouth	50.3636	-4.1413	intertidal	OR633527	
15		BAU 4529	<i>zizyphinum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633528	
16		BAU 4629.1	<i>zizyphinum</i>	2. France, Roscoff	48.7261	-3.9908	0–1 m	OR633529	OR632474
17		BAU 4629.3	<i>zizyphinum</i>	2. France, Roscoff	48.7261	-3.9908	0–1 m	OR633530	OR632475
18		MNCN-15.05/200521	<i>conulus</i>	6. Spain, Alboran Island	35.9980	-2.9178	68–70 m	OR633531	OR632476
19		MNCN-15.05/200526	<i>zizyphinum</i>	5. Spain, Marbella	36.5055	-4.8758	5 m	OR633532*	
20		MNHN-IM-2019-13695	<i>zizyphinum</i>	11. Corsica, St. Antonio shoal	41.3400	9.1817	43 m	OR633533	
21		MNHN-IM-2019-14568	<i>zizyphinum</i>	11. Corsica	41.3400	9.1800	42 m	OR633534	
22		MZUB-Call A	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528836
23		MZUB-Call B	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528837
24		MZUB-Call 22	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528838
25		MZUB-Call 23	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528839
26		MZUB-Call 24	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528840
27		MZUB-Call 25	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m	PP528827	PP528841
28		MZUB-Call 26	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528842
29		MZUB-Call 31	<i>zizyphinum</i>	25. Italy, Chioggia, Tegnue	45.2304	12.4894	22 m		PP528843
30		MZUB-Call AT1	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528844
31		MZUB-Call AT2	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m	PP528828*	PP528845
32		MZUB-Call AT3	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528846
33		MZUB-Call AT4	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528847
34		MZUB-Call AT5	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528848
35		MZUB-Call AT6	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528849

<i>C. laugieri</i>		MZUB-Call AT7	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528850	
		MZUB-Call AT8	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528851	
		MZUB-Call AT9	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528852	
		MZUB-Call AT12	<i>zizyphinum</i>	2. France, Ploubazlanec	48.9776	-3.0004	0 m		PP528853	
		13012-4701-1	<i>funiculatum</i>	19. Sicily, Egadi Islands	37.7972	12.3336	80 m		OR632477	
		BAU 1614	"elegant"	10. Corsica, Calcatoggio, Tour d'Ancone	42.0431	8.7256	5 m	OR633535	OR632478	
	<i>r</i>	BAU 1830.0	<i>laugieri</i>	18. Sicily, Barcarello	38.2111	13.2811	2 m	OR633536	OR632479	
		BAU 1830.1	<i>laugieri</i>	18. Sicily, Barcarello	38.2111	13.2811	2 m	OR633537*		
		BAU 1830.3	<i>laugieri</i>	18. Sicily, Barcarello	38.2111	13.2811	2 m	OR633538	OR632480	
		BAU 2320.1	"big-cord"	12. Italy, Giannutri Island, Punta San Francesco	42.2517	11.1164	10–20 m	OR633539	OR632481	
		BAU 2320.2	"big-cord"	12. Italy, Giannutri Island, Punta San Francesco	42.2517	11.1164	10–20 m	OR633540	OR632482	OR678237
		BAU 2320.3	"big-cord"	12. Italy, Giannutri Island, Punta San Francesco	42.2517	11.1164	10–20 m	OR633541	OR632483	OR678238
		BAU 2494	<i>spongiarum</i>	20. Tunisia, Gharbi Island	34.6744	11.0353	0–1 m	OR633542	OR632484	OR678239
		BAU 2527.1	<i>spongiarum</i>	20. Tunisia, Gremdi Island	34.7639	11.3319	0–1 m	OR633543	OR632485	
	<i>r</i>	BAU 2527.2	<i>spongiarum</i>	20. Tunisia, Gremdi Island	34.7639	11.3319	0–1 m	OR633544	OR632486	
		BAU 2726.1	<i>laugieri</i>	22. Italy, Scilla	38.2544	15.7189	12 m	OR633545*	OR632487	
		BAU 2726.3	<i>qualterianum</i>	22. Italy, Scilla	38.2544	15.7189	24 m	OR633546*	OR632488	
		BAU 2919.1	<i>qualterianum</i>	12. Italy, Giannutri Island	42.2542	11.1083	30 m	OR633547	OR632489	
		BAU 2919.2	<i>laugieri</i>	12. Italy, Giannutri Island	42.2542	11.1083	12 m	OR633548	OR632490	OR678240
		BAU 2919.3	<i>laugieri</i>	12. Italy, Giannutri Island	42.2542	11.1083	12 m	OR633549	OR632491	OR678241
		BAU 2919.4	<i>qualterianum</i>	12. Italy, Giannutri Island	43.2542	11.1083	35 m	OR633550	OR632492	OR678242
		BAU 3052	<i>laugieri</i>	Sicily	-	-	-	OR633551	OR632493	OR678243
		BAU 3080.1	"big-cord"	24. Croatia, Trsteno	42.7094	17.9764	0–5 m	OR633552	OR632494	OR678244
		BAU 3094.1	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633553	OR632495	
		BAU 3094.2	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633554	OR632496	OR678245
	<i>r</i>	BAU 3094.3	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633555	OR632497	OR678246
		BAU 3098.1	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633556	OR632498	
		BAU 3098.2	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633557	OR632499	
		BAU 3098.3	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5839	26.4233	1–7 m	OR633558	OR632500	
		BAU 3111	<i>laugieri</i>	26. Greece, Astypalaea Is., Agrilidi	36.5644	26.3531	0–1 m	OR633559	OR632501	
		BAU 3159.1	<i>funiculatum</i>	19. Sicily, off Marsala	37.7972	12.3336	70–80 m	OR633560	OR632502	

1									
2		BAU 3159.2	<i>funiculatum</i>	19. Sicily, off Marsala	37.7972	12.3336	70–80 m	OR633561	OR632503
3		BAU 3159.3	<i>funiculatum</i>	19. Sicily, off Marsala	37.7972	12.3336	70–80 m	OR633562	OR632504
4		BAU 3403	"elegant"	17. Italy, Cetraro	39.5092	15.9308	5 m	OR633563	OR632505
5	r	BAU 3515.1	<i>laugieri</i>	12. Italy, Giannutri Island	42.2453	11.1089	11 m	OR633564	OR632506 OR678247
6		BAU 3515.2	<i>laugieri</i>	12. Italy, Giannutri Island	42.2453	11.1089	11 m	OR633565	OR632507 OR678248
7		BAU 3515.3	<i>laugieri</i>	12. Italy, Giannutri Island	42.2453	11.1089	11 m	OR633566	OR632508
8		BAU 3532.1	<i>laugieri</i>	12. Italy, Giannutri Island	42.2542	11.1083	11 m	OR633567	OR632509 OR678249
9		BAU 3650.1	<i>laugieri</i>	12. Italy, Giannutri Island	42.2542	11.1083	11 m	OR633568	OR632510
10		BAU 3650.2	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	11 m	OR633569	OR632511
11		BAU 3650.3	<i>seriopunctatus</i>	12. Italy, Giannutri Island	42.2542	11.1083	11 m	OR633570	OR632512
12		BAU 3697	<i>neretinum</i>	8. Italy, Gallinara Island	44.0269	8.2264	10 m	OR633571	OR632513
13		BAU 3698.1	"big-cord"	15. Sardinia, San Teodoro, Lu Impostu	40.8250	9.6833	0–3 m	OR633572	OR632514
14		BAU 3699	"big-cord"	15. Sardinia, San Teodoro, Lu Impostu	40.7667	9.6917	0–3 m	OR633573	OR632515
15		BAU 3700.1	"stepped"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633574	OR632516
16		BAU 3700.3	"big-cord"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633575	OR632517 OR678250
17		BAU 3700.4	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633576	OR632518 OR678251
18		BAU 3700.5	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633577	OR632519
19		BAU 3700.6	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633578	OR632520
20		BAU 3700.7	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633579	OR632521 OR678252
21		BAU 3700.8	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633580	OR632522 OR678253
22		BAU 3700.9	"uniform"	15. Sardinia, San Teodoro, Lu Impostu	40.8500	9.6583	0–3 m	OR633581*	
23		BAU 3702.1	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	1–30 m	OR633582	OR632523
24		BAU 3702.2	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	1–30 m	OR633583	OR632524 OR678254
25		BAU 3702.3	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	1–30 m	OR633584	OR632525 OR678255
26		BAU 3702.4	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	1–30 m	OR633585	OR632526
27		BAU 3706.1	<i>kochi</i>	5. Spain, Malaga	36.7058	-4.3756		OR633586	OR632527
28	r	BAU 3708	<i>gualterianum</i>	16. Italy, Ponza Island	40.5944	12.9728	35 m	OR633587	OR632528
29		BAU 3709.1	<i>gualterianum</i>	12. Italy, Giannutri Island	42.2542	11.1083	30 m	OR633588	OR632529
30	r	BAU 3709.2	<i>gualterianum</i>	12. Italy, Giannutri Island	42.2542	11.1083	30 m	OR633589	OR632530
31	r	BAU 3710	"big-cord"	5. Spain, Getares, La Ballenera	36.1050	-5.4306	3 m	OR633590	OR632531 OR678257
32		BAU 3711	<i>gualterianum</i>	12. Italy, Giannutri Island	42.2542	11.1083	25 m	OR633591	OR632532 OR678258
33	r	BAU 3716.1	<i>laugieri</i>	21. Malta, Gozo	36.0619	14.2864	15 m	OR633592*	OR632533
34	r	BAU 3717.1	"elegant"	23. Italy, Santa Maria al Bagno	40.1300	17.9936	5 m	OR633593	OR632534
35									
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									
46									

BAU 3717.2	"elegant"	23. Italy, Santa Maria al Bagno	40.1300	17.9936	5 m	OR633594	OR632535	
BAU 3717.3	"elegant"	23. Italy, Santa Maria al Bagno	40.1300	17.9936	5 m	OR633595	OR632536	
BAU 3717.4	"elegant"	23. Italy, Santa Maria al Bagno	40.1300	17.9936	5 m	OR633596	OR632537	
BAU 3720.1	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633597	OR632538	OR678259
BAU 3720.2	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633598	OR632539	
BAU 3720.3	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633599	OR632540	
BAU 3720.4	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633600*		
BAU 3720.5	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633601	OR632541	OR678260
BAU 3720.6	"elegant"	23. Italy, Porto Cesareo	40.2578	17.8967	90–100 m	OR633602	OR632542	OR678261
BAU 3721.1	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	3 m	OR633603	OR632543	
BAU 3721.2	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	3 m	OR633604	OR632544	
BAU 3721.3	"big-cord"	12. Italy, Giannutri Island	42.2542	11.1083	3 m	OR633605	OR632545	
BAU 3779	<i>laugieri</i>	10. Corsica, Scandola Reserve, Palazzino	42.3800	8.5500	15 m	OR633606	OR632546	OR678262
BAU 3780	<i>laugieri</i>	10. Corsica, Scandola Reserve, Palazzino	42.3800	8.5500	15 m	OR633607	OR632547	
<i>r</i> BAU 3781	<i>laugieri</i>	10. Corsica, Scandola Reserve, Palazzino	42.3800	8.5500	15 m	OR633608	OR632548	
BAU 3849	"big-cord"	15. Sardinia, Olbia, Porto San Paolo	40.8783	9.6389	1 m	OR633609	OR632549	OR678263
BAU 3850.1	"big-cord"	23. Italy, Porto Cesareo	40.2578	17.8967	2 m	OR633610	OR632550	
<i>r</i> BAU 3850.2	<i>laugieri</i>	23. Italy, Porto Cesareo	40.2578	17.8967	2 m	OR633611		
BAU 4183	<i>laugieri</i>	19. Sicily, Marettimo	37.9667	12.0767	10 m	OR633612		
<i>r</i> BAU 4222	<i>conulus</i>	14. Sardinia, Bosa	40.3067	8.4622	110 m	OR633613*	OR632551	
BAU 4290	<i>laugieri</i>	12. Italy, Elba, Scoglietto di Portoferraio	42.8281	10.3311	15 m	OR633614		
BAU 4507	"big-cord"	15. Sardinia, Capo dell'Orso	41.1756	9.4194	0–1 m	OR633615		
BAU 4523.1	"chessboard"	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633616*		
BAU 4523.2	<i>funiculatum</i>	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633617*		
BAU 4523.3	"elegant"	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633618		
BAU 4523.4	"elegant"	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633619		
BAU 4523.5	<i>funiculatum</i>	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633620		
BAU 4523.6	<i>funiculatum</i>	19. Sicily, Trapani	38.0264	12.4403	10–20 m	OR633621*		
BAU 4524	"elegant"	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633622		
BAU 4525.1	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633623		
BAU 4525.2	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633624		
BAU 4525.3	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633625		
BAU 4525.4	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633626		

1										
2	BAU 4525.5	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633627			
3	BAU 4525.6	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633628			
4	BAU 4525.7	<i>funiculatum</i>	19. Sicily, off Mazara del Vallo	37.4172	11.9422	200 m	OR633629			
5										
6	CY011	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633630	OR632552		
7	CY012	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633631			
8	CY013	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633632			
9	CY104	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5645	33.0470	20 m	OR633633	OR632553	OR678264	
10	CY139	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5596	33.0377	15 m	OR633634			
11	CY175	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5596	33.0377	15 m	OR633635			
12										
13	CY270	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	10 m	OR633636	OR632554		
14	CY271	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	10 m	OR633637			
15	CY272	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	10 m	OR633638			
16	CY277	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	10 m	OR633639			
17										
18	CY309	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	5 m	OR633640			
19	CY327	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	5 m	OR633641	OR632555		
20	CY371	<i>laugieri</i>	27. Cyprus, Akrotiri	34.5638	33.0124	10 m	OR633642			
21	CY545	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	15 m	OR633643	OR632556		
22	CY558	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633644			
23										
24	CY560	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633645			
25	CY562	<i>laugieri</i>	27. Cyprus, Cape Greco	34.9860	34.0786	20 m	OR633646			
26	MNCN-15.05/200523.1	<i>kochi</i>	5. Spain, Marbella	36.5055	-4.8758	0 m	OR633647	OR632557	OR678265	
27	MNCN-15.05/200523.2	<i>kochi</i>	5. Spain, Marbella	36.5055	-4.8758	0 m	OR633648			
28	MNCN-15.05/200523.3	<i>kochi</i>	5. Spain, Marbella	36.5055	-4.8758	0 m	OR633649			
29	MNCN-15.05/200523.4	<i>kochi</i>	5. Spain, Marbella	36.5055	-4.8758	0 m	OR633650			
30	MNCN-15.05/200523.5	<i>kochi</i>	5. Spain, Marbella	36.5055	-4.8758	0 m	OR633651			
31										
32	MNCN-15.05/200524	<i>planatum</i>	5. Spain, Calahonda	36.4900	-4.6967	3 m	OR633652	OR632558	OR678266	
33	MNCN-15.05/200525	<i>kochi</i>	5. Spain, Benalmádena	36.5883	-4.5283	1–5 m	OR633653			
34	MNCN-15.05/200527.1	<i>planatum</i>	6. Spain, Playa de los Genoveses	36.7433	-2.1067	12–13 m	OR633654	OR632559		
35	MNCN-15.05/200527.2	<i>planatum</i>	6. Spain, Playa de los Genoveses	36.7433	-2.1067	12–13 m	OR633655	OR632560		
36	MNCN-15.05/200527.3	<i>planatum</i>	6. Spain, Playa de los Genoveses	36.7433	-2.1067	12–13 m	OR633656			
37										
38	MNCN-15.05/200528	"elegant"	5. Spain, Fuengirola	36.5372	-4.6206	35 m	OR633657*			
39	MNHN-IM-2019-4729	"elegant"	9. Corsica, Lavasina	42.7550	9.4783	53 m	OR633658			
40	MNHN-IM-2019-4736	<i>laugieri</i>	9. Corsica, Cap Corse	42.8933	9.5050	40 m	OR633659			
41										
42										
43										
44										
45										
46										

MNHN-IM-2019-4762	"elegant"	9. Corsica, St. Severa	42.8650	9.5100	80 m	OR633660	
MNHN-IM-2019-5652	"elegant"	9. Corsica, Negru shoal	42.7683	9.3333	26 m	OR633661	
MNHN-IM-2019-5910	"uniform"	9. Corsica	43.0017	9.3117	110 m	OR633662	
MNHN-IM-2019-12469	<i>laugieri</i>	11. Corsica, Toru Island	41.5083	9.3933	13–29 m	OR633663	
MNHN-IM-2019-12693	<i>laugieri</i>	11. Corsica, Toru Island	41.5083	9.3933	26 m	OR633664	
MNHN-IM-2019-12777	<i>laugieri</i>	11. Corsica, L'Arche	41.5283	9.4017	38 m	OR633665	
MNHN-IM-2019-13319	<i>kochi</i>	11. Corsica	41.3633	9.1933	15 m	OR633666	
MNHN-IM-2019-13320	"big-cord"	11. Corsica	41.3633	9.1933	15 m	OR633667	
MNHN-IM-2019-13539	<i>laugieri</i>	11. Corsica, Ratino Island	41.3467	9.2750	20–25 m	OR633668	
MNHN-IM-2019-13540	"elegant"	11. Corsica, Ratino Island	41.3467	9.2750	20–25 m	OR633669	
MNHN-IM-2019-14567	"big-cord"	11. Corsica	41.3617	9.2067	35–40 m	OR633670	
MNHN-IM-2019-16420	<i>laugieri</i>	10. Corsica, Cala Vecchia	42.3483	8.5850	5–21 m	OR633671	
MNHN-IM-2019-16850	"big-cord"	10. Corsica, Cargèse	42.1200	8.5900	45–48 m	OR633672	
MNHN-IM-2019-17918	<i>gualterianum</i>	10. Corsica	42.2367	8.5317	56 m	OR633673*	
MNHN-IM-2019-18098	<i>laugieri</i>	10. Corsica, Punta Palazzu, Phoque-moine	42.3767	8.5517	10–15 m	OR633674	
MNHN-IM-2019-18101	<i>gualterianum</i>	10. Corsica, Capu Rossu	42.2367	8.5317	56 m	OR633675	
MNHN-IM-2019-18139	<i>gualterianum</i>	10. Corsica, Garganellu Island	42.3650	8.5383	3–51 m	OR633676	
MZUB-Call 2	<i>laugieri</i>	25. Italy, Trieste, Sistiana	45.7695	13.6271	4 m	PP528820	PP528829
MZUB-Call 3	<i>laugieri</i>	25. Italy, Trieste, Sistiana	45.3007	12.3071		PP528821*	PP528830
MZUB-Call 4	<i>virescens</i>	25. Italy, Venice, Pellestrina	45.3007	12.3071		PP528822*	PP528831
MZUB-Call 5	<i>virescens</i>	25. Italy, Venice, Pellestrina	45.3007	12.3071		PP528823*	PP528832
MZUB-Call 6	<i>virescens</i>	25. Italy, Venice, Pellestrina	45.3007	12.3071		PP528824*	PP528833
MZUB-Call 7	<i>virescens</i>	25. Italy, Venice, Pellestrina	45.3007	12.3071		PP528825*	PP528834
MZUB-Call 8	<i>virescens</i>	25. Italy, Venice, Pellestrina	45.3007	12.3071		PP528826*	PP528835

1
2
3
4
5
6
7
89
10
11
1213
14
15
16
17
18
19
20
2122
23
24
25
26
27
28
29
30
31
32
3334
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
5455
56
57
58
59
60
1242

Figure 1. Map of the collection sites. Numbers refer to the localities listed in Table 1. Species in the legend correspond to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Figure 3).

Figure 2. The *Its2* folding of *Calliostoma*. Apical STEM highlighted in red. Nucleotide colour legend: A, red; C, green; U, blue; G, yellow.

Figure 3. Bayesian Inference phylogenetic reconstruction of Calliostomatinae using a combined *cox1*, *16S rRNA* and *its2* alignment. White dots at nodes stand for maximum posterior probability and likelihood scores. Numbers in square brackets refer to the localities shown in Figure 1. Searchable file in Supplementary material 1.

Figure 4. A–J *Calliostoma conulus*. **A.** BAU 3705 (Giannutri Is.); **B.** BAU 3713 protoconch detail; **C.** BAU 3704 innermost marginal tooth; **D.** BAU 3841 rachidian tooth; **E.** BAU 3713 radula; **F.** MNHN-IM-2019-16536 (Corsica). Photo credit: Gilles Devauchelle; **G.** MNHN-IM-2019-4735 (Corsica) juv.; **H.** BAU 2730 (Sicily); **I.** BAU 3778 (Corsica); **J.** BAU 3841 (Malpica); **K–S** *Calliostoma granulatum*. **K.** BAU 3718.3 (off Fiumicino); **L–N** BAU 3718.1 innermost marginal teeth, rachidian teeth, and radula; **O.** MNHN-IM-2019-13088 (Corsica). Photo credit: Gilles Devauchelle; **P.** BAU 4627.1 (Roscoff); **Q.** MNHN-IM-2019-12422 (Corsica). Photo credit: Andrea Nappo; **R.** MNCN-15.05/200522 (Malaga); **S.** BAU 4517.2 (Catalonia). Scale bars: **A** = 3 mm; **B** = 100 µm; **C–D** = 50 µm; **E** = 100 µm; **F** = 3 mm; **G** = 1 mm; **H–J** = 3 mm; **K** = 3 mm; **L–M** = 50 µm; **N** = 100 µm; **O** = 3 mm; **P–Q** = 1 mm; **R–S** = 3 mm.

Figure 5. *Calliostoma zizyphinum*, according to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Fig. 3). **A–I** *zizyphinum* morphotype. **A.** BAU 2042.1 (Roscoff); **B.** BAU 3712 protoconch detail; **C.** BAU 765.2 innermost marginal tooth; **D.** BAU 2042.4 rachidian tooth; **E.** BAU 2042.2 radula; **F.** MNHN-IM-2019-14568 (Corsica). Photo credit: Gilles Devauchelle; **G.** MNHN-IM-2019-13695 (Corsica). Photo credit: Andrea Nappo; **H.** MNHN-IM-2019-14568 (Corsica). Photo credit: Andrea Nappo; **I.** BAU 3840.1 (Cambados); **J–M** *lividum* morphotype. **J.** BAU 3623 (Biscoitos); **K.** BAU 765.2 (San Miguel Is.); **L.** BAU 703.1 (San Miguel Is.); **M.** BAU 703.2 (San Miguel Is.); **N.** BAU 2864.2 (Sicily) *alexandrinum* morphotype, juv.; **O.** MNCN-15.05/200521 (Alboran Is.) *conulus*-like morphotype; **P.** BAU 3715 (Asia shipwreck) *dubium* morphotype; **Q.** BAU 4480.1 (Aveiro) *kochi* morphotype. Scale bars: **A** = 3 mm; **B** = 100 µm; **C–D** = 50 µm; **E** = 150 µm; **F** = 3 mm; **G** = 1 mm; **H–I** = 3 mm; **J–K** = 1 mm; **L–M** = 3 mm; **N** = 1 mm; **O–P** = 3 mm; **Q** = 1 mm.

Figure 6. *Calliostoma laugierii*, according to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Fig. 3). **A.** BAU 3700.4 (Sardinia); **B.** BAU 3717.1 protoconch detail; **C.** BAU 3710 innermost marginal tooth; **D.** BAU 3403 rachidian tooth; **E.** BAU 3781 radula; **F.** MNHN-IM-2019-14567 (Corsica). Photo credit: Gilles Devauchelle; **G–K.** *laugierii* and “big-cord” morphotypes. **G.** BAU 2726.3 (Sicily) juv.; **H.** BAU 2919.2 (Giannutri Is.) juv.; **I.** BAU 1830.3 (Sicily); **J.** BAU 1830.0 (Sicily); **K.** BAU 3779 (Corsica); **L.** BAU 4222 *conulus*-like morphotype (Sardinia); **M–N** *gualterianum* morphotype, juv; **M.** MNHN-IM-2019-17918 (Corsica). Photo credit: Andrea Nappo; **N.** BAU 2919.4 (Giannutri Is.); **O.** BAU 3700.7 (Sardinia) “uniform” morphotype; **P–Q** *gualterianum* morphotype; **P.** BAU 3709.1 (Giannutri Is.); **Q.** BAU 3711 (Giannutri Is.); **R.** BAU 3700.1 (Sardinia) “stepped” morphotype; **S–T** *spongium* morphotype; **S.** BAU 2527.2 (Gremdi Is.); **T.** BAU 2494 (Gharbi Is.); **U.** BAU 4523.1 (Sicily) “chessboard” morphotype; **V–X** *funiculatum* morphotype; **V.** BAU 3159.2 (off Marsala); **W.** BAU 4523.3 (Sicily); **X.** BAU 4523.2 (Sicily); **Y–d** “elegant” morphotype; **Y.** MNHN-IM-2019-5652 (Corsica) juv. Photo credit: Andrea Nappo; **Z.** BAU 3717.1 (Santa Maria al Bagno); **a.** BAU 3720.1 (Porto Cesareo); **b.** MNHN-IM-2019-4729 (Corsica). Photo credit: Andrea Nappo; **c.** BAU 3720.4 (Porto Cesareo); **d.** BAU 3403 (Cetraro); **e.** *seriopunctatus* morphotype BAU 3650.3 (Giannutri Is.); **f.** BAU 3710 (La Ballenera); **g.** *planatum* morphotype. MNCN-15.05/200527.2 (Playa de los Genoveses); **h.** *virescens* morphotype MZUB-Call 4 (Venice); **i–j** *kochi* morphotype. **i.** MNCN-15.05/200523.2 (Marbella); **j.** BAU 3850.2 (Porto Cesareo); **k–p** “big-cord” morphotype. **k.** BAU 3098.3 (Astypalaea Is.); **l.** BAU 3699 (Sardinia); **m.** BAU 3080.1 (Trsteno); **n.** CY371 (Cyprus); **o.** CY558 (Cyprus); **p.** BAU 4507 (Sardinia). Scale bars: **A** = 2 mm; **B** = 100 µm; **C–D** = 50 µm; **E** = 100 µm; **F** = 2 mm; **G–J** = 1 mm; **K–L** = 2 mm; **M–P** = 1 mm; **Q–R** = 2 mm; **S–U** = 1 mm; **V–X** = 2 mm; **Y** = 1 mm; **Z–d** = 2 mm; **e** = 1 mm; **f–j** = 2 mm; **k–p** = 1 mm.

Figure 7. Iconography of *Calliostoma*. Additional information in Supplementary Materials 3. **A.** *Calliostoma conulus*, syntype. LSL-509; **B.** *Calliostoma zizyphinum*, syntype. LSL-510; **C.** *Calliostoma granulatum*, holotype. NHMW-MO-14000 (Fig. S3-8). Photo credits: Paolo G. Albano; **D.** *Calliostoma gubbiolii*, holotype. MCZR-M-TYPE-00005/H (Fig. S3-9); **E.** *Calliostoma agrigentinum*, holotype. HUJ56283 (Fig. S3-1); **F.** *Calliostoma inopinatum*, holotype. MNHN-IM-2000-31504. Photo credit: Manuel Caballer - 2016; **G–L.** *Calliostoma laugierii*. **G.** Typical morphotype. MCZR-M-11643 (Fig. S3-11); **H.** morphotype *neretinum*, syntype of *C. neretinum*. MBMPP-259.02 (Fig. S3-20); **I.** morphotype

spongiarum. MCZR-M-11628 (Fig. S3-23); **J.** morphotype *planatum*, syntype of *C. planatum*. MNHN-IM-2000-38224. Photo credit: Mélanie Van Weddingen - 2022; **K.** morphotype *kochi*, syntype of *C. kochi*. MNHN-IM-2000-31510. Photo credit: Manuel Caballer - 2016; **L.** morphotype *seriopunctatus*. MCZR-M-11637 (Fig. S3-23); **M–O.** *Calliostoma zizyphinum*. **M.** morphotype *dubium*, possible syntype of *T. dubius*. MCZR-M-11572 (Fig. S3-6); **N.** morphotype *alexandrinum*, syntype of *C. alexandrinum*. MCZR-M-11667 (Fig. S3-3); **O.** morphotype *lividum*, syntype of *C. lividum*. MCZR-M-11654 (S3-19). Scale bars: **A–B** = 2 mm; **B** = 5 mm; **C** = 2 mm; **E** = 5 mm; **F** = 2 mm; **G–K** = 2 mm; **L** = 1 mm; **M** = 2 mm; **N** = 1 mm; **O** = 2 mm.

For Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Supplementary materials

- S1.** Bayesian Inference phylogenetic reconstruction of Calliostomatinae using a combined *cox1*, *16S rRNA* and *its2* alignment. White dots at nodes stand for maximum posterior probability and likelihood scores. Numbers in square brackets refer to the localities shown in Figure 1.
- S2.** List of accession numbers and references for the used sequences.
- S3.** Iconography of *Calliostoma* from the Mediterranean and eastern Atlantic.
- S4.** List of used primers.
- S5.** Ten best ASAP partitions based on the *cox1* alignment. Each column represents a partition hypothesis and each coloured box a putative species. The third column (rank = 1, 22 species) corresponds to this study SSH.
- S6.** Maximum Likelihood and Bayesian Inference reconstructions using single genes (*cox1*, *16s rDNA*, and *its2*).
- S7.** K2P distance matrices generated with Hooty according to the SSH, PSH and ASAP partitions.

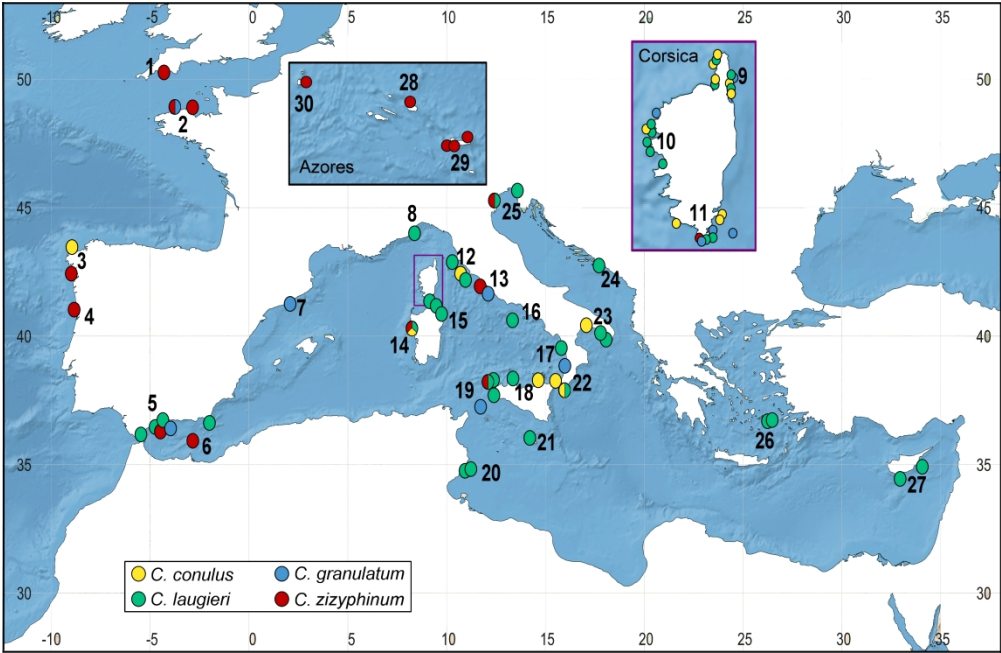


Figure 1. Map of the collection sites. Numbers refer to the localities listed in Table 1. Species in the legend correspond to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Figure 3).

572x372mm (300 x 300 DPI)

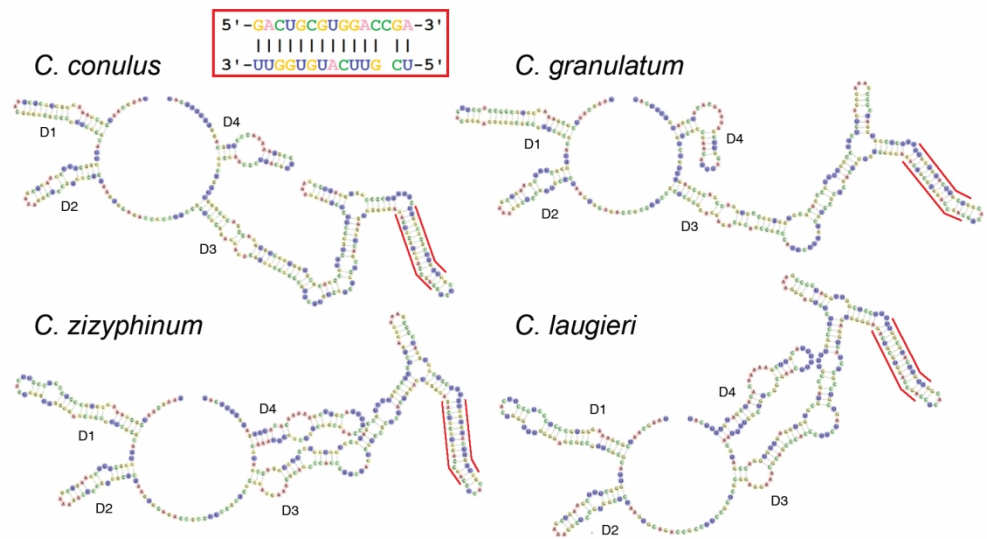


Figure 2. The Its2 folding of Calliostoma. Apical STEM highlighted in red. Nucleotide colour legend: A, red; C, green; U, blue; G, yellow.

156x87mm (300 x 300 DPI)

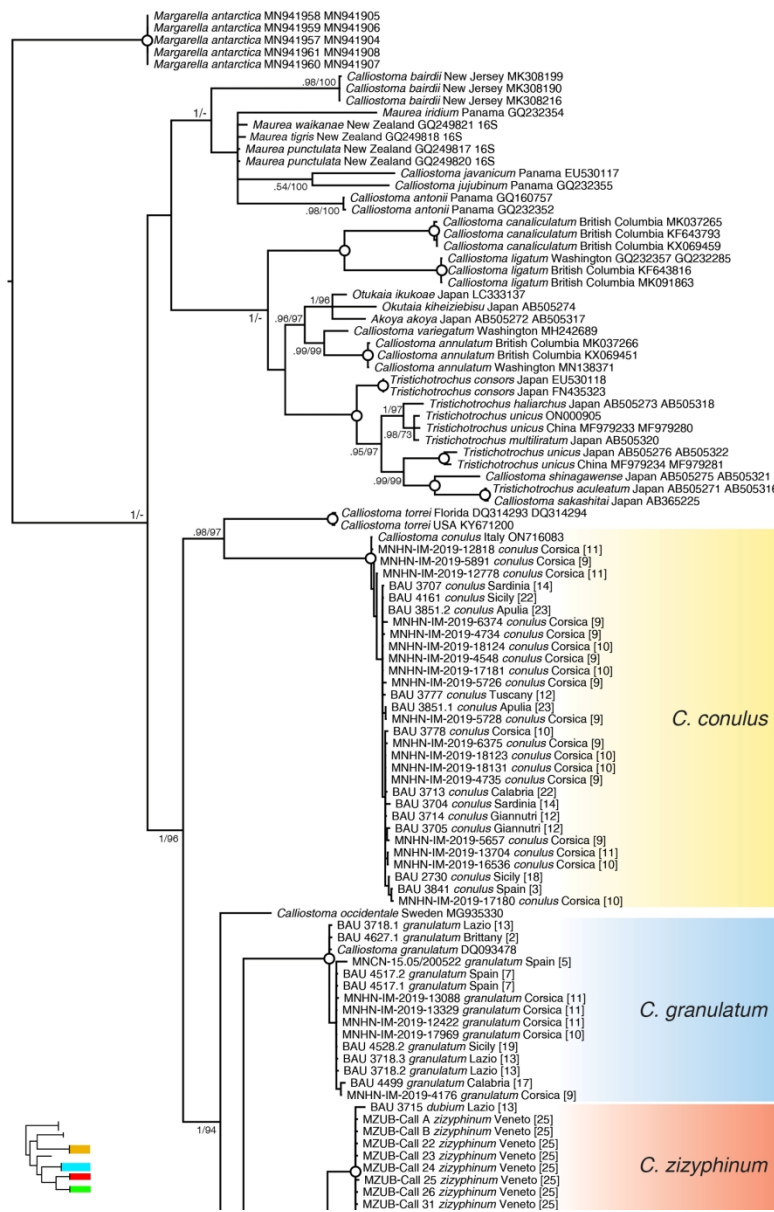


Figure 3. Bayesian Inference phylogenetic reconstruction of Calliostomatinae using a combined cox1, 16S rRNA and its2 alignment. White dots at nodes stand for maximum posterior probability and likelihood scores. Numbers in square brackets refer to the localities shown in Figure 1. Searchable file in Supplementary material 1.

167x257mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

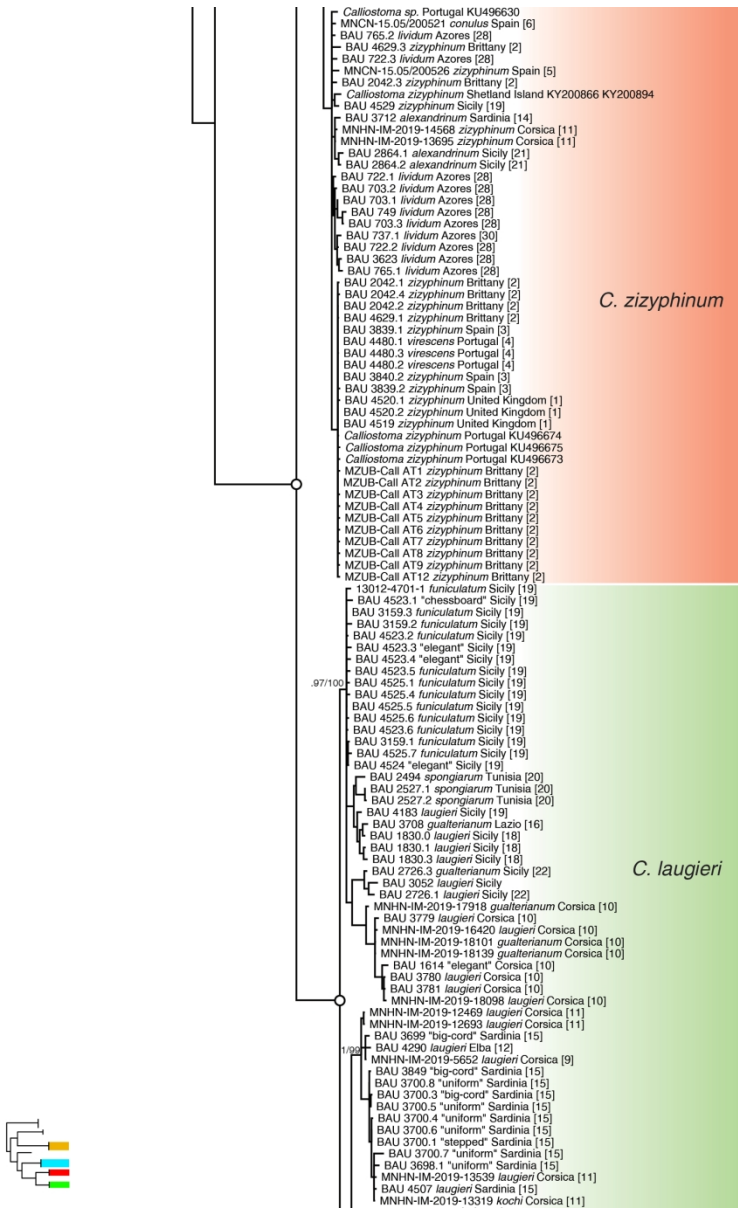


Figure 3. Bayesian Inference phylogenetic reconstruction of Calliostominae using a combined cox1, 16S rRNA and its2 alignment. White dots at nodes stand for maximum posterior probability and likelihood scores. Numbers in square brackets refer to the localities shown in Figure 1. Searchable file in Supplementary material 1.

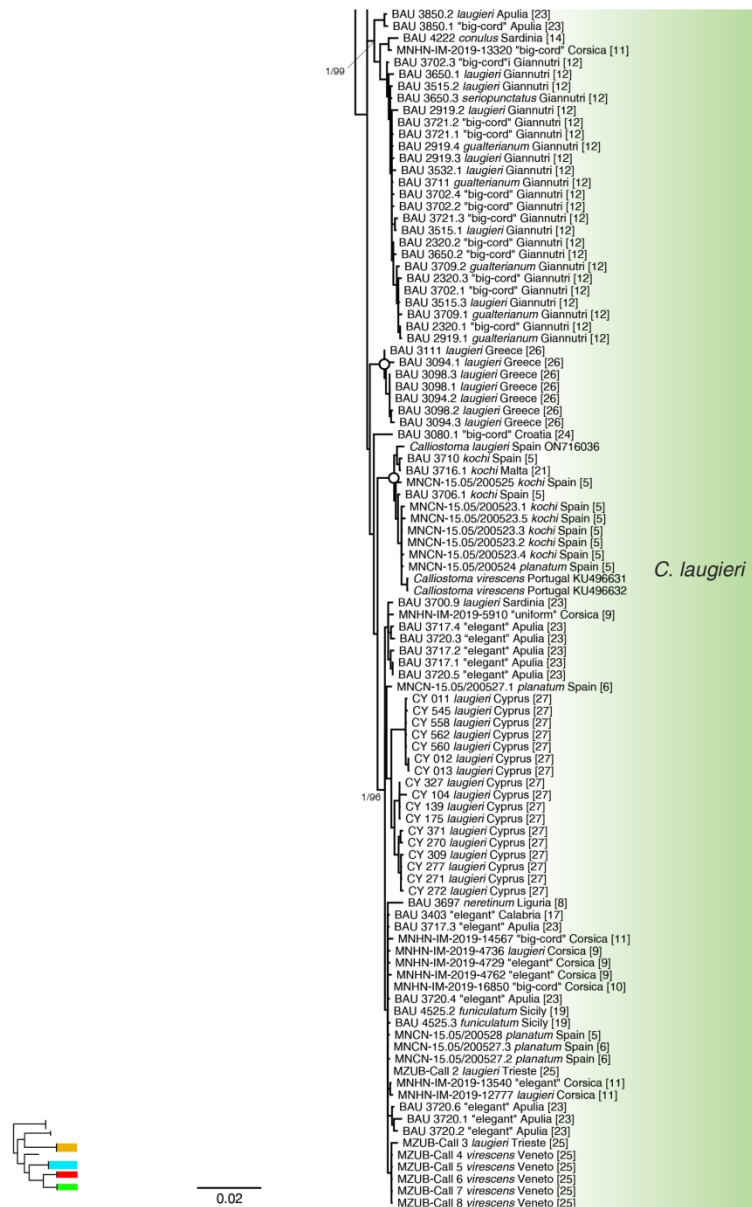


Figure 3. Bayesian Inference phylogenetic reconstruction of Calliostominae using a combined *cox1*, 16S rRNA and *its2* alignment. White dots at nodes stand for maximum posterior probability and likelihood scores. Numbers in square brackets refer to the localities shown in Figure 1. Searchable file in Supplementary material 1.

166x260mm (300 x 300 DPI)

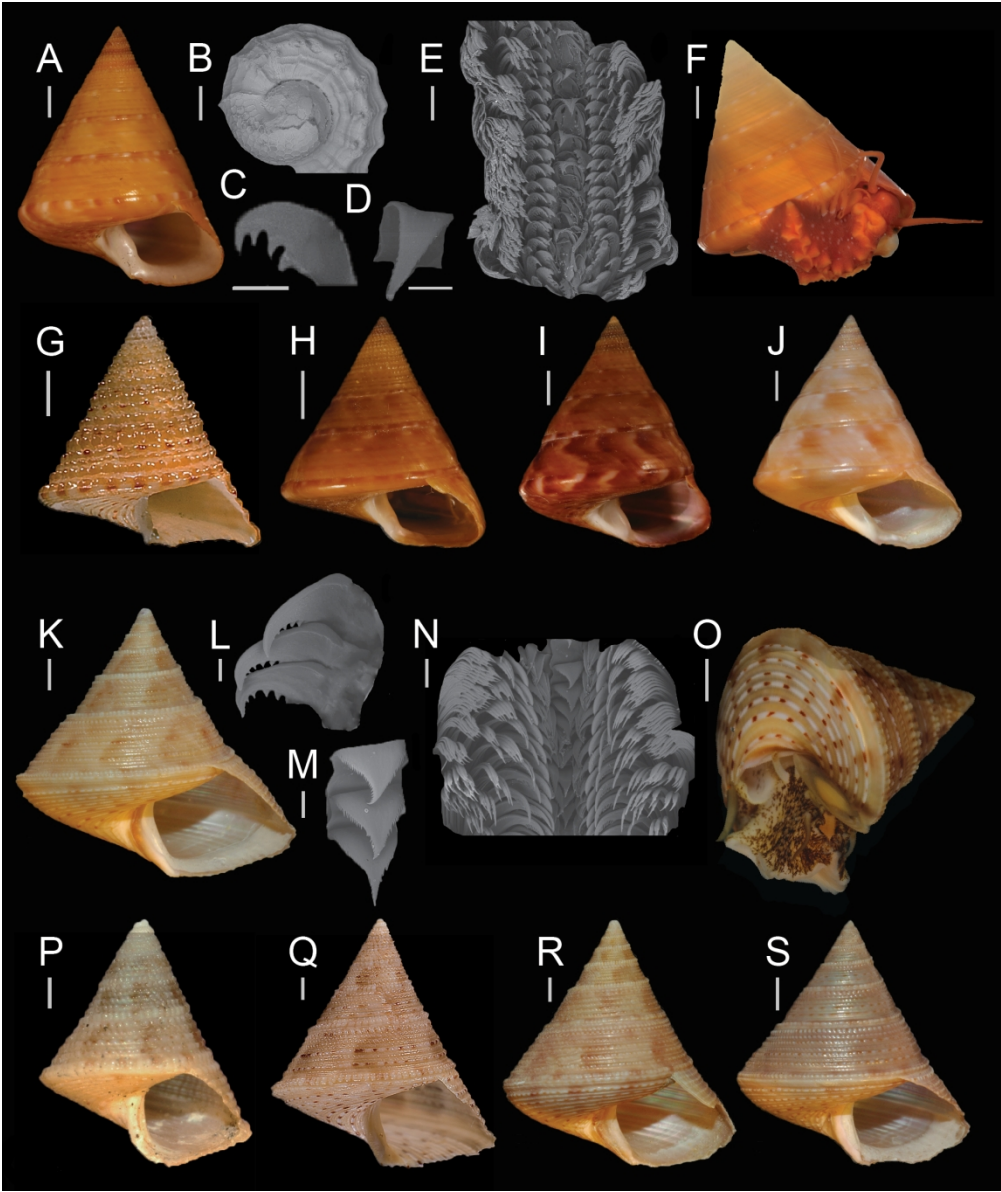


Figure 4. A–J *Calliostoma conulus*. A. BAU 3705 (Giannutri Is.); B. BAU 3713 protoconch detail; C. BAU 3704 innermost marginal tooth; D. BAU 3841 rachidian tooth; E. BAU 3713 radula; F. MNHN-IM-2019-16536 (Corsica). Photo credit: Gilles Devauchelle; G. MNHN-IM-2019-4735 (Corsica) juv.; H. BAU 2730 (Sicily); I. BAU 3778 (Corsica); J. BAU 3841 (Malpica); K–S *Calliostoma granulatum*. K. BAU 3718.3 (off Fiumicino); L–N BAU 3718.1 innermost marginal teeth, rachidian teeth, and radula; O. MNHN-IM-2019-13088 (Corsica). Photo credit: Gilles Devauchelle; P. BAU 4627.1 (Roscoff); Q. MNHN-IM-2019-12422 (Corsica). Photo credit: Andrea Nappo; R. MNCN-15.05/200522 (Malaga); S. BAU 4517.2 (Catalonia). Scale bars: A = 3 mm; B = 100 µm; C–D = 50 µm; E = 100 µm; F = 3 mm; G = 1 mm; H–J = 3 mm; K = 3 mm; L–M = 50 µm; N = 100 µm; O = 3 mm; P–Q = 1 mm; R–S = 3 mm.

177x210mm (300 x 300 DPI)

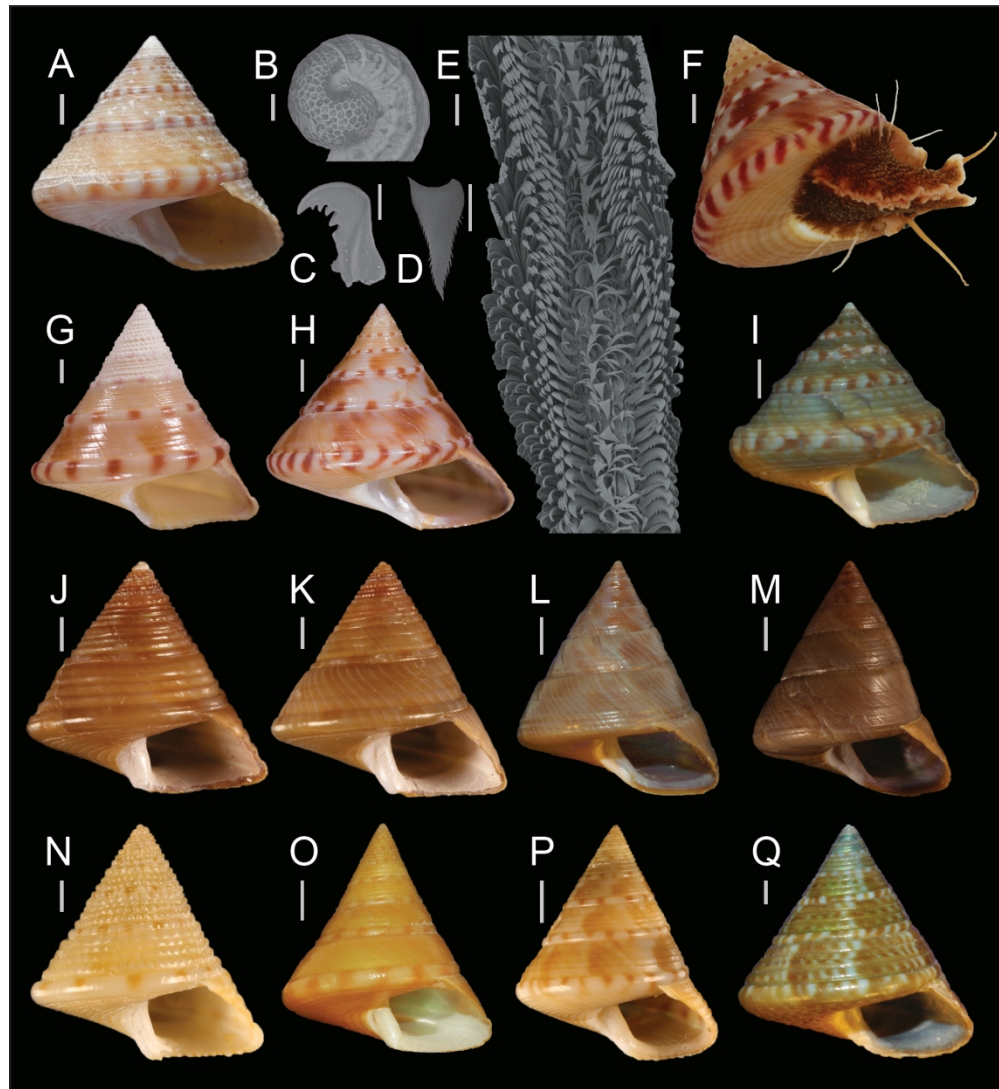


Figure 5. *Calliostoma zizyphinum*, according to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Fig. 3). A–I *zizyphinum* morphotype. A. BAU 2042.1 (Roscoff); B. BAU 3712 protoconch detail; C. BAU 765.2 innermost marginal tooth; D. BAU 2042.4 rachidian tooth; E. BAU 2042.2 radula; F. MNHN-IM-2019-14568 (Corsica). Photo credit: Gilles Devauchelle; G. MNHN-IM-2019-13695 (Corsica). Photo credit: Andrea Nappo; H. MNHN-IM-2019-14568 (Corsica). Photo credit: Andrea Nappo; I. BAU 3840.1 (Cambados); J–M *lividum* morphotype. J. BAU 3623 (Biscoitos); K. BAU 765.2 (San Miguel Is.); L. BAU 703.1 (San Miguel Is.); M. BAU 703.2 (San Miguel Is.); N. BAU 2864.2 (Sicily) *alexandrinum* morphotype, juv.; O. MNCN-15.05/200521 (Alboran Is.) *conulus*-like morphotype; P. BAU 3715 (Asia shipwreck) *dubium* morphotype; Q. BAU 4480.1 (Aveiro) *kochi* morphotype. Scale bars: A = 3 mm; B = 100 µm; C–D = 50 µm; E = 150 µm; F = 3 mm; G = 1 mm; H–I = 3 mm; J–K = 1 mm; L–M = 3 mm; N = 1 mm; O–P = 3 mm; Q = 1 mm.

177x193mm (300 x 300 DPI)

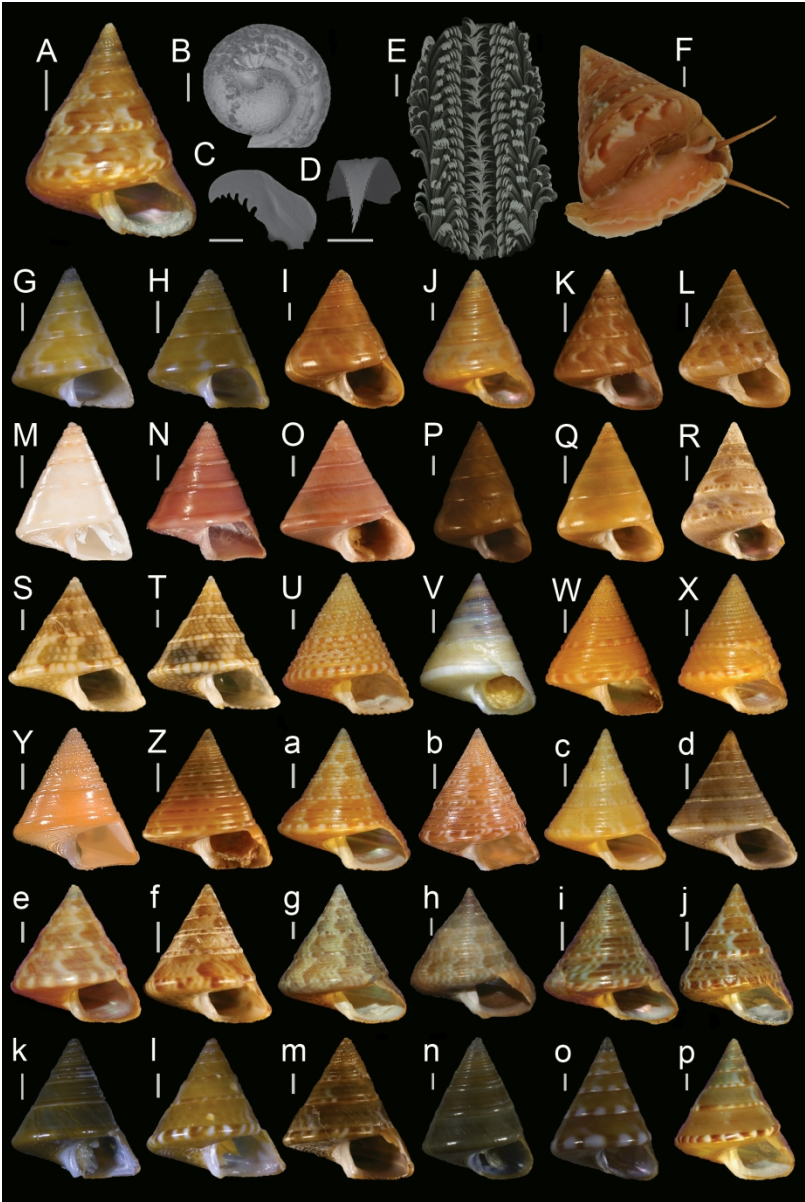


Figure 6. *Calliostoma laugieri*, according to the secondary species hypotheses (SSH) as derived after the species delimitation process (see Fig. 3). A. BAU 3700.4 (Sardinia); B. BAU 3717.1 protoconch detail; C. BAU 3710 innermost marginal tooth; D. BAU 3403 rachidian tooth; E. BAU 3781 radula; F. MNHN-IM-2019-14567 (Corsica). Photo credit: Gilles Devauchelle; G–K. *laugieri* and “big-cord” morphotypes. G. BAU 2726.3 (Sicily) juv.; H. BAU 2919.2 (Giannutri Is.) juv.; I. BAU 1830.3 (Sicily); J. BAU 1830.0 (Sicily); K. BAU 3779 (Corsica); L. BAU 4222 conulus-like morphotype (Sardinia); M–N *gualterianum* morphotype, juv; M. MNHN-IM-2019-17918 (Corsica). Photo credit: Andrea Nappo; N. BAU 2919.4 (Giannutri Is.); O. BAU 3700.7 (Sardinia) “uniform” morphotype; P–Q *gualterianum* morphotype; P. BAU 3709.1 (Giannutri Is.); Q. BAU 3711 (Giannutri Is.); R. BAU 3700.1 (Sardinia) “stepped” morphotype; S–T *spongiorum* morphotype; S. BAU 2527.2 (Gremdi Is.); T. BAU 2494 (Gharbi Is.); U. BAU 4523.1 (Sicily) “chessboard” morphotype; V–X *funiculatum* morphotype; V. BAU 3159.2 (off Marsala); W. BAU 4523.3 (Sicily); X. BAU 4523.2 (Sicily); Y–d “elegant” morphotype; Y. MNHN-IM-2019-5652 (Corsica) juv. Photo credit: Andrea Nappo; Z. BAU 3717.1 (Santa Maria al Bagno); a. BAU 3720.1 (Porto Cesareo); b. MNHN-IM-2019-4729 (Corsica). Photo credit: Andrea Nappo; c. BAU 3720.4 (Porto Cesareo); d. BAU 3403 (Cetraro); e. *seriopunctatus* morphotype BAU

3650.3 (Giannutri Is.); f. BAU 3710 (La Ballenera); g. planatum morphotype. MNCN-15.05/200527.2 (Playa de los Genoveses); h. virescens morphotype MZUB-Call 4 (Venice); i-j kochi morphotype. i. MNCN-15.05/200523.2 (Marbella); j. BAU 3850.2 (Porto Cesareo); k-p "big-cord" morphotype. k. BAU 3098.3 (Astypalaea Is.); l. BAU 3699 (Sardinia); m. BAU 3080.1 (Trsteno); n. CY371 (Cyprus); o. CY558 (Cyprus); p. BAU 4507 (Sardinia). Scale bars: A = 2 mm; B = 100 μ m; C-D = 50 μ m; E = 100 μ m; F = 2 mm; G-J = 1 mm; K-L = 2 mm; M-P = 1 mm; Q-R = 2 mm; S-U = 1 mm; V-X = 2 mm; Y = 1 mm; Z-d = 2 mm; e = 1 mm; f-j = 2 mm; k-p = 1 mm.

177x264mm (300 x 300 DPI)

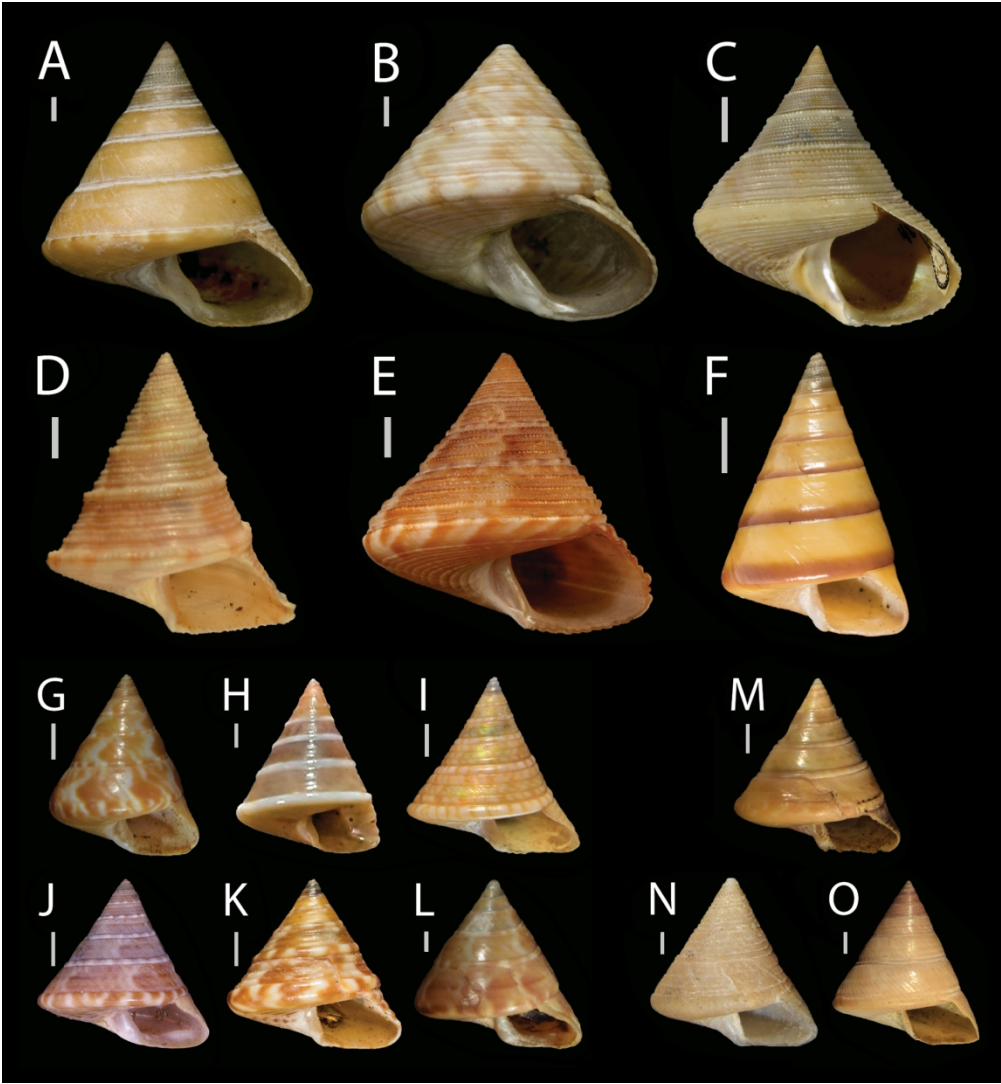


Figure 7. Iconography of *Calliostoma*. Additional information in Supplementary Materials 3. A. *Calliostoma conulus*, syntype. LSL-509; B. *Calliostoma zizyphinum*, syntype. LSL-510; C. *Calliostoma granulatum*, holotype. NHMW-MO-14000 (Fig. S3-8). Photo credits: Paolo G. Albano; D. *Calliostoma gubbiolii*, holotype. MCZR-M-TYPE-00005/H (Fig. S3-9); E. *Calliostoma agrigentinum*, holotype. HJ56283 (Fig. S3-1); F. *Calliostoma inopinatum*, holotype. MNHN-IM-2000-31504. Photo credit: Manuel Caballer - 2016; G-L. *Calliostoma laugieri*. G. Typical morphotype. MCZR-M-11643 (Fig. S3-11); H. morphotype *neretinum*, syntype of *C. neretinum*. MBMPP-259.02 (Fig. S3-20); I. morphotype *spongiorum*. MCZR-M-11628 (Fig. S3-23); J. morphotype *planatum*, syntype of *C. planatum*. MNHN-IM-2000-38224. Photo credit: Mélanie Van Weddingen - 2022; K. morphotype *kochi*, syntype of *C. kochi*. MNHN-IM-2000-31510. Photo credit: Manuel Caballer - 2016; L. morphotype *seriopunctatus*. MCZR-M-11637 (Fig. S3-23); M-O. *Calliostoma zizyphinum*. M. morphotype *dubium*, possible syntype of *T. dubius*. MCZR-M-11572 (Fig. S3-6); N. morphotype *alexandrinum*, syntype of *C. alexandrinum*. MCZR-M-11667 (Fig. S3-3); O. morphotype *lividum*, syntype of *C. lividum*. MCZR-M-11654 (S3-19). Scale bars: A-B = 2 mm; B = 5 mm; C = 2 mm; E = 5 mm; F = 2 mm; G-K = 2 mm; L = 1 mm; M = 2 mm; N = 1 mm; O = 2 mm.

178x192mm (300 x 300 DPI)

SUPPLEMENTARY DATA OF

The polymorphic top-shell puzzle: iterative taxonomy of *Calliostoma* Swainson, 1840 (Gastropoda: Calliostomatidae) in the Mediterranean Sea

Giacomo Chiappa¹, Giulia Fassio¹, Carlo Smriglio², Paolo Mariottini², Paolo G. Albano³, Maria Vittoria Modica⁴, Dario Zuccon⁵, Nicolas Puillandre⁵ & Marco Oliverio¹

¹Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Viale dell'Università, 32, 00185 Rome, Italy; giacomo.chiappa@uniroma1.it, giulia.fassio@uniroma1.it, marco.oliverio@uniroma1.it

²Department of Science, University RomaTre, Viale Marconi 446, 00146 Rome, Italy; csmriglio@alice.it, paolo.mariottini@uniroma3.it

³Department of Marine Animal Conservation and Public Engagement, Stazione Zoologica Anton Dohrn, Villa Comunale, Naples, Italy; pgalbano@gmail.com

⁴Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Via Gregorio Allegri 1, 00198 Rome, Italy; mariavittoria.modica@szn.it

⁵Institut Systématique Evolution Biodiversité (ISYEB), Muséum National d'Histoire Naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, CP 51, 75005 Paris, France; dario.zuccon@mnhn.fr, nicolas.puillandre@mnhn.fr

Correspondence: G. Chiappa; email: giacomo.chiappa@uniroma1.it

CONTENTS:

S1. Bayesian Inference phylogenetic reconstruction of Calliostomatinae.

S2. Sequences downloaded from Genbank.

S3. Iconography of *Calliostoma*.

S4. List of used primers.

S5. ASAP species delimitation hypotheses.

S6. Single gene phylogenetic reconstructions.

Table S2-1. Calliostomatid sequences downloaded from Genbank.

Name	Species	Location	cox1	16S	Source
NSMT:Mo76812	<i>Akoya akoya</i>	Japan, Chiba, off Yasuda	AB505272	AB505317	Williams et al. 2010
MIB1451	<i>Calliostoma annulatum</i>	Canada, British Columbia, Strathcona	KX069451	-	Castelin et al. 2016
16_STFX_005	<i>Calliostoma annulatum</i>	Canada, British Columbia, Barkley Sound	MK037266	-	Wierenga & Maccagno 2018*
BFHL2045	<i>Calliostoma annulatum</i>	Washington, San Juan Island	MN138371	-	Leray et al. 2019*
MCZ DNA102415	<i>Calliostoma antonii</i>	Panama, El Chumical	GQ160757	GQ160691	Aktipis & Giribet
NHMUK:20080936	<i>Calliostoma antonii</i>	Panama	GQ232352	GQ232281	Williams et al. 2010
USNM:I:1446286	<i>Calliostoma bairdii</i>	USA, New Jersey, off Barnegat Light	MK308190	-	Aguilar et al. 2018*
USNM:I:1446287	<i>Calliostoma bairdii</i>	USA, New Jersey, off Barnegat Light	MK308216	-	Aguilar et al. 2018*
USNM:I:1446288	<i>Calliostoma bairdii</i>	USA, New Jersey, off Barnegat Light	MK308199	-	Aguilar et al. 2018*
10BCMOL-00114	<i>Calliostoma canaliculatum</i>	Canada, British Columbia, Haida Gwaii	KF643793	-	Layton et al. 2014
MIB1489	<i>Calliostoma canaliculatum</i>	Canada, British Columbia, Strathcona	KX069459	-	Castelin et al. 2016
16_STFX_003	<i>Calliostoma canaliculatum</i>	Canada, British Columbia, Barkley Sound	MK037265	-	Willies & Rohovie 2018*
SEAMoBB_Italy_AK	<i>Calliostoma conulus</i>	Italy	ON716083	-	Mugnai et al. 2023
MCZ:DNA100086	<i>Calliostoma granulatum</i>	-	-	DQ093478	Huff et al. 2005*
CJAV.BDT.1	<i>Calliostoma javanicum</i>	Panama	EU530117	-	Williams et al. 2008
NHMUK:20080938	<i>Calliostoma jujubinum</i>	Panama	GQ232355	-	Williams et al. 2010
SEAMoBB-Murcia-D	<i>Calliostoma laugierii</i>	Spain, Murcia	ON716036	-	Mugnai et al. 2023
NHMUK:20080939	<i>Calliostoma ligatum</i>	USA	GQ232357	GQ232285	Williams et al. 2010
10BCMOL-00283	<i>Calliostoma ligatum</i>	Canada, British Columbia, Bamfield	KF643816	-	Layton et al. 2014
2016-WCHS-006	<i>Calliostoma ligatum</i>	Canada, British Columbia, Bamfield	MK091863	-	Smith & Mowatt 2018*
Gastr 9013V	<i>Calliostoma occidentale</i>	Sweden, Skagerrak	MG935330	-	Lundin 2018*
-	<i>Calliostoma sakashitai</i>	Japan, Kanagawa, Misaki	AB365225	-	Kano 2007
NSMT:Mo76816	<i>Calliostoma shinagawense</i>	Japan, Kanagawa, Miura, off Zyogasima	AB505275	AB505321	Williams et al. 2010
USNM:1069300	<i>Calliostoma torrei</i>	USA, Florida, off Jacksonville	DQ314293	DQ314294	Harasewych 2006
SERTC/SCDNR S1005	<i>Calliostoma torrei</i>	USA	-	KY671200	Harasewych 2017*
BMBM-0459	<i>Calliostoma variegatum</i>	Washington, San Juan Island	MH242689	-	Leray et al. 2019*
LMBAG35-002	<i>Calliostoma virescens</i>	Portugal, Algarve	KU496631	-	Borges et al. 2016
LMBAG35-003	<i>Calliostoma virescens</i>	Portugal, Algarve	KU496632	-	Borges et al. 2016
MT09277	<i>Calliostoma zizyphinum</i>	North Sea	KR084401	-	Barco et al. 2016
TR1-15	<i>Calliostoma zizyphinum</i>	Portugal, Alentejo	KU496674	-	Borges et al. 2016
NHMUK:20160315	<i>Calliostoma zizyphinum</i>	Shetland Islands	KY200866	KY200894	Uribe et al. 2017
NHMUK:20050731	<i>Maurea iridium</i>	Panama	GQ232354	GQ232283	Williams et al. 2010
NMNZ:M.287110	<i>Maurea punctulata</i>	New Zealand, Cornwallis Manukau Harbour	-	GQ249817	Williams et al. 2010
NMNZ:M.287111	<i>Maurea punctulata</i>	New Zealand, Cape Wanbrow	-	GQ249820	Williams et al. 2010
NMNZ:M.287112	<i>Maurea tigris</i>	New Zealand, Whatipu	-	GQ249818	Williams et al. 2010
NMNZ:M.287114	<i>Maurea waikanae</i>	New Zealand, Snares Islands	-	GQ249821	Williams et al. 2010
KIV724-BC1-19	<i>Otukaia ikukooae</i>	Japan: Okinawa Trough	LC333137	-	Chen et al. 2017
T45	<i>Otukaia kiheziebisu</i>	Japan: Miyagi, off Kesenuma	AB505274	-	Williams et al. 2010

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

NSMT:Mo76813	<i>Tristichotrochus aculeatum</i>	Japan, Kanagawa, Miura, off Zyogasima	AB505271	AB505316	Williams et al. 2010
OT22	<i>Tristichotrochus consors</i>	Japan, Mie, Sugashima	EU530118	-	Williams et al. 2008
NHM:20080940	<i>Tristichotrochus consors</i>	Japan, Mie, Sugashima	FN435323	-	Williams et al. 2010
NSMT:Mo76814	<i>Tristichotrochus haliarchus</i>	Japan, Iwate, Off Yagi	AB505273	AB505318	Williams et al. 2010
NSMT:Mo76815	<i>Tristichotrochus multirilatam</i>	Japan, Iwate, Otsuchi	-	AB505320	Williams et al. 2010
NSMT:Mo76817	<i>Tristichotrochus unicus</i>	Japan, Kumamoto, Amakusa, Tsuzizima	AB505276	AB505322	Williams et al. 2010
	<i>Tristichotrochus unicus</i>	-	ON000905	-	Sun et al. 2022*
DYLLK2	<i>Tristichotrochus unicus</i>	China	MF979234	MF979281	Zhang & Kong 2017*
DYLLK1	<i>Tristichotrochus unicus</i>	China	MF979233	MF979280	Zhang & Kong 2017*
ANTARXXIV-2017-1	<i>Margarella antarctica</i>	Antarctica, South Shetland Islands	MN941957	MN941904	Merino Valencia 2020
ANTARXXIV-2017-5	<i>Margarella antarctica</i>	Antarctica, South Shetland Islands	MN941958	MN941905	Merino Valencia 2020
ANTARXXIV-2017-4	<i>Margarella antarctica</i>	Antarctica, South Shetland Islands	MN941959	MN941906	Merino Valencia 2020
ANTARXXIV-2017-3	<i>Margarella antarctica</i>	Antarctica, South Shetland Islands	MN941960	MN941907	Merino Valencia 2020
ANTARXXIV-2017-2	<i>Margarella antarctica</i>	Antarctica, South Shetland Islands	MN941961	MN941908	Merino Valencia 2020

*paper not published

Table S2-2. Vetigastropoda ITS2 sequences downloaded from Genbank.

Species	ITS2	Source
<i>Oxystele variegata</i>	JX303637	Muteveri et al. 2012*
<i>Oxystele sinensis</i>	JX145383	Muteveri et al. 2012*
<i>Oxystele tigrina</i>	JX303580	Muteveri et al. 2012*
<i>Phorcus lineatus</i>	OV121087	Darwin Tree of Life* (www.darwintreeoflife.org)
<i>Steromphala cineraria</i>	OU744727	Darwin Tree of Life* (www.darwintreeoflife.org)
<i>Tegula atra</i>	EU636217	Aguilera-Munoz & Gallardo-Escarate 2008*
<i>Tegula viridula</i>	AY682095	Lopes et al. 2004*
<i>Turbo sarmaticus</i>	JX303660	Muteveri et al. 2012*
<i>Haliotis tubercularis</i>	AF296862	Coleman & Vacquier, 2000
<i>Megathura crenulata</i>	AF296849	Coleman & Vacquier, 2000

*paper not published

FIGURE S3. ICONOGRAPHY OF *CALLIOSTOMA*

- S3-1.** *Calliostoma agrigentinum* Coen, 1936 (Holotype)
- S3-2.** *Calliostoma agrigentinum* Coen, 1936
- S3-3.** *Calliostoma alexandrinum* Pallary, 1912 (Syntype) [= *C. zizyphinum*]
- S3-4.** *Calliostoma chemnitzii* (R. A. Philippi, 1848) [= *C. zizyphinum*]
- S3-5.** *Calliostoma conulus* (Linnaeus, 1758)
- S3-6.** *Calliostoma dubium* (R. A. Philippi, 1844) (possible syntypes) [= *C. zizyphinum*]
- S3-7.** *Calliostoma floreanae* Parenzan, 1970 (Syntypes) [= *C. laugieri*]
- S3-8.** *Calliostoma granulatum* (Born, 1778) (Holotype)
- S3-9.** *Calliostoma gubbioli* Nofroni, 1984 (Holotype)
- S3-10.** *Calliostoma kochi* Pallary, 1902 (Syntypes) [= *C. laugieri*]
- S3-11.** *Calliostoma laugieri* (Payraudeau, 1826)
- S3-12.** *Calliostoma laugieri* (Payraudeau, 1826)
- S3-13.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “stepped morphotype” in the paper
- S3-14.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “elegant morphotype” in the paper
- S3-15.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “uniform morphotype” in the paper
- S3-16.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “uniform morphotype” in the paper
- S3-17.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “uniform morphotype” in the paper
- S3-18.** *Calliostoma laugieri* (Payraudeau, 1826), referred to as “uniform morphotype” in the paper
- S3-19.** *Calliostoma lividum* Dautzenberg, 1927 (Syntypes) [= *C. zizyphinum*]
- S3-20.** *Calliostoma neretinum* Parenzan, 1970 (Holotype) [= *C. laugieri*]
- S3-21.** *Calliostoma planatum* (Pallary, 1900) (Syntypes) [= *C. laugieri*]
- S3-22.** *Calliostoma seriopunctatus* (Blainville, 1830) [= *C. laugieri*]
- S3-23.** *Calliostoma spongiorum* (Bucquoy, Dautzenberg & Dollfus, 1885) [= *C. laugieri*]
- S3-24.** *Calliostoma zizyphinum* (Linnaeus, 1758)
- S3-25.** *Calliostoma zizyphinum* (Linnaeus, 1758)
- S3-26.** *Calliostoma zizyphinum* (Linnaeus, 1758)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



FIGURE S3-1. *Calliostoma agrigentinum* Coen, 1936. The Steinhardt Museum of Natural History, Tel Aviv, Israel, Coen collection. **A–D.** HUJ56283, holotype, H 34.0 x D 37.0 mm; Agrigento, Sicily. **E.** HUJ56283, original label handwritten by Coen. **F.** HUJ56283, museum label.

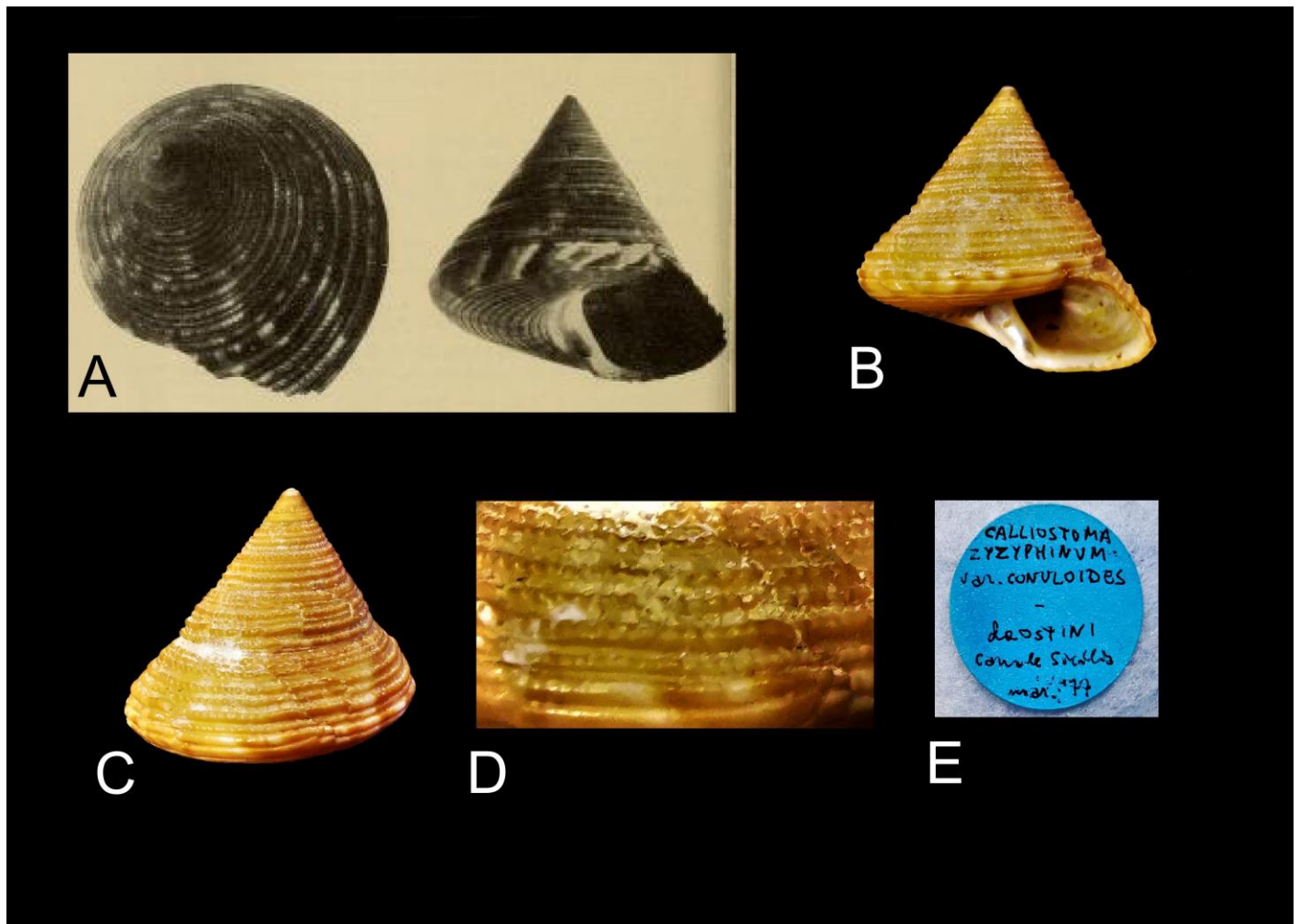


FIGURE S3-2. *Calliostoma agrigentum* Coen, 1936. Ex M. Pizzini collection, Museo Civico di Zoologia, Rome, Italy. **A.** Original illustration from Coen (1936: 274). **B–D.** Sine numera, H 23.2 x D 23.0 mm; Sicily Channel. **E.** Sine numera, original label handwritten by Pizzini.

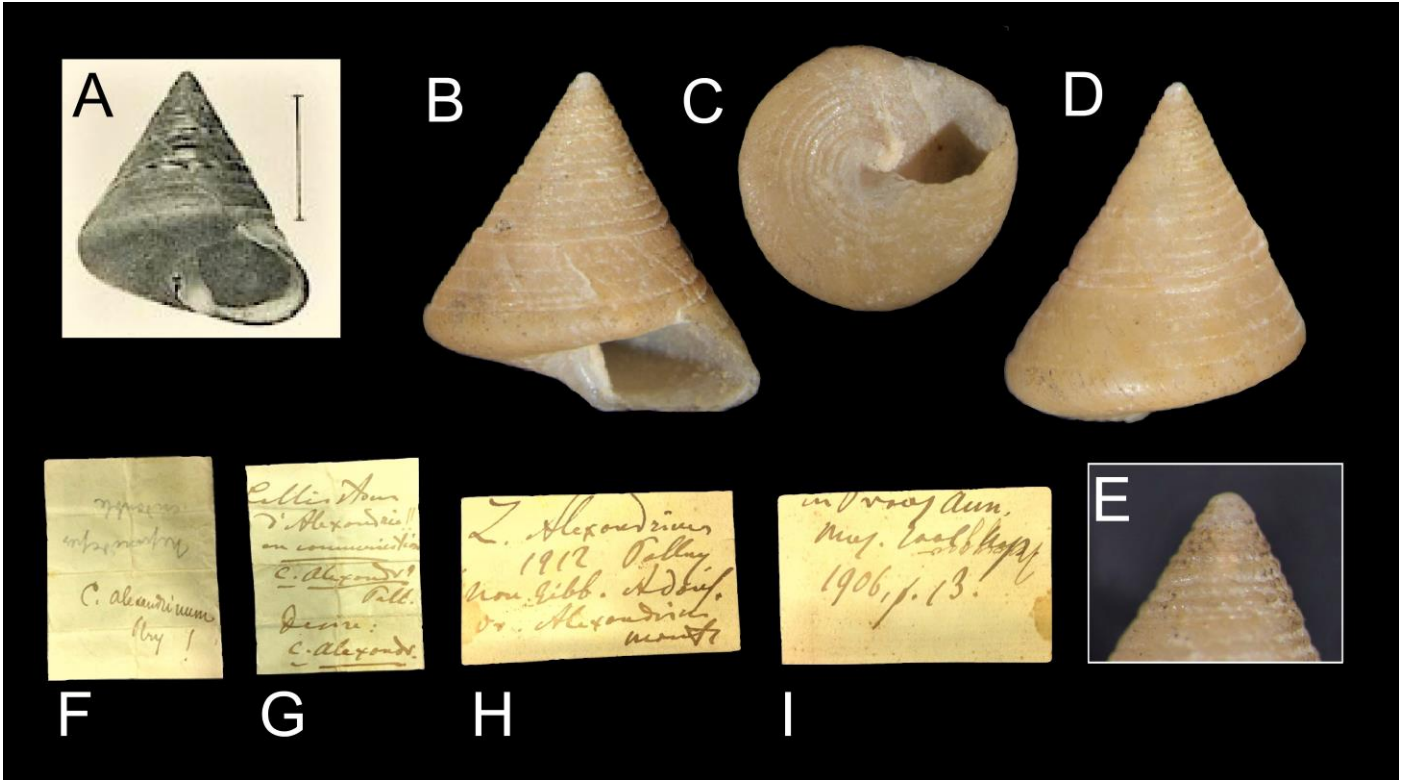


FIGURE S3-3. *Calliostoma alexandrinum* (Pallary, 1912) = *C. zizyphinum*. Museo Civico di Zoologia, Rome, Italy. **A.** Original illustration from Pallary (1912: pl. XVI, fig. 11). **B–E.** MCZR-M-11667, syntype, H 7.8 x D 7.0 mm; Alexandria, Egypt. **F.** Original label handwritten by Pallary. **G–I.** MCZR-M-11667, original labels handwritten by Monterosato.

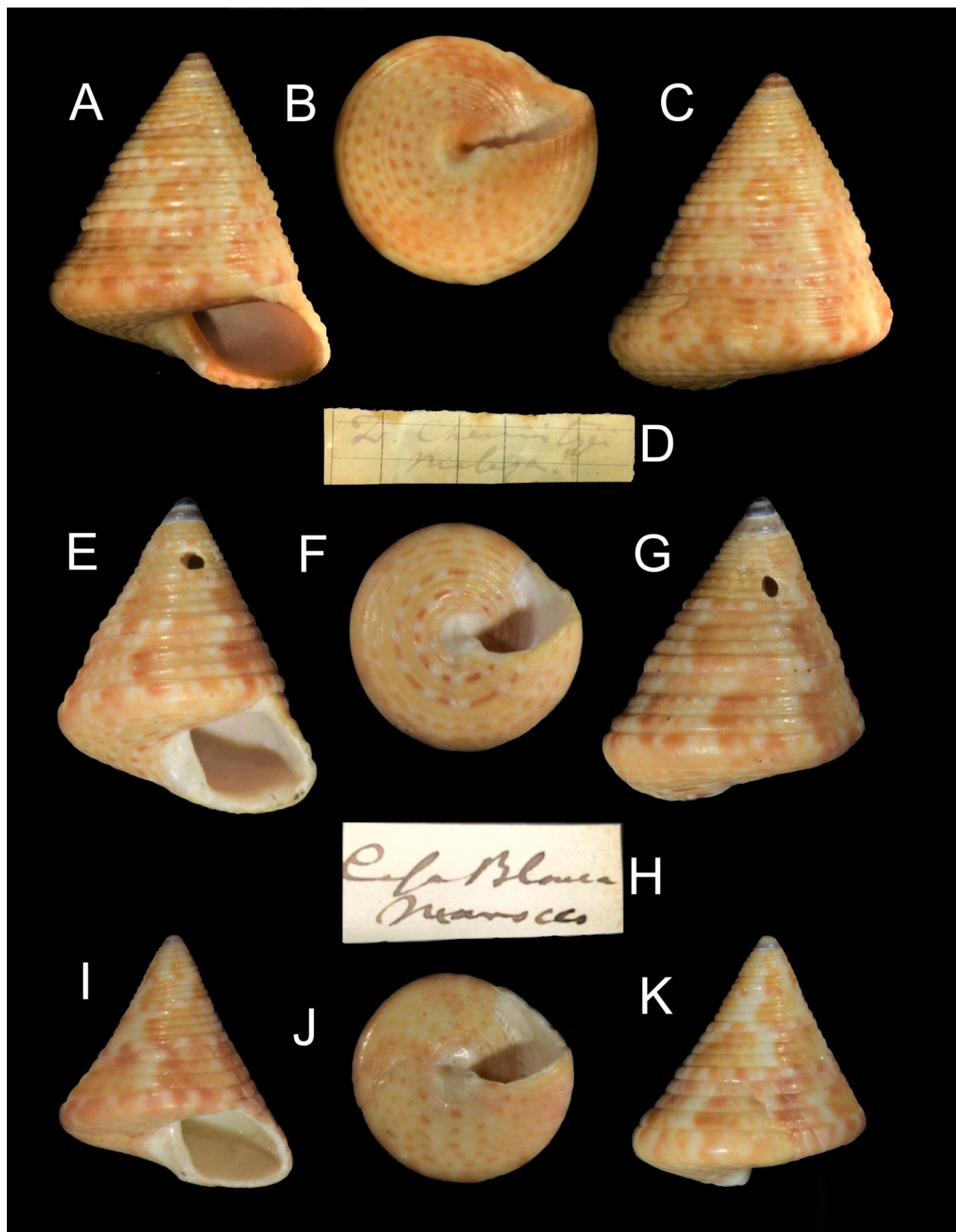


FIGURE S3-4. *Calliostoma chemnitzii* (R. A. Philippi, 1848) = *C. zizyphinum*. Museo Civico di Zoologia, Rome, Italy. **A–C.** MCZR-M-11657, H 16.8 x D 14.2 mm; Malaga, Spain. **D.** MCZR-M-11657, original label handwritten by Monterosato. **E–G.** MCZR-M-11657, H 10.9 x D 9.9 mm; Casablanca, Morocco. **H.** MCZR-M-11657, original label handwritten by Monterosato. **I–K.** MCZR-M-11657, H 8.5 x D 8.0 mm; Casablanca, Morocco.

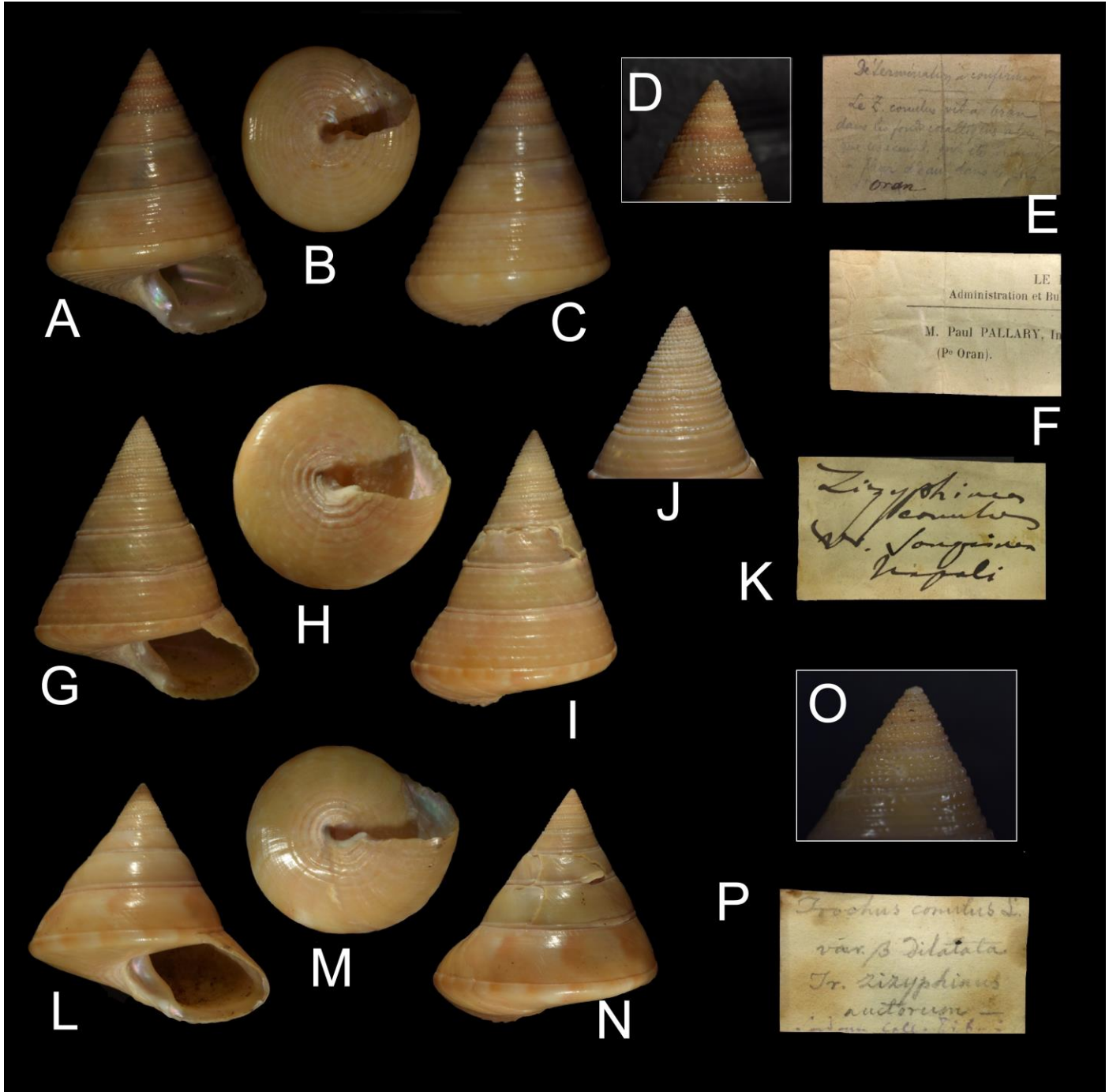


FIGURE S3-5. *Calliostoma conulus* (Linnaeus, 1758). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11536, sculptured form, H 17.3 x D 14.2 mm; Oran, Algeria. **E–F.** MCZR-M-11536, original labels handwritten by Pallary. **G–J.** MCZR-M-11536, elated form, H 24.0 x D 19.3 mm; ventral, basal and dorsal views, Naples, Italy. **K.** MCZR-M-11536, original label handwritten by Monterosato. **L–P.** Sine numera, inflated form, H 24.2 x D 23.5 mm; Sardinia, Italy. **Q.** Sine numera, original label handwritten by Tiberi, ex Tiberi collection.

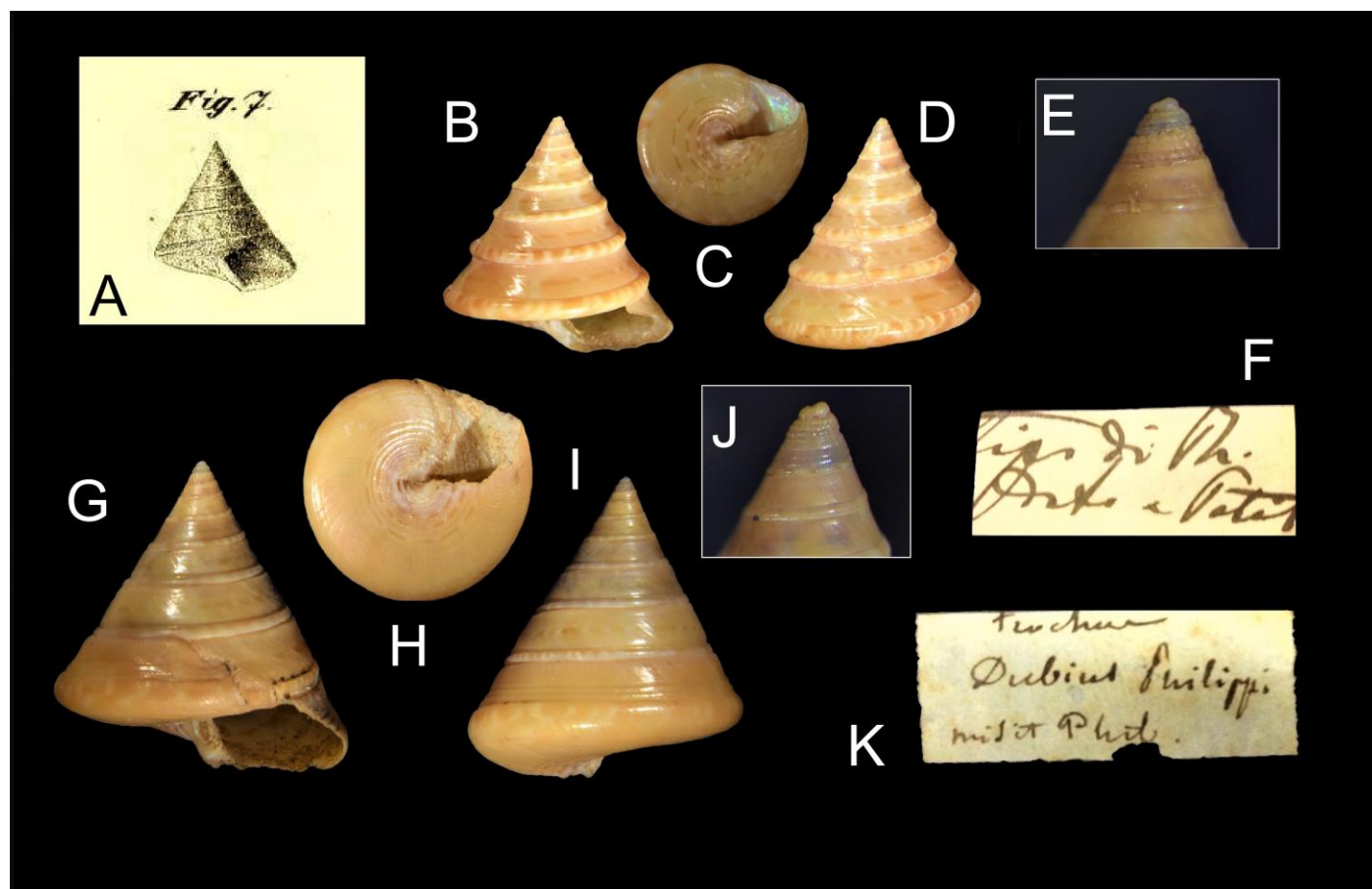


FIGURE S3-6. *Calliostoma dubium* (Philippi, 1844) = *C. zizyphinum*. Museo Civico di Zoologia, Rome, Italy. **A.** Original illustration from Philippi (1844: pl. XXV, fig. 7). **B–E.** MCZR-M-11572, possible syntype, H 10.5 x D 9.1 mm; ex Coen collection. **F.** MCZR-M-11572, original label handwritten by Monterosato. **G–J.** MCZR-M-11572, possible syntype, H 12.2x D 11.2 mm; ex Coen collection. **K.** MCZR-M-11572, original label handwritten by R.A. Philippi.



FIGURE S3-7. *Calliostoma floreanae* Parensan, 1970 = *C. laugier*. Parensan collection, Università del Salento, Museo di Biologia marina "Pietro Parensan", Porto Cesareo, Lecce, Italy. **A–C.** Sine numera, syntype, beached, H 9.0 mm; Agrigento, Sicily. **D.** Original label handwritten by Parensan. **E.** Box containing the beached syntypes; Agrigento, Sicily; **F–J.** Sine numera, syntype, beached, H 8.0 mm; Agrigento, Sicily. **K.** Sine numera, syntypes, beached; Agrigento, Sicily.



FIGURE S3-8. *Calliostoma granulatum* Born, 1778. NHMW-MO-14000, Holotype, H 31.61 mm; Natural History Museum, Vienna, Austria ©NHMW, Mollusca Collection; Photo credits: P.G. Albano.

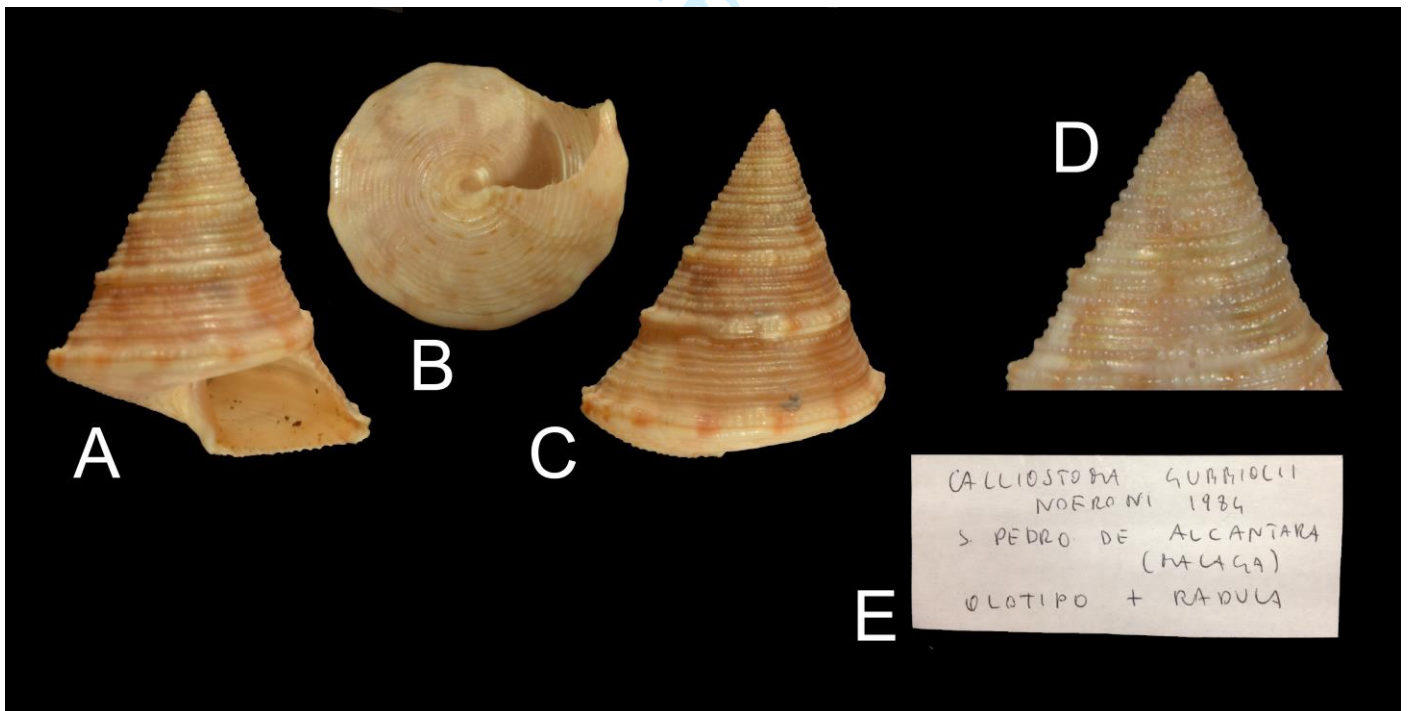


FIGURE S3-9. *Calliostoma gubbiolii* Nofroni, 1984. Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-TYPE-00005/H, holotype, H 14.5 x D 13.4 mm; San Pedro de Alcantara (Malaga), Spain. **E.** Original label handwritten by Nofroni.

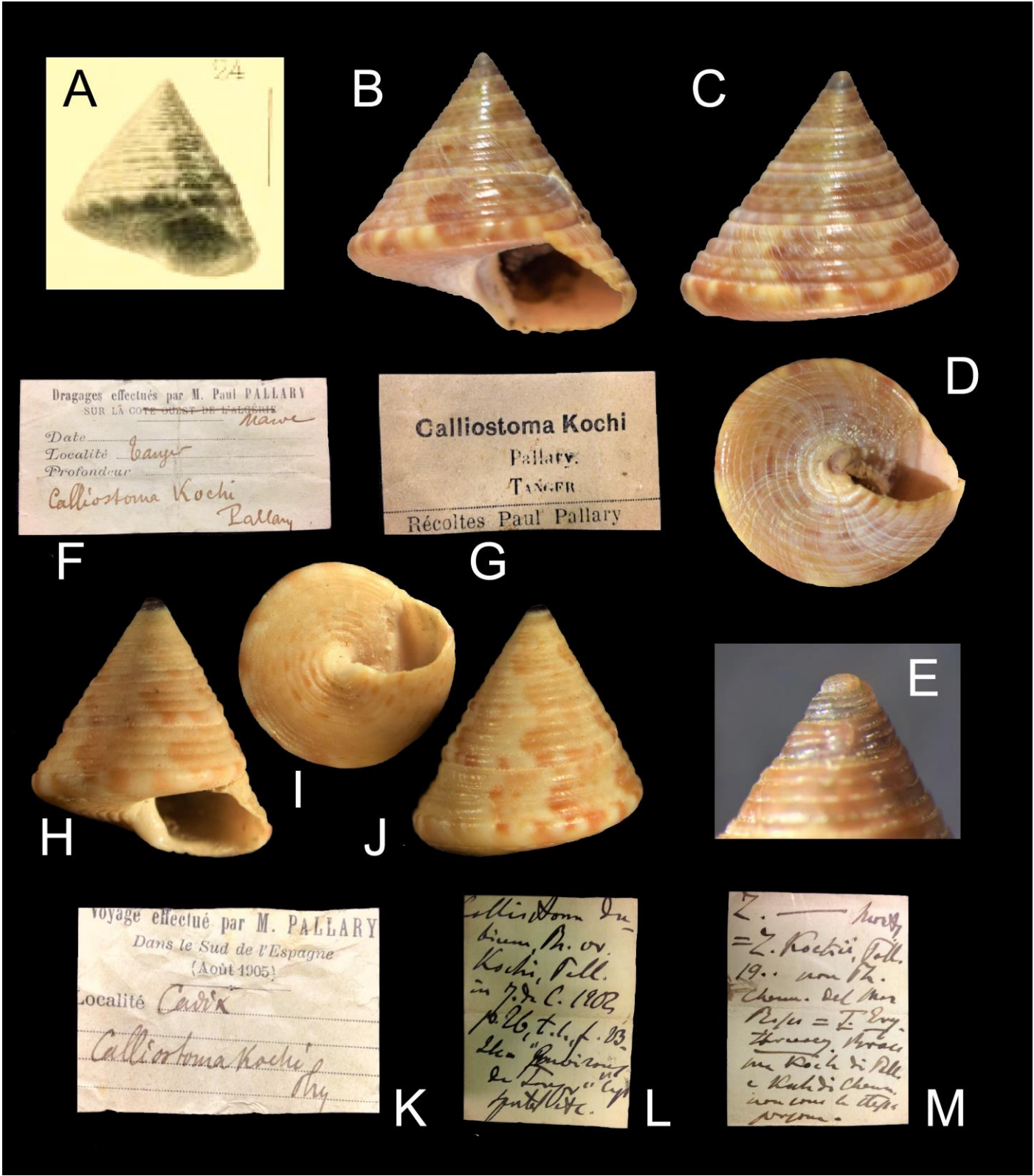


FIGURE S3-10. *Calliostoma kochi* Pallary, 1902 = *C. laugier*. Museo Civico di Zoologia, Rome, Italy. **A.** Original illustration from Pallary (1902: pl. 1, fig. 24). **B–E.** MCZR-M-11618, syntype, H 10.0 x D 10.2 mm; Tanger, Algeria. **F–G.** MCZR-M-11618, original labels handwritten by Pallary. **H–J.** MCZR-M-11618, syntype, H 11.9 x D 9.7 mm; Cadiz, Spain. **K.** Original labels handwritten by Pallary. **L–M.** Original labels handwritten by Monterosato.

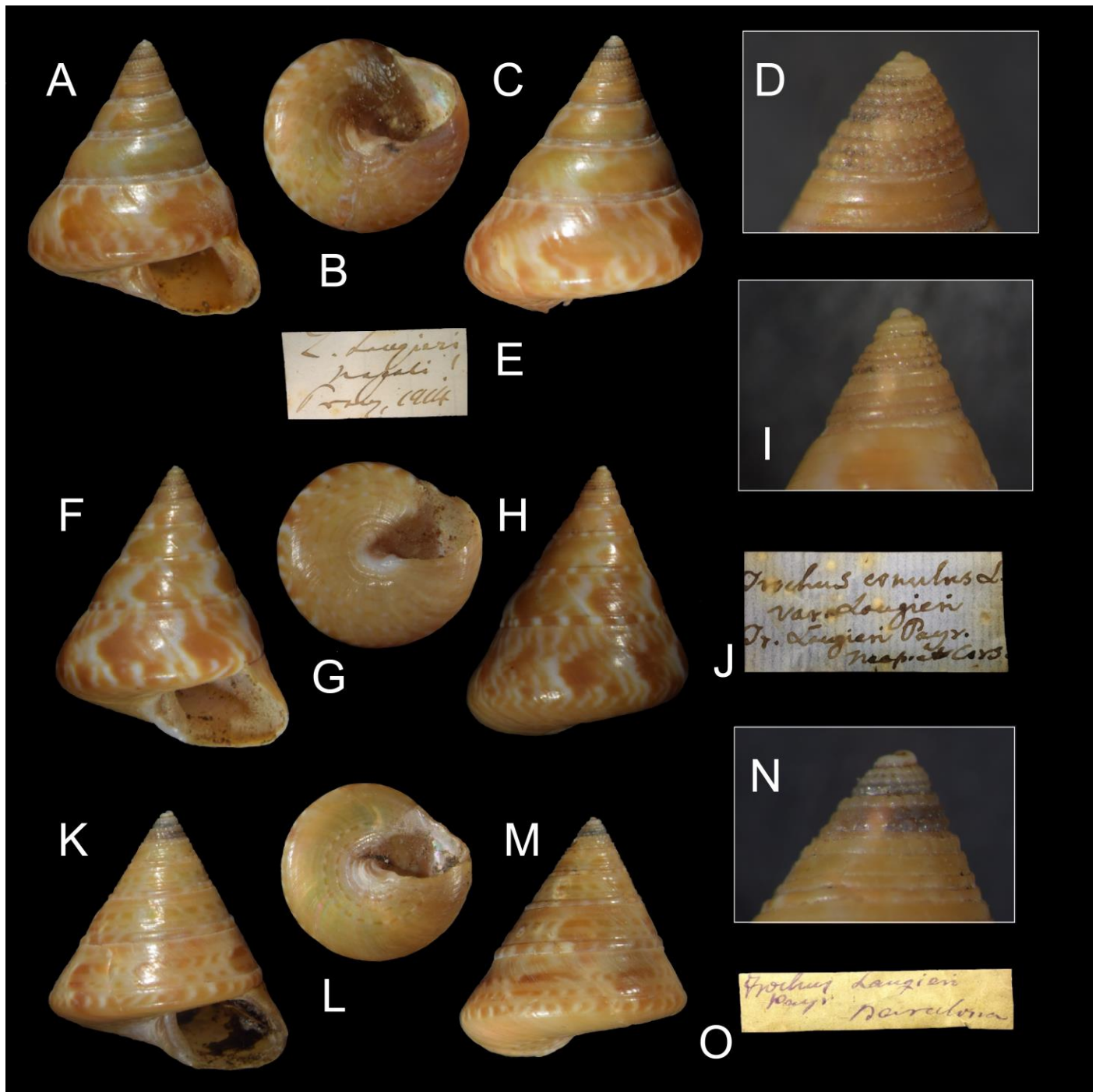


FIGURE S3-11. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11630, H 10.3 x D 8.4 mm; Naples, Italy. **E.** MCZR-M-11630, original label handwritten by Monterosato. **F–I.** MCZR-M-11643, H 10.2 x D 8.4 mm; Naples, Italy. **J.** MCZR-M-11643, original label. **K–N.** MCZR-M-11643, H 9.1 x D 8.6 mm; Barcelona, Spain. **O.** MCZR-M-11643, original label.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

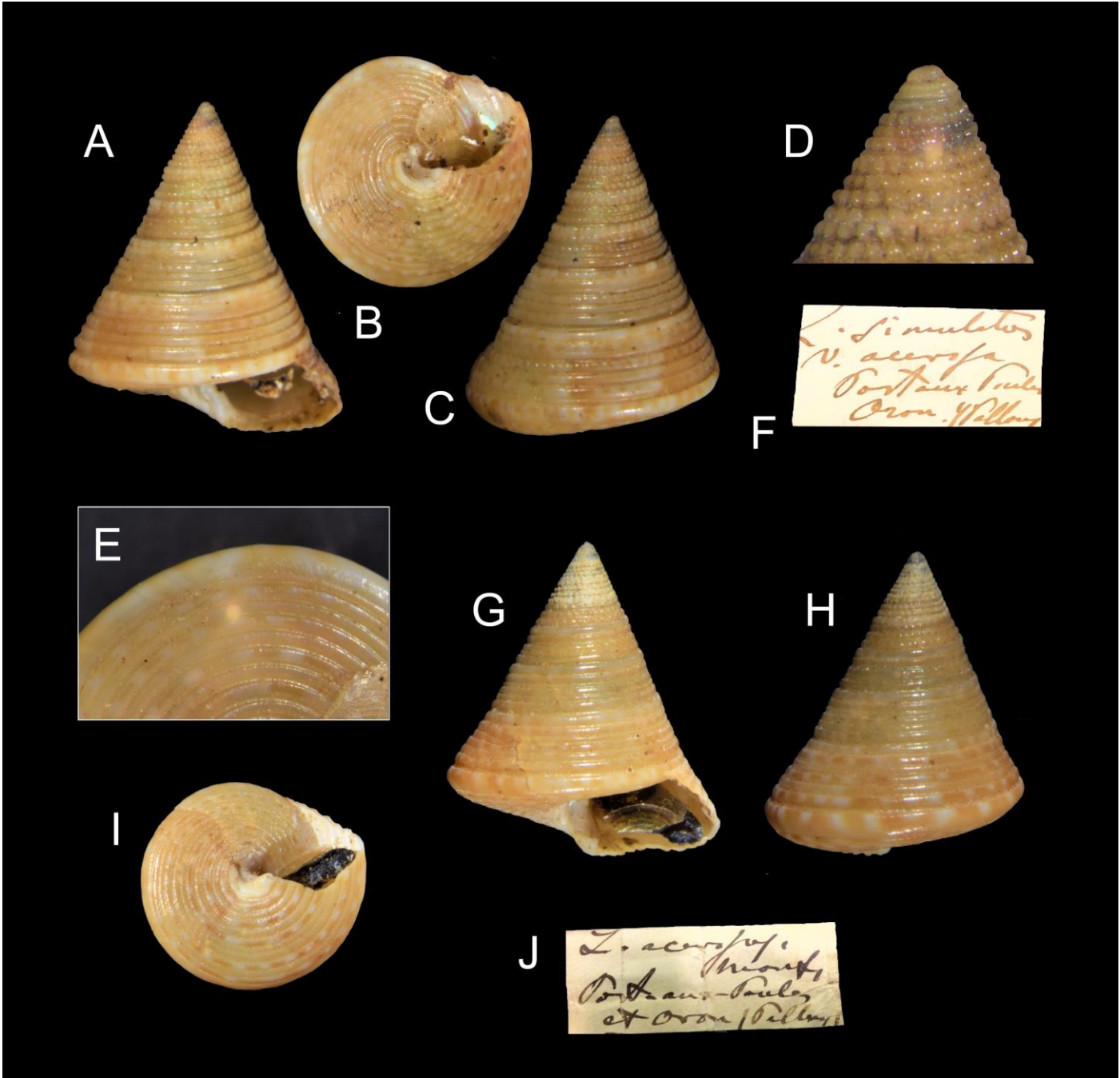


FIGURE S3-12. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–E.** MCZR-M-11620, H 10.4 x D 8.3 mm; Port aux Paules, Oran, Algeria. **F.** MCZR-M-11620, original label handwritten by Monterosato. **G–I.** MCZR-M-11620, H 10.4 x D 8.8 mm; Port aux Paules, Oran, Algeria. **J.** MCZR-M-11620, original label handwritten by Monterosato.

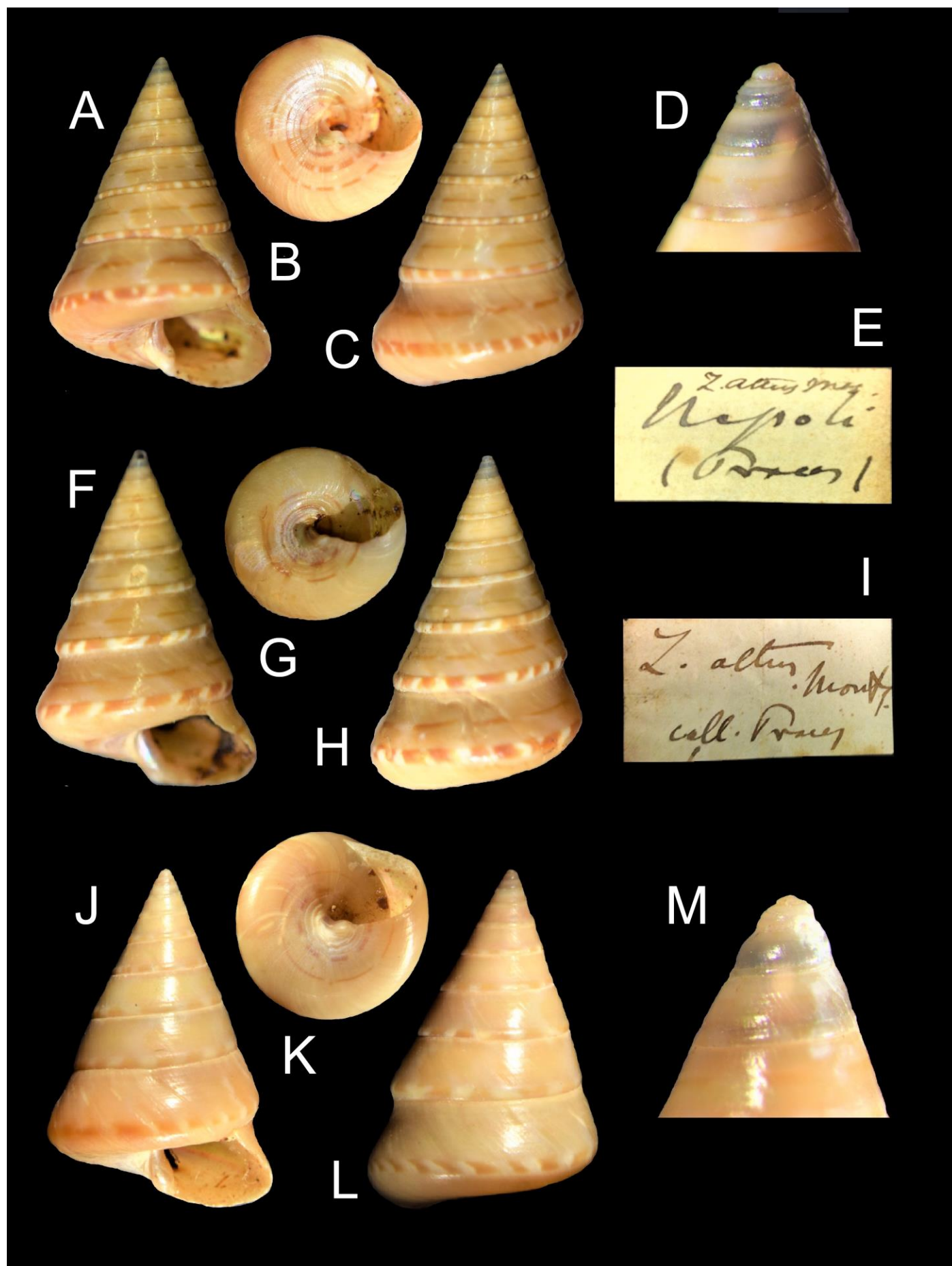


FIGURE S3-13. *Calliostoma laugierii* (Payraudeau, 1826). Ex Praus collection, Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11631, H 16.7 x D 11.8 mm, Naples. **E.** MCZR-M-11631, original label handwritten by Monterosato. **F–H.** MCZR-M-11631, H 16.9 x D 11.9 mm, Naples. **I.** MCZR-M-11631, original labels handwritten by Monterosato. **J–M.** MCZR-M-11631, H 15.8 x D 10.9 mm, Naples.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

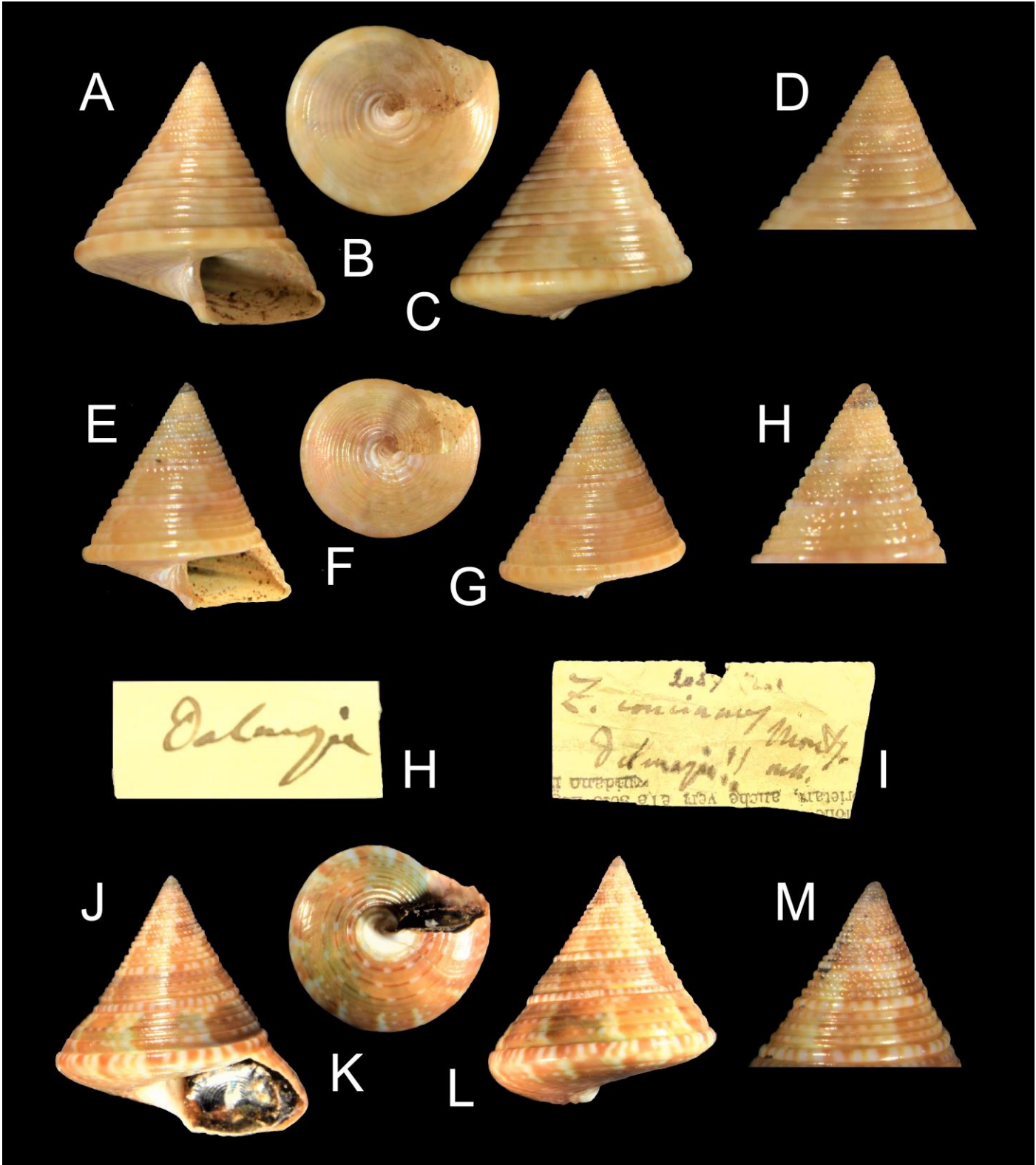


FIGURE S3-14. *Calliostoma laugierii* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11633, H 9.9 x D 9.8 mm; “*Dalmazia*”, historical-geographical region of the eastern coast of the Adriatic Sea. **E–H.** MCZR-M-11633, H 7.5 x D 7.0 mm; “*Dalmazia*”, historical-geographical region of the eastern coast of the Adriatic Sea. **I–J.** MCZR-M-11633, original labels handwritten by Monterosato. **K–N.** CS-PM, H 11.5 x D 10.7 mm; Porto Cesareo, Lecce, Apulia, Italy; fishing boats, 90 m.

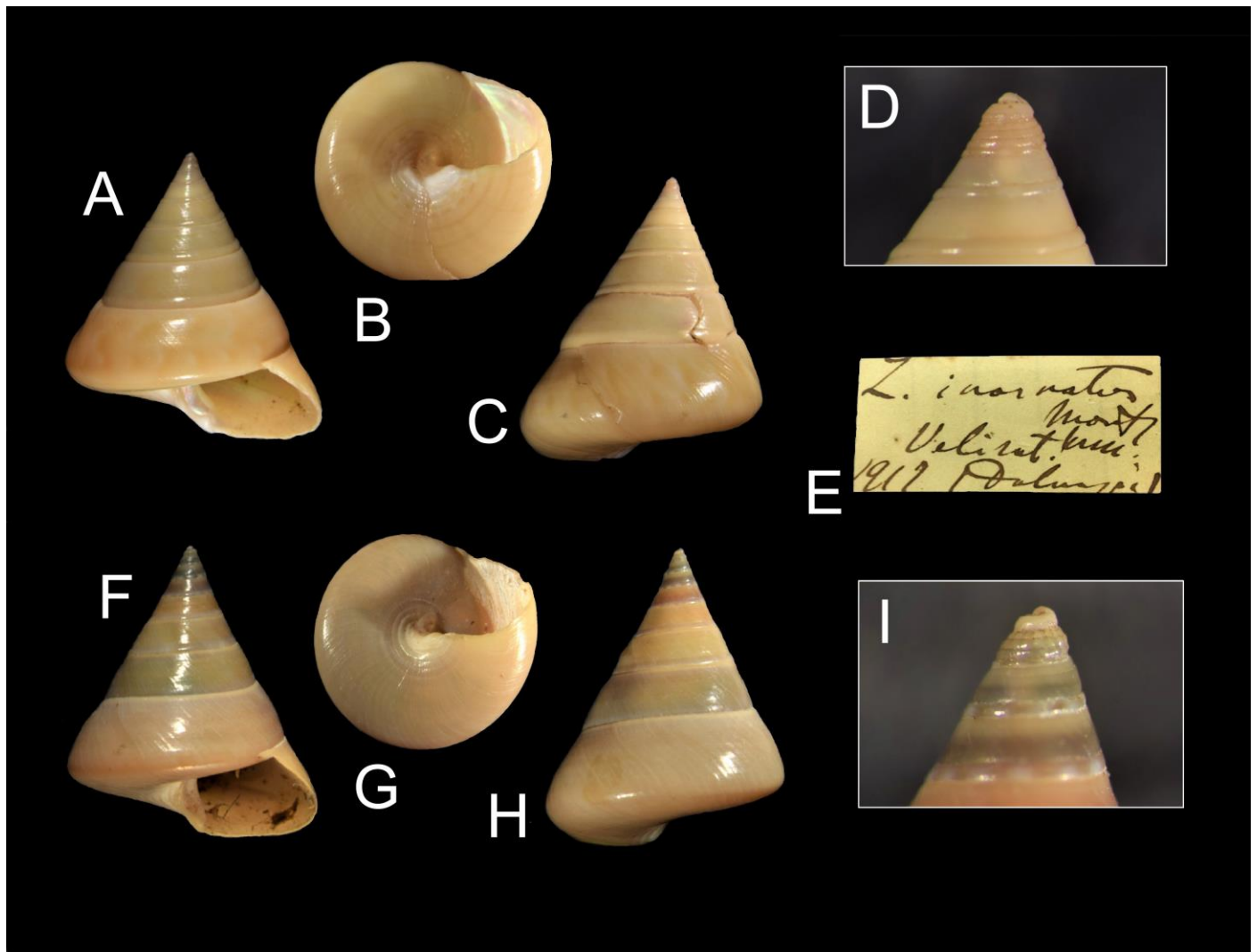


FIGURE S3-15. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11633, H 14.4 x D 11.9 mm; Veli Rat, Croatia. **E.** MCZR-M-11633, original label handwritten by Monterosato. **F–I.** MCZR-M-11633, H 12.9 x D 11.2 mm; Veli Rat, Croatia.

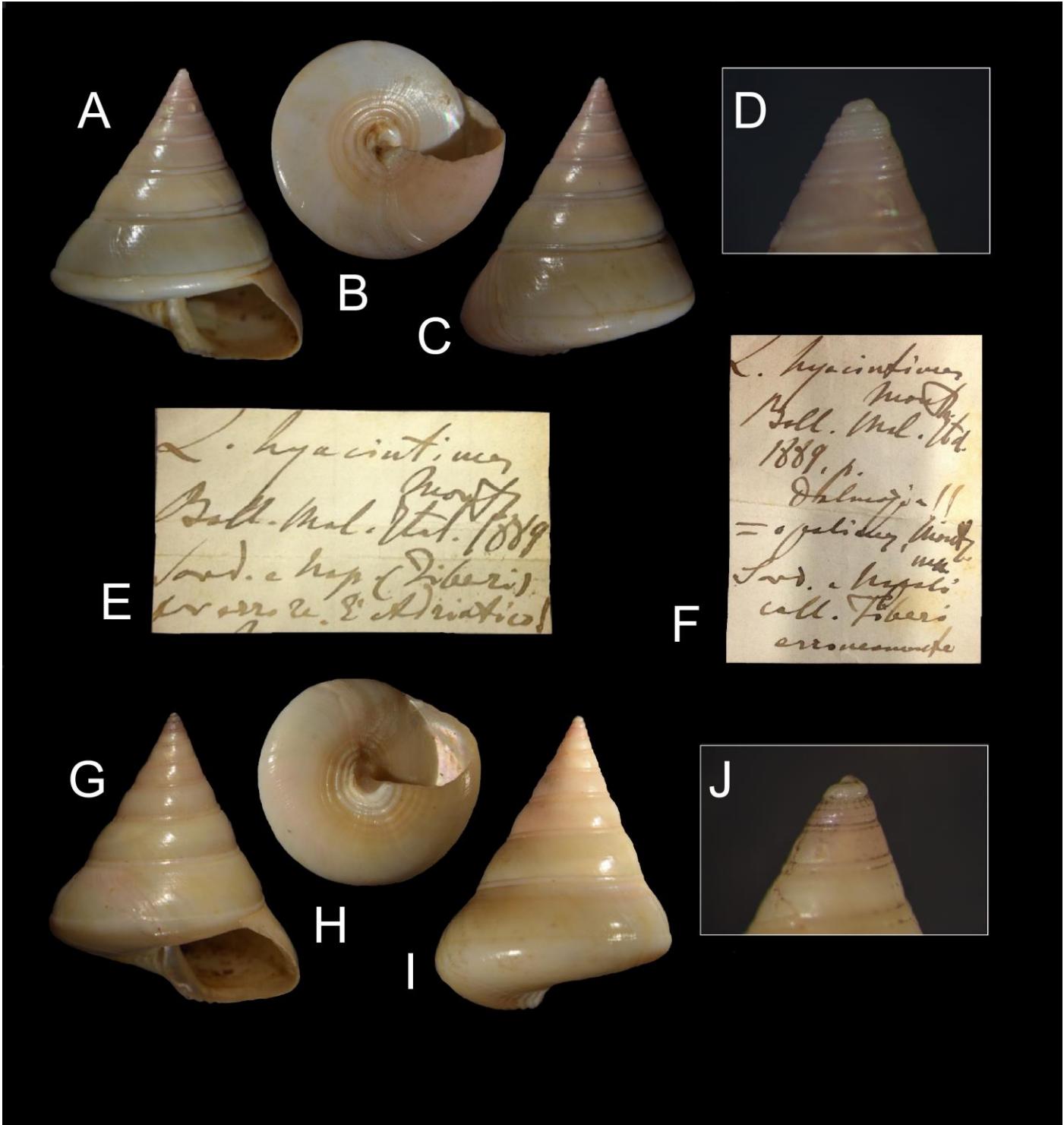


FIGURE S3-16. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11621, H 12.6 x D 11.1 mm; “*Dalmazia*”, historical-geographical region of the eastern coast of the Adriatic Sea. **E–F.** MCZR-M-11621, original labels handwritten by Monterosato. **G–J.** MCZR-M-11621, H 13.3 x D 11.6 mm; “*Dalmazia*”, historical-geographical region of the eastern coast of the Adriatic Sea.

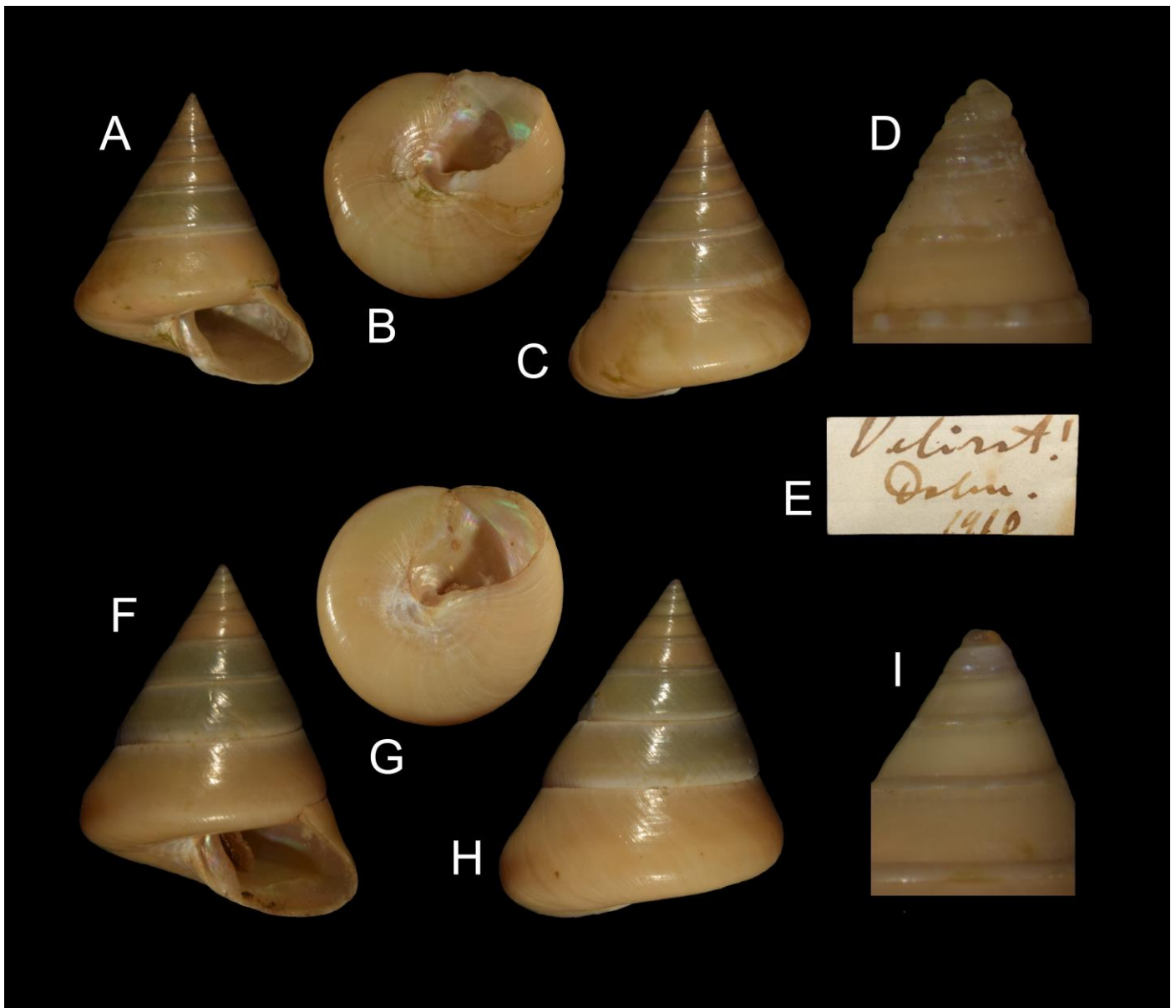


FIGURE S3-17. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11661, H 17.9 x D 15.0 mm; Veli Rat, Croatia. **E.** MCZR-M-11661, original label handwritten by Monterosato. **F–I.** MCZR-M-11661, H 19.9 x D 16.1 mm; Veli Rat, Croatia.

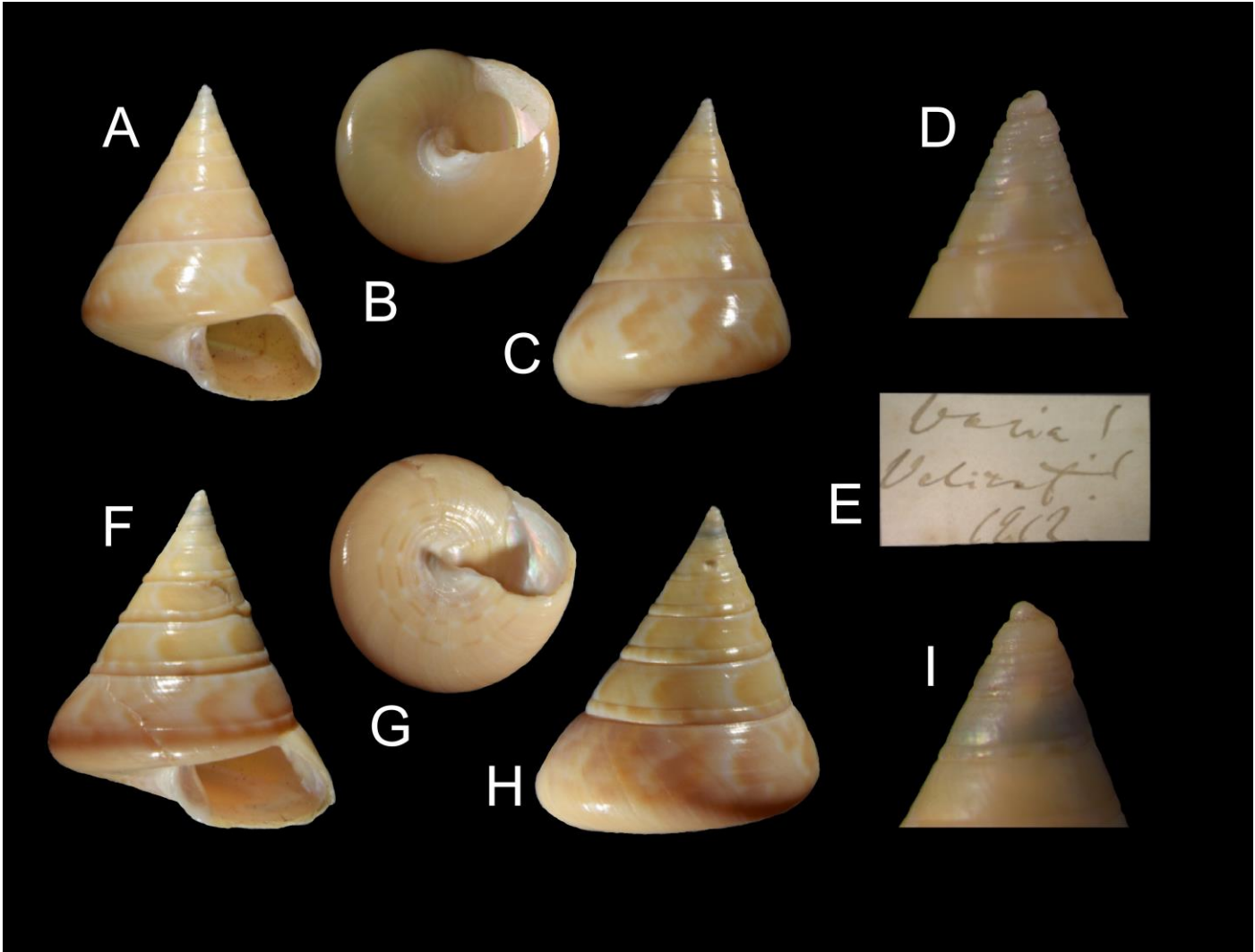


FIGURE S3-18. *Calliostoma laugieri* (Payraudeau, 1826). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11633, H 17.8 x D 14.9 mm; Veli Rat, Croatia. **E.** MCZR-M-11633, original label handwritten by Monterosato. **F–I.** MCZR-M-11633, H 19.9 x D 15.9 mm; Veli Rat, Croatia.

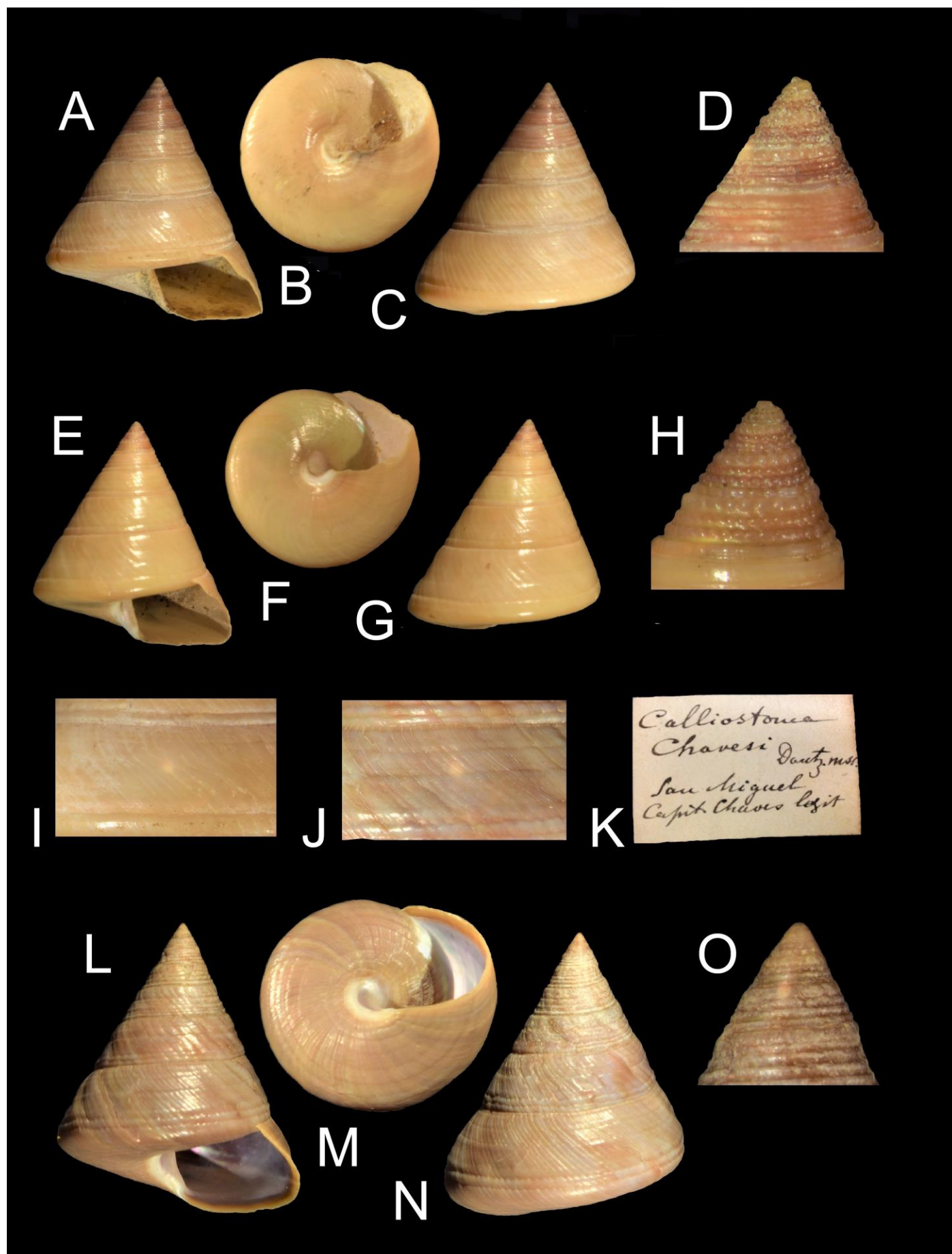


FIGURE S3-19. *Calliostoma lividum* Dautzenberg, 1927 = *C. zizyphinum*. Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11654, syntype, H 15.4 x D 13.6 mm; Island of Sao Miguel, Azores Islands, Portugal. **E–J.** MCZR-M-11654, syntype, H 20.3 x D 17.7 mm; Island of Sao Miguel, Azores Islands, Portugal. **K.** MCZR-M11654, original label handwritten by Dautzenberg. **L–O.** CS-PM, H 22.3 x D 19.4 mm; Castelo Branco, Faial Island, Azores Islands, Portugal; 20–24 m, on rocks, specimen with exalted sculpture.



FIGURE S3-20. *Calliostoma neretinum* Parenzan, 1970 = *C. laugieri*. Parenzan collection, Università del Salento, Museo di Biologia marina "Pietro Parenzan", Porto Cesareo, Lecce, Italy. **A–E.** 259,02, Holotype, H 15.0 mm; Porto Cesareo, Apulia, Italy. **F–G.** Original labels.

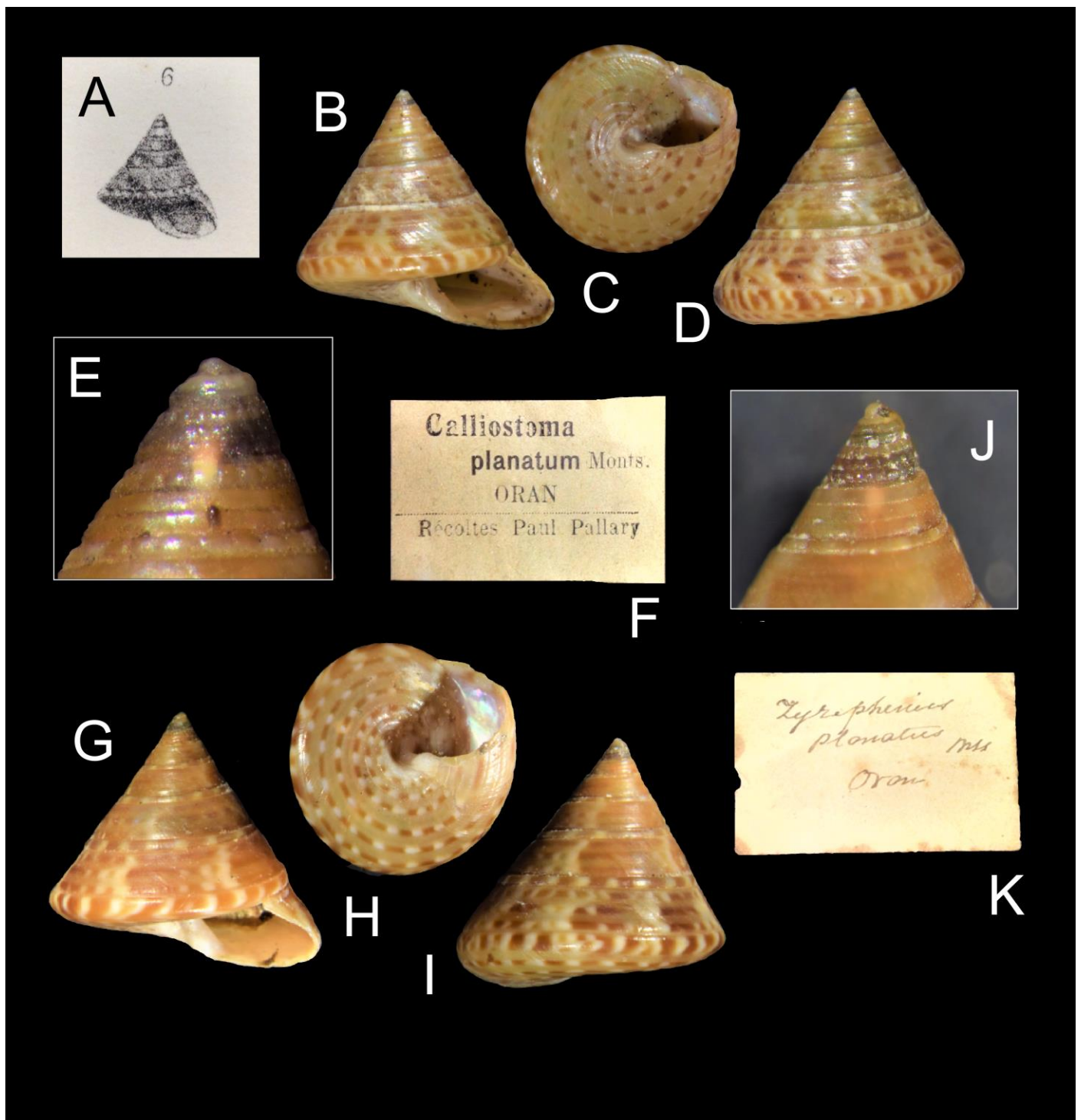


FIGURE S3-21. *Calliostoma planatum* (Pallary, 1900) = *C. laugier*. Museo Civico di Zoologia, Rome, Italy. **A.** Original illustration from Pallary (1900: pl. VIII, fig. 6). **B–E.** MCZR-M-30093, syntype, H 9.8 x D 9.7 mm; Oran, Algeria. **F.** MCZR-M-30093, original label by Pallary. **G–J.** MCZR-M-30093, syntype, H 9.5 x D 9.6 mm; Oran, Algeria. **K.** MCZR-M-30093, original label handwritten by Pallary.

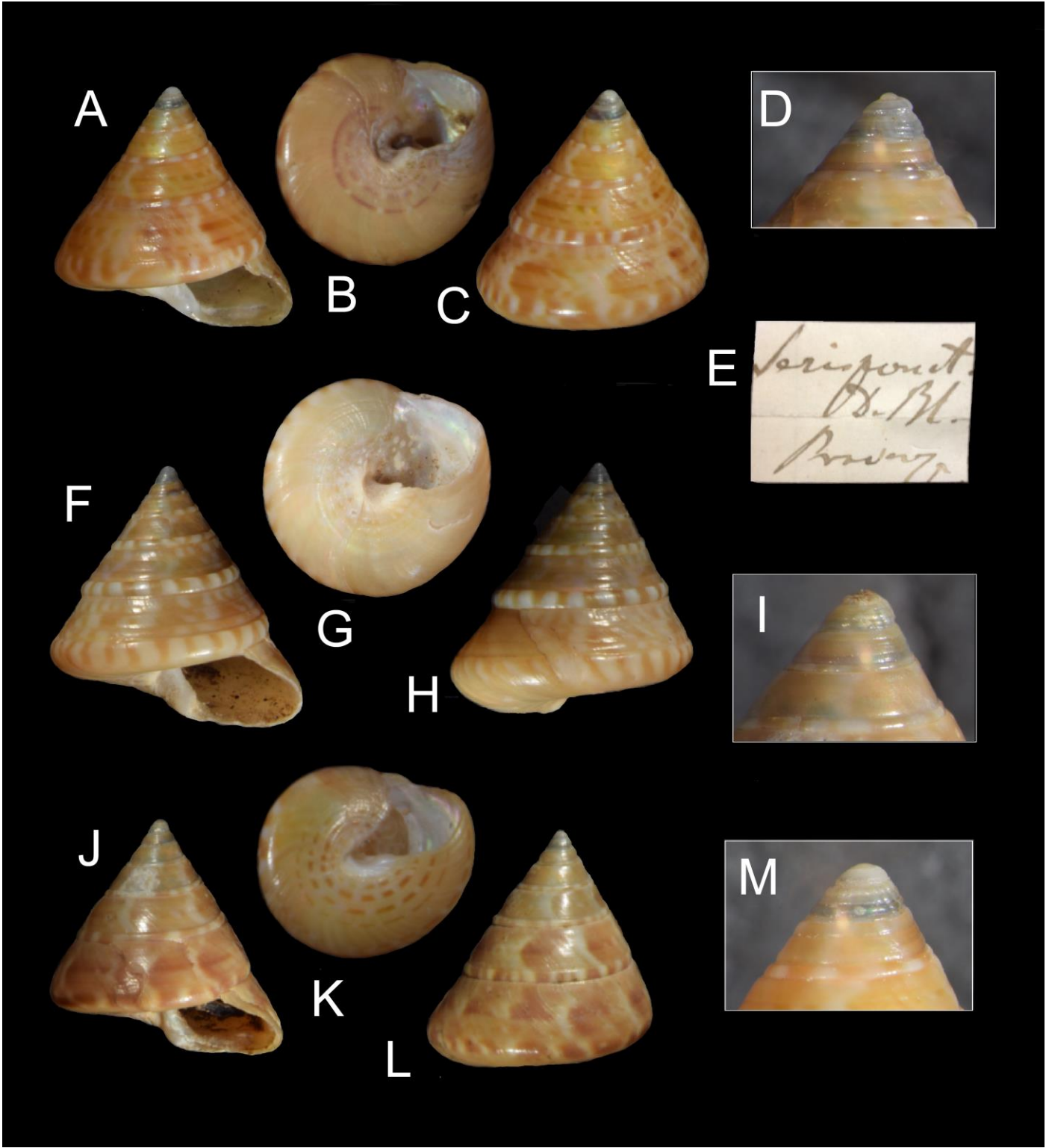


FIGURE S3-22. *Calliostoma seriopunctatus* (Blainville, 1830) = *C. laugieri*. Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11637, H 11.4 x D 10.6 mm; Provenza, France. **E.** MCZR-M-11637, Original label handwritten by Monterosato. **F–I.** MCZR-M-11637, H 10.6 x D 10.1 mm; Provenza, France. **J–M.** MCZR-M-11637, H 8.7 x D 8.4 mm; Provenza, France.

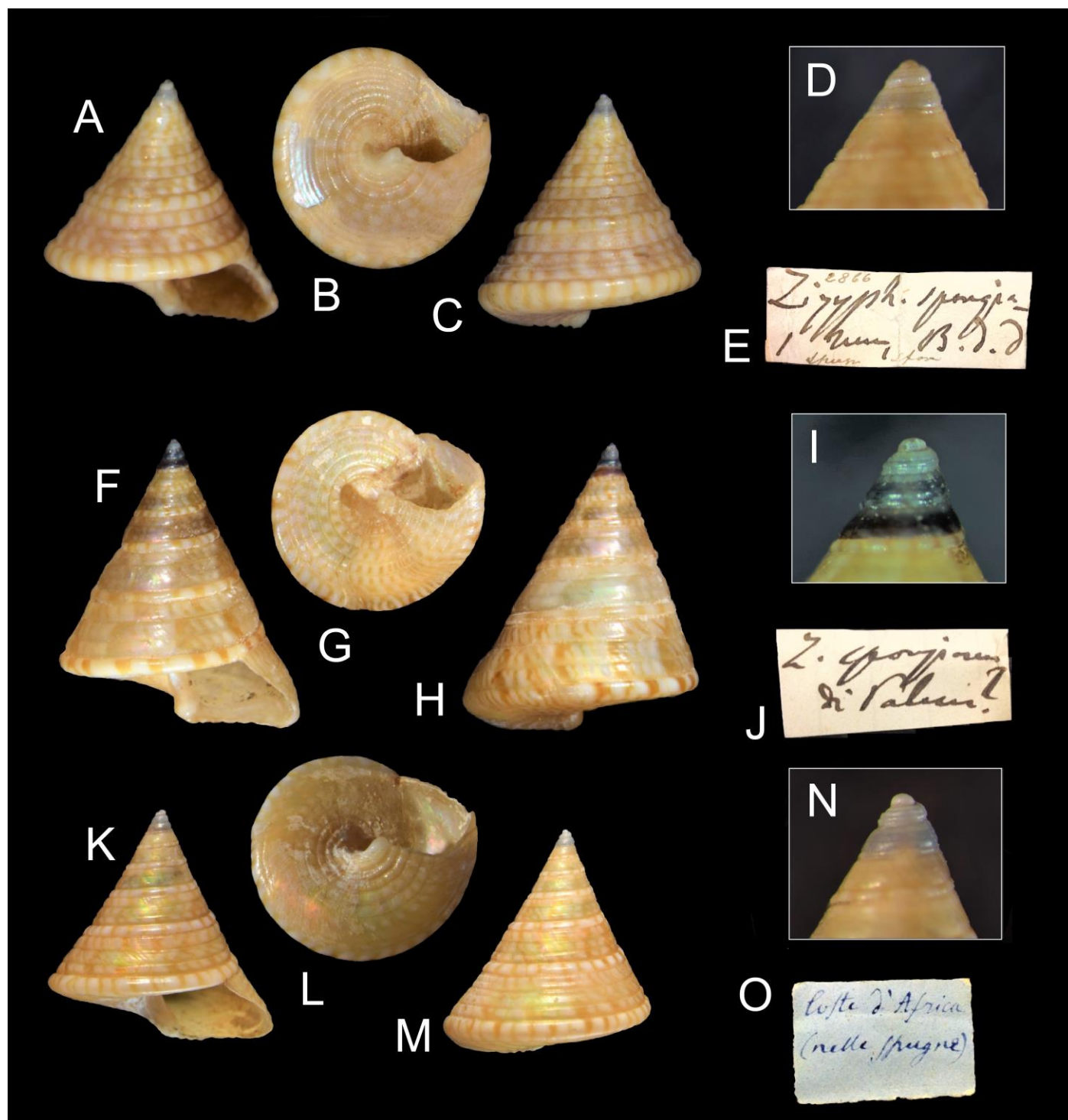


FIGURE S3-23. *Calliostoma spongiarum* (Bucquoy, Dautzenberg e Dollfus, 1885) = *C. laugeri*. Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11645, H 8.9 x D 7.7 mm; Sfax, Tunisia; on sponges. **E.** MCZR-M-11645, original label handwritten by Monterosato. **F–I.** MCZR-M-11628, H 13.6 x D 11.0 mm; Palermo?, Sicily. **J.** MCZR-M-11628, original label handwritten by Monterosato. **K–N.** MCZR-M-11628, H 11.0 x D 10.3 mm; “Coste D’Africa (nelle spugne)”, Tunisia; on sponges. **O.** MCZR-M-11628, original label handwritten by Brugnone.

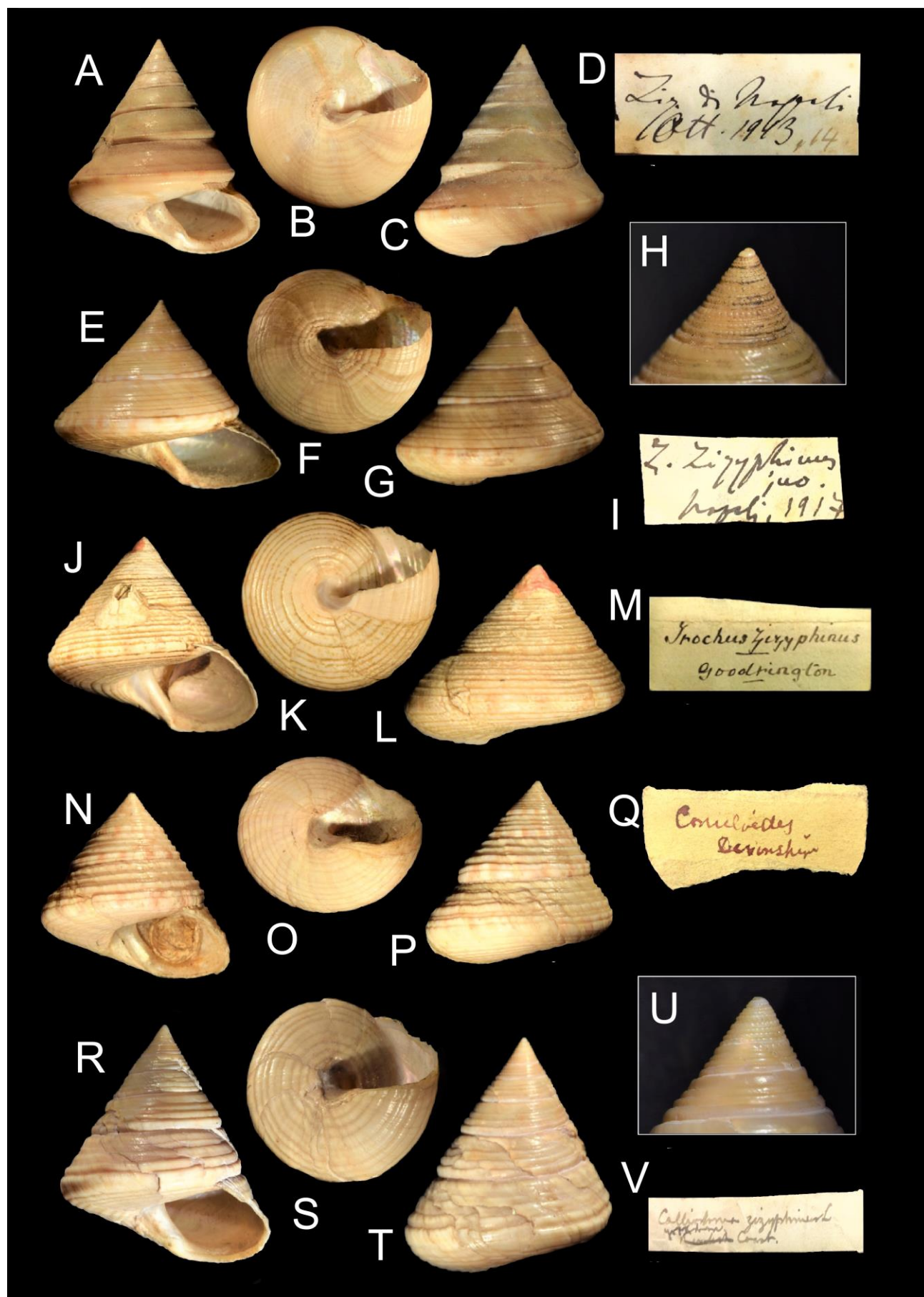


FIGURE S3-24. *Calliostoma zizyphinum* (Linnaeus, 1758). Museo Civico di Zoologia, Rome, Italy. **A–C.** MCZR-M-11595, smooth form, H 33.7 x D 31.2 mm; Naples. **D.** MCZR-M-11595, original label handwritten by Monterosato. **E–H.** MCZR-M-11539, vaguely sculptured form, H 26.3 x D 30.3 mm; Naples. **I.** MCZR-M-11539, original label handwritten by Monterosato. **J–L.** MCZR-M-11604, sculptured form, H 22.7 x D 24.6 mm; Goodrington, Great Britain. **M.** MCZR-M-11604, original label. **N–P.** MCZR-M-11593, evidently sculptured form, H 22.8 x D 24.8 mm; Devonshire, Great Britain. **Q.** MCZR-M-11593, original label, MCZR-M-11593. **R–U.** MCZR-M-11594, very similar to *C. delonguevilleae* (Vilvens & Swinnen, 2017), H 29.4 x D 27.5 mm; Yorkshire, Great Britain. **V.** MCZR-M-11594, original label.

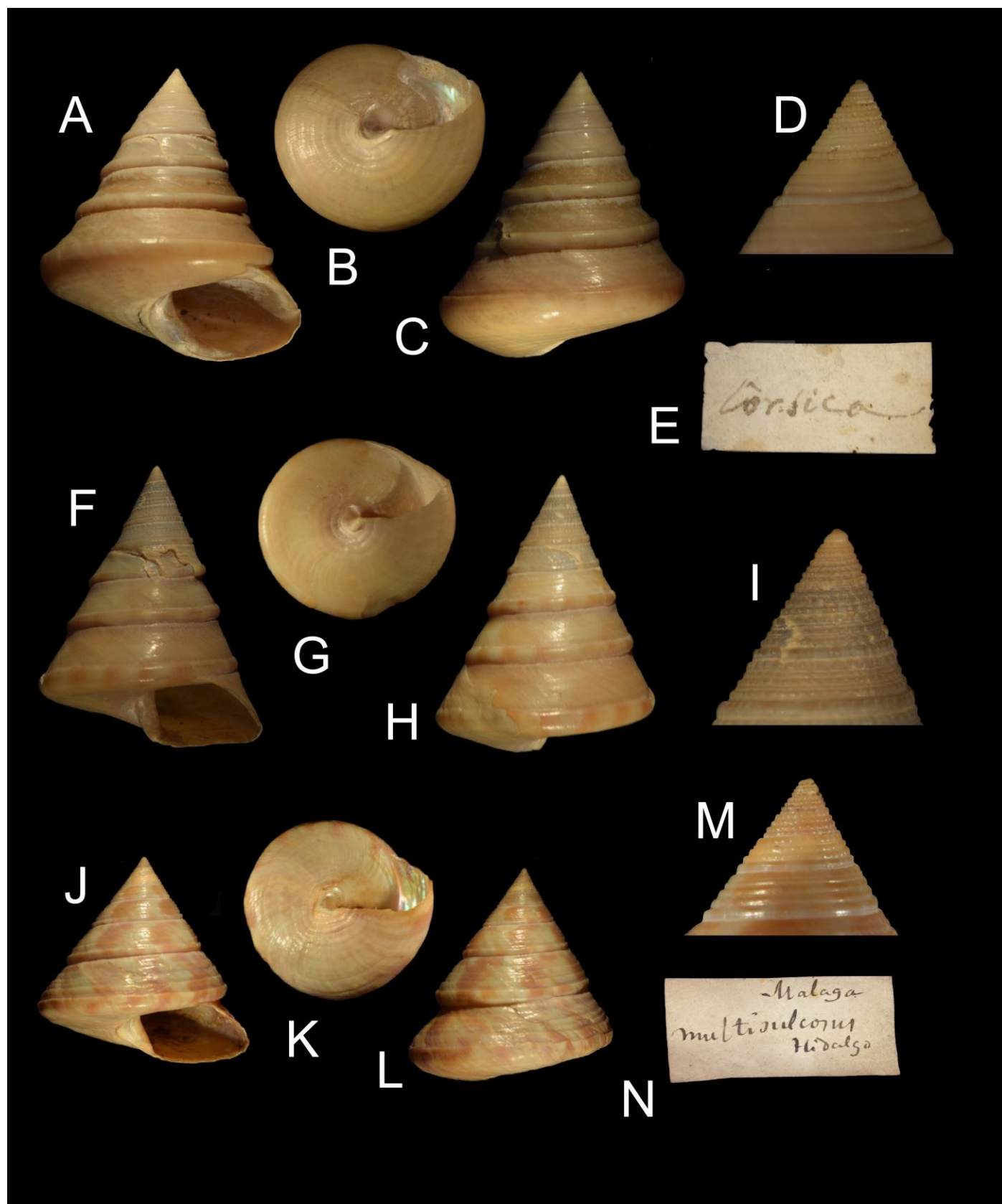


FIGURE S3-25. *Calliostoma zizyphinum* (Linnaeus, 1758). Museo Civico di Zoologia, Rome, Italy. **A–D.** sine numera, smooth and teratological form, H 32.7 x D 29.1 mm; Corsica. **E.** sine numera, original label. **F–I.** sine numera, smooth and teratological form, H 21.3 x D 18.1 mm; Corsica. **J–M.** MCZR-M-11619, corded form, H 25.0 x D 23.3 mm; Malaga, Spain. **N.** MCZR-M-11619, original label handwritten by Hidalgo.

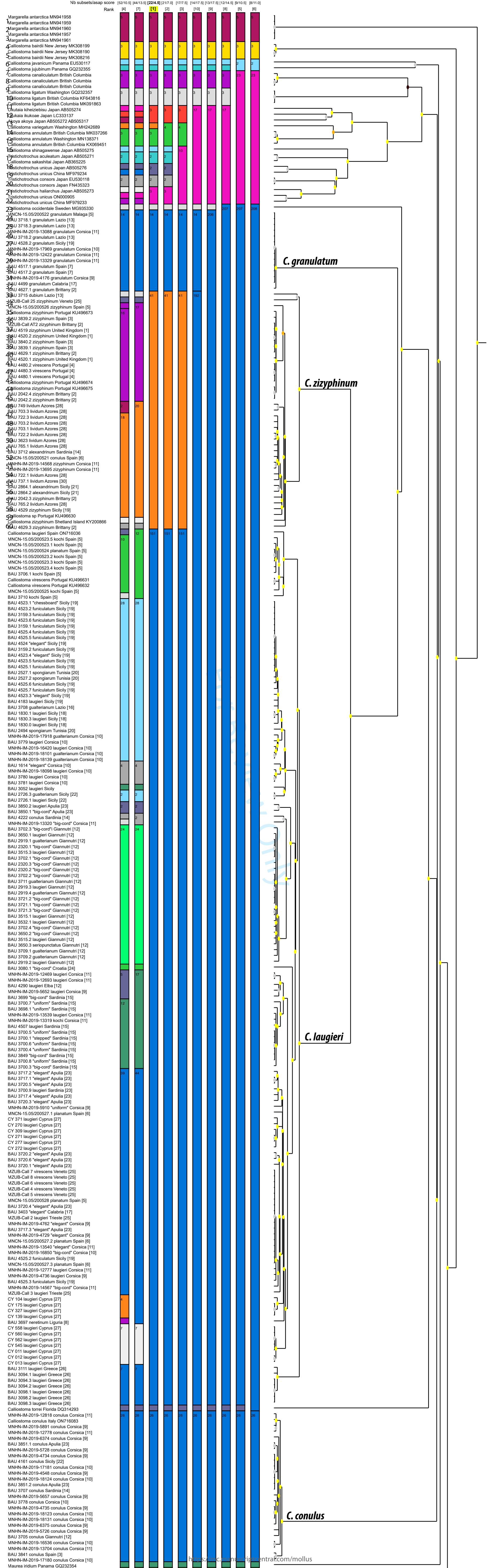
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



FIGURE S3-26. *Calliostoma zizyphinum* (Linnaeus, 1758). Museo Civico di Zoologia, Rome, Italy. **A–D.** MCZR-M-11608, juvenile specimen, H 7.0 x D 7.6 mm; Saint Jean de Luz, France. **E–H.** MCZR-M-11608, juvenile specimen, H 8.2 x D 8.7 mm; Saint Jean-de Luz, France. **I–J.** MCZR-M-11608, original label handwritten by Monterosato.

Table S4. List of used primers.

Gene	Primer name	F/R	Primer sequence	Source
COI	LCO1490	For	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
COI	HCO2198	Rev	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
COI	jgLCO1490	For	TITCIACIAAYCAYAARGAYATTGG	Geller et al. 2013
COI	jgHCO2198	Rev	TAIACYTCIGGRTGICCRAARAAYCA	Geller et al. 2013
COI (mini)	mICOLintF	For	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al. 2013
COI (mini)	mICOLintR	Rev	GGRGGRTASACSGTTCASCCSGTSCC	Leray et al. 2013
16S	16SA	For	CGCCTGTTTATCAAAAACAT	Palumbi 1996
16S (long)	CGleur	Rev	TATTTAGGGCTTAAACCTAATGCAC	Hayashi 2005
16S (short)	16SH	Rev	CCGGTCTGAACTCAGATCAC	Espirito et al. 2001
ITS2	3d	For	GCATCGATGAAGAACGCAG	Oliverio & Mariottini 2001
ITS2	4R	Rev	AGTTTCTTTCTCCGCTTA	Oliverio & Mariottini 2001



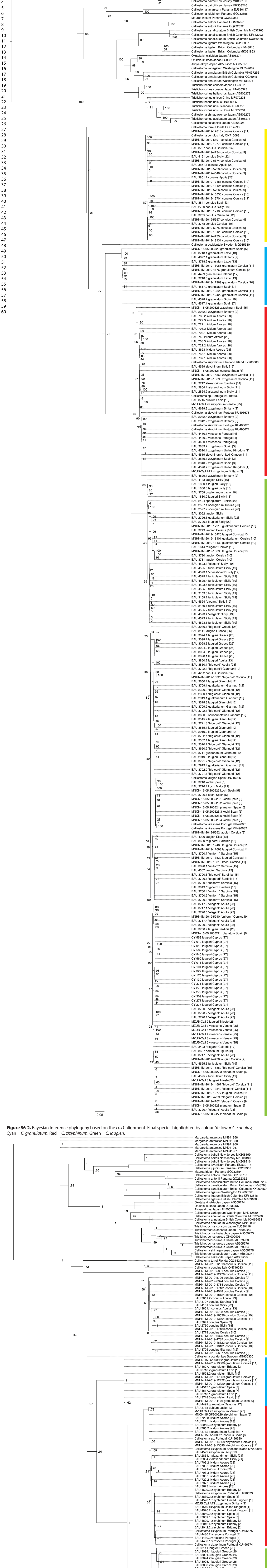


Figure S6-2. Bayesian Inference phylogeny based on the *cox1* alignment. Final species highlighted by colour. Yellow = *C. conulus*; Cyan = *C. granulatum*; Red = *C. zizyphinum*; Green = *C. laugieri*.

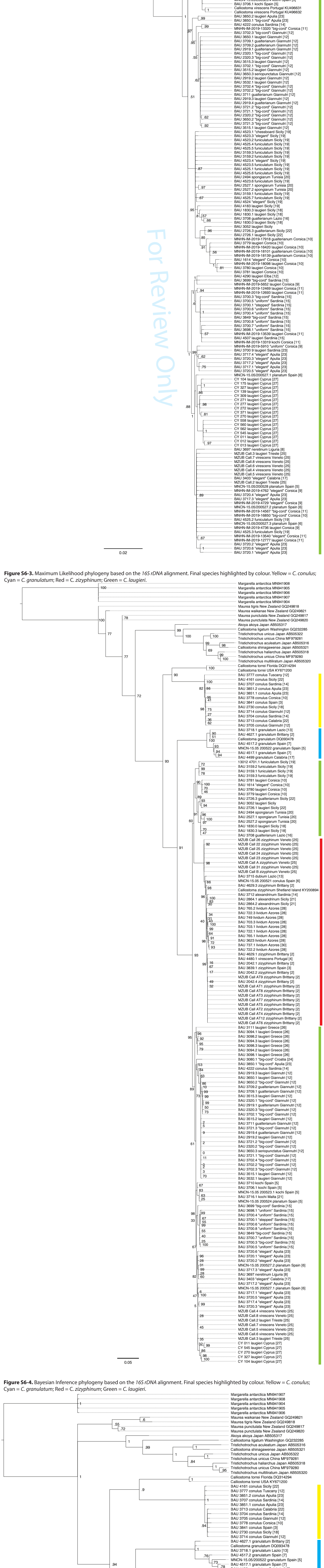


Figure S6-3. Maximum Likelihood phylogeny based on the 16S rDNA alignment. Final species highlighted by colour. Yellow = *C. conulus*; Cyan = *C. granulatum*; Red = *C. zizyphinum*; Green = *C. laugieri*.

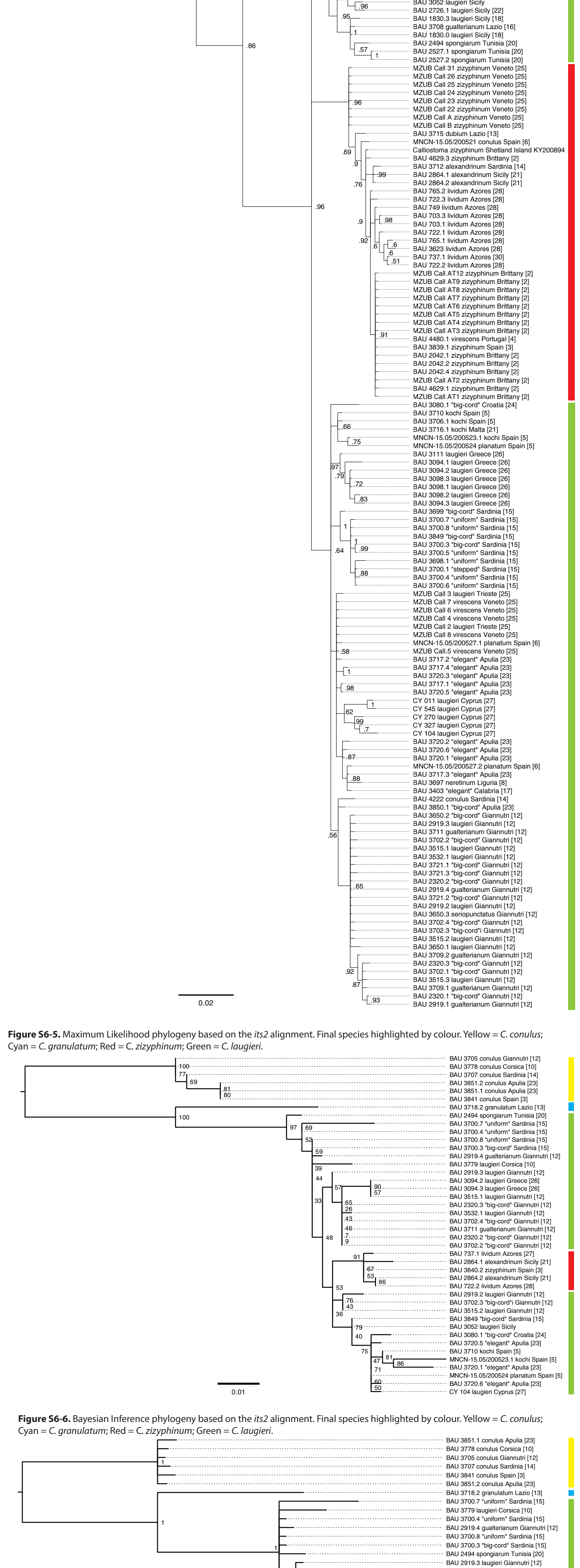


Figure S6-4. Bayesian Inference phylogeny based on the 16S rDNA alignment. Final species highlighted by colour. Yellow = *C. conulus*; Cyan = *C. granulatum*; Red = *C. zizyphinum*; Green = *C. laugieri*.

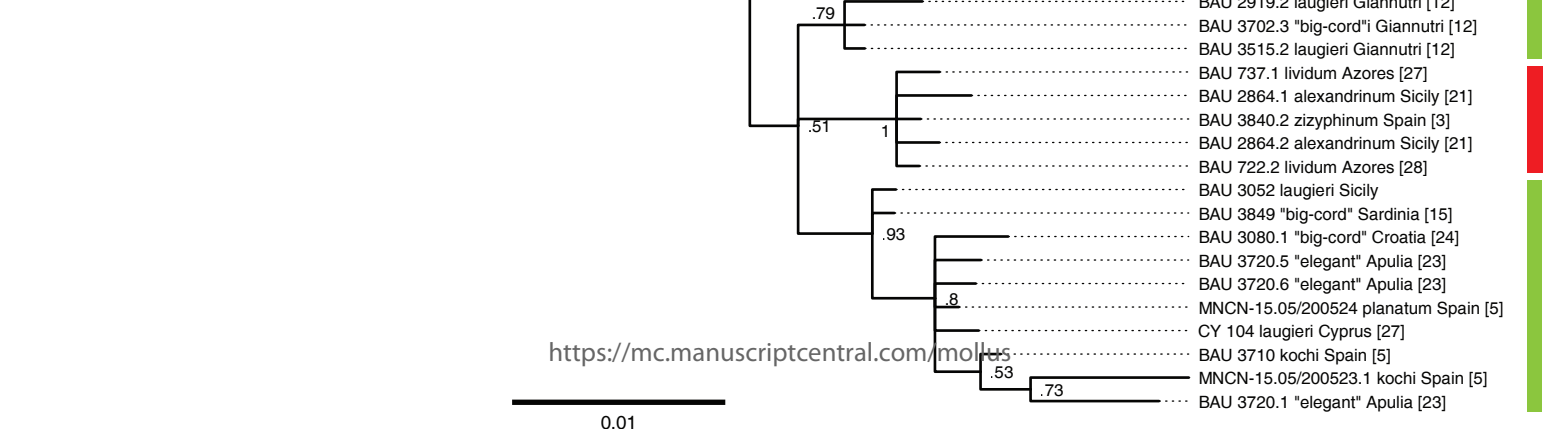


Figure S6-5. Maximum Likelihood phylogeny based on the *its2* alignment. Final species highlighted by colour. Yellow = *C. conulus*; Cyan = *C. granulatum*; Red = *C. zizyphinum*; Green = *C. laugieri*.

Figure S6-6. Bayesian Inference phylogeny based on the *its2* alignment. Final species highlighted by colour. Yellow = *C. conulus*; Cyan = *C. granulatum*; Red = *C. zizyphinum*; Green = *C. laugieri*.

