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# Molecular phylogeny of European Runcinida (Gastropoda, Heterobranchia): the discover of an unexpected pool of complex species, with special reference to the case of *Runcina coronata*

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Runcinida is a small heterobranch order of sea slugs with 61 known species distributed worldwide across temperate and tropical latitudes with two established families (Ilbiidae and Runcinidae). Little is known about the phylogenetic relationships within Runcinida. Here, we present the first molecular phylogeny of the order with an emphasis on European species and we discuss the taxonomic status of the type species *Runcina coronata*. Molecular phylogenetics based on the mitochondrial genes cytochrome *c* oxidase subunit I and 16S rRNA and nuclear gene histone *H3* is used to investigate relationships between species. Detailed morpho-anatomical worked was additionally employed to study *Runcina coronata*. Our results suggest the monophyly of Runcinida and showed that *Runcina coronata* is a complex of four species, namely: *R. coronata* proper, *R. aurata* and two new species here formally described (***R. caletensis*** **sp. nov.** and ***R. tingensis*** **sp. nov.**).

ADDITIONAL KEYWORDS: biodiversity – cryptic species – Ilbiidae – Runcinidae – sea slugs – species delimitation.

## INTRODUCTION

The order Runcinida Burn, 1963 includes two recognized families, namely Ilbiidae Burn, 1963 and Runcinidae H.Adams & A.Adams, 1854 (Bouchet *et al.*, 2017), AQ6 with 61 valid species distributed in the Atlantic and Mediterranean Sea (46 species) and the Indian and Pacific Ocean (15 species) (MolluscaBase, 2021). Members of this taxon are mostly characterized by the presence of an undivided dorsal shield (notum), an undivided foot, small external gills around, or to the right side of, the anus (sometimes absent), lack of parapodial lobes and four gizzard plates (Burn, 1963; Miller & Rudman, 1968; Thompson, 1976; Burn & Thompson, 1998).

The family Ilbiidae is composed of three genera: *Fofinha* Moro & Ortea, 2015, *Ilbia* Burn, 1963 and

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*Pseudoilbia* Miller & Rudman, 1968. Members of this family are characterized by the absence of shell, gills (*Ilbia* and *Pseudoilbia*) and/or gizzard plates (*Pseudoilbia* and *Fofinha*) and presence of a pedal furrow (*Ilbia* and *Pseudoilbia*).

The family Runcinidae includes eight genera: *Edmundsina* Ortea, 2013, *Ildica* Bergh, 1889, *Karukerina* Ortea, 2013, *Lapinura* Marcus & Marcus, 1970, *Metaruncina* Baba, 1967, *Runcina* Forbes in Forbes & Hanley, 1853, *Runcinella* Odhner, 1924 and *Runcinida* Burn, 1963. Members of this family are characterized by having a radula with a bilobed rachidian tooth, presence of gills (with up to four pinnulae) and four gizzard plates. The shell can be present or absent.

The systematics of Runcinida is confusing due to the lack of synapomorphic traits uniting all members in each family. Earlier referred by Odhner (1939) as Peltacea and subsequently named Runcinacea by Burn, 1963, the group was considered a suborder – the most 'derived' – within Cephalaspidea only sharing the presence of an external seminal groove (Odhner, 1939; Burn, 1963; Schmekel, 1985). Colosi (1915) first challenged the position of runcinids within Cephalaspidea and later Odhner (Odhner, 1968) suggested that Runcinacea should be ranked as an order. Recently, Bouchet *et al.* (2017: 330, paragraph e) in their revised *Nomenclator and typification of gastropod and monoplacophoran families* suggested the ending-ida for orders and proposed the name Runcinida to replace Runcinacea.

Over the years, many authors have continued to consider runcinids as part of Cephalaspidea (Marcus & Marcus, 1970; Kress, 1977; Schmekel, 1985; Thompson & Brodie, 1988; Vaught, 1989; Gosliner, 1990, 1991; Cervera *et al.*, 1991; Mikkelsen, 1993, 1996; Millard, 1997; Schmekel & Cappellato, 2001, 2002), while several phylogenetic studies on Heterobranchia based on morphological (Dayrat & Tillier, 2002; Wägele & Klussmann-Kolb, 2005) and molecular data (Dayrat *et al.*, 2001; Grande *et al.*, 2004a, b; Vonnemann *et al.*, 2005) questioned the inclusion of Runcinida in the Cephalaspidea. Malaquias *et al.* (2009), based on molecular phylogenetics, produced the first sound evidence for the inclusion of runcinids in their own order as early proposed by Odhner (in Franc, 1968). This was later supported by the molecular phylogenetic studies by Jörger *et al.* (2010), Wägele *et al.* (2014) and Oskars *et al.* (2015).

Most known runcinids species are concentrated in European waters (Atlantic Ocean, including the Mediterranean Sea) and belong to the genera *Pseudoilbia*, *Runcina* and *Runcinella*. Among these genera, *Runcina* is the most species-rich (31 species). However, the definition of its type species, *Runcina coronata* (Quatrefages, 1844), is problematic and remains a matter of debate. The species *R. coronata* is reported from England southwards to the Mediterranean coast of France (Quatrefages, 1844; Alder & Hancock, 1846; Vayssière, 1883; Schmekel & Cappellato, 2002; Cervera *et al.*, 2004; Ballesteros *et al.*, 2016). It is defined by having an almost elliptical body with two small lobes on the front of the head and a rounded posterior end of the body. The notum is brown in colour with lighter edges and with characteristic small, dull, white spots forming a semicircle on the head zone behind the eyes and at the posterior end of the notum. Three small and slightly pinnate gills are situated on the right side of the anus (Quatrefages, 1844; Alder & Hancock, 1846; Forbes & Hanley, 1851).

*Runcina coronata* (as *Pelta coronata*) was described from Brehat, Bretagne (Atlantic coastline of France). Two years later, Alder & Hancock (1846) found specimens at Torbay (southern England) similar to those described by Quatrefages and also to *Limapontia nigra* Johnston, 1835, but differing from the former by the presence of gills. Alder & Hancock (1846) believed that the lack of gills in *R. coronata* was an erroneous observation by Quatrefages but, nevertheless, they did not designate any specific name for these specimens. Based on additional specimens also collected at Torbay, Forbes (1853) described the species *Runcina hancocki*, which mainly differed from *R. coronata* by the presence of external gills. Vayssière (1883), in his 'Monographie du *Pelta*', considered *Runcina* a synonym of *Pelta*, but in 1951, the genus name *Pelta* Quatrefages, 1844 was suppressed and only the name *Runcina* Forbes (in Forbes & Hanley, 1853) was considered valid (see Lemche, 1967: opinion n. 811).

*Runcina coronata* was first reported from the Mediterranean Sea (Marseille, France) by Vayssière (1883). Pruvot-Fol (1954) suggested that the Mediterranean species *R. calaritana* Colosi, 1915 was conspecific with *R. coronata* and Burn (1963), after comparing the original description of *R. coronata* and the specimens studied by Vayssière, pointed out differences in the shape of the body, coloration and number of crests in the gizzard plates, suggesting these specimens to belong to *R. calaritana*. The species *Runcina aurata* García *et al.*, 1986 (type locality: Club La Hacienda, Bay of Algeciras, Strait of Gibraltar) was synonymized with *R. coronata* by Cervera *et al.* (1991) after a detailed comparison of specimens of *Runcina aurata* with the original description of *R. coronata*.

This work provides the first approach to the study of the diversity and systematics of the European species of runcinids based on a molecular phylogenetic framework. The taxonomic status of the type species *Runcina coronata* (Quatrefages, 1844) is investigated combining molecular phylogenetics and morpho-anatomical characters.

## MATERIAL AND METHODS

### Taxon sampling

Samples were obtained from fieldwork conducted by the authors and colleagues, and through the study of museum collections. Voucher specimens are held in the collections of the Zoologische Staatssammlung München, ZSM (Munich, Germany), Museum Victoria, MV (Melbourne, Australia), Museu de Zoologia da Universidade de São Paulo, MZUSP (São Paulo, Brazil), University Museum of Bergen, ZMBN (Bergen, Norway) and Museo Nacional de Ciencias Naturales (Madrid, Spain). Seventy-seven specimens of Runcinida were included for molecular analyses, representing five genera: *Ilbia* (one specimen), *Lapinura* (two specimens), *Metaruncina* (one specimen), *Runcina* (71 specimens) and *Runcinida* (one specimen). The remaining two specimens were referred as 'runcinid' sp.. All the species used in this study are listed in Table 1 and were identified by comparison with primary literature. The aplysiid *Aplysia dactylomela* Rang, 1828 and the acteonoid *Micromelo undatus* (Bruguière, 1792) were used as outgroup taxa. In total, 189 novel sequences were generated during the present work and 91 were obtained from GenBank (Table 1).

For the study of the *Runcina coronata* species-complex, we included specimens from Swanage in southern England (*c.* 210 km from the type locality of *R. coronata*, which is Brehat in France), Cádiz in Andalusia, south-western Spain and Tangier in north-western Morocco.

### DNA extraction, amplification and sequencing

Tissue samples were taken from the foot and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) and 16S rRNA (16S) and nuclear histone H3 (*H3*) genes were amplified by polymerase chain reaction (PCR) using the universal primers: LCO1490 and HCO2198 (Folmer *et al.*, 1994 for *COI*); 16S ar-L and 16br-H (Palumbi *et al.*, 1991 for 16S); and H3aF and H3aR (Colgan *et al.*, 1998 for *H3*). Polymerase chain reactions were conducted in a 25 µL reaction volume containing 1 µL of both forward and reverse primers (10 µmol/L), 2.5 µL of dNTP (2 mmol/L), a gene-dependent amount of magnesium chloride (25 mmol/L), 0.25 µL of Qiagen DNA polymerase (5 units/µL), 5 µL of 'Q-solution' (5×), 2.5 µL of Qiagen buffer (10×) (Qiagen Taq PCR Core Kit) and 2 µL of genomic DNA. Amplification of *COI* was performed with an initial denaturation for 5 min at 94 °C, followed by 35–36 cycles of 1 min at 94 °C, 30 s at 45 °C (annealing temperature) and 1 min at 72 °C, with a final extension of 10 min at 72 °C. The 16S amplification began with an initial denaturation for 5 min at 94 °C, followed by 35–36 cycles of 1 min at 94 °C, 30 s at 42 and 49 °C (annealing temperatures) and 1 min at 72 °C, with a final extension of 10 min at 72 °C. Amplification of *H3* was performed with an initial denaturation for 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 30 s at 52 °C (annealing temperature) and 1 min at 72 °C, with a final extension of 10 min at 72 °C. Successful PCR products were sent to Macrogen, Inc for purification and sequencing on a 3730XL DNA sequencer (Applied Biosystems).

### Phylogenetic analyses

Sequences were edited in GENEIOUS v.10.2.3 (Kearse *et al.*, 2012) and aligned using MAFFT (Katoh *et al.*, 2009) implemented in GENEIOUS v.10.2.3 with default settings. Sequences from the protein-coding genes *COI* and *H3* were translated into amino acids to check for stop-codons. Hypervariable regions of the 16S alignment where homology could not be confidently established were removed using GBlocks under relaxed settings (Talavera & Castresana, 2007). Nevertheless, analyses including and excluding these regions provided similar results. Therefore, final analyses were performed including all nucleotides. Sequences of the *COI*, 16S and *H3* genes were trimmed to 658, 489 and 328 nucleotides, respectively. Single gene and concatenated (*H3* + *COI*+ 16S) analyses were performed. Saturation for the first-, second- and third-codon positions of the *COI* and *H3* genes was calculated in MEGA X (Kumar *et al.*, 2018).

The best-fit evolutionary model for each gene was determined in jModelTest v.2.1.6 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012), under the Akaike information criterion (AIC) (Akaike, 1974). The GTR+G+I model was selected for the *COI* and 16S genes, and K80+G for the *H3* gene. Bayesian inference (BI) analyses were performed in MrBayes v.3.2.1 (Ronquist & Huelsenbeck, 2003) with random starting trees and two parallel runs of $10^7$ generations. The models implemented were those estimated with jModelTest v.2.1.6. The combined dataset was partitioned among genes and the 'unlink' command was used to allow all parameters to vary independently within each partition. Convergence was checked in TRACER v.1.7.1 (Rambaut *et al.*, 2018) with a burn-in of 25%. Nodes with a posterior probability (PP) ≥ 0.95 (Alfaro *et al.*, 2003) were considered well supported and discussed. Maximum likelihood (ML) analysis was executed using RAxML v.8 (Stamatakis, 2014) and node support was assessed with nonparametric bootstrapping (BS) with 5000 replicates. Nodes with bootstrap values (BS) ≥ 70 (Hillis & Bull, 1993) were considered significant and were discussed. Both BI AQ5

**Table 1.** List of specimens used for phylogenetic analysis. (*) New sequences generated for this study. Museum abbreviations: Museum Victoria collections (NMVF), University Museum of Bergen (ZMBN), Zoologische Staatssammlung München, Germany (ZSM), Museo Nacional de Ciencias Naturales (MNCN), Museu de Zoologia da Universidade de São Paulo (MZUSP) and The Natural History Museum, London, United Kingdom (NHMUK)

| Species Preliminary Ids | Revised Ids | Locality | Voucher no. | 16S | *COI* | *H3* |
|---|---|---|---|---|---|---|
| *Ilbia ilbi* Burn, 1963 | | Hamers Haven, Australia | NMVF234189 | MK323019 | - | MK322992 |
| *Lapinura divae* (Marcus & Marcus, 1963) | | Bermuda (GB) | ZMBN 82997 | KJ022825 | KF992195 | KJ022893 |
| *Lapinura divae* | | Bermuda | ZMBN 82997 | MW144769* | - | MW182586* |
| *Metaruncina nhatrangiensis* Chernyshev, 2005 | | Vietman (GB) | - | KP313664 | KP313668 | - |
| *Metaruncina setoensis* (Baba, 1954) | | Okinawa, Japan | ZMBN 88231 | MW144770* | MW172338* | - |
| *Pseudoilbia avellana* (Schmekel & Cappellato, 2001) | *R. avellana* | Palamos, Spain | MNCN 15.05/90415 | - | MW172346* | MW182594* |
| *Runcina adriatica* T. Thompson, 1980 | | Azores Islands, Portugal | MNCN 15.05/91518 | MW144774* | MW172339* | MW182587* |
| *Runcina adriatica* | | Azores Islands, Portugal | ZMBN81756 | - | MW172341* | MW182589* |
| *Runcina adriatica* | | Azores Islands, Portugal | ZMBN81756 | MW144773* | MW172344* | MW182591* |
| *Runcina adriatica* | | Iz Island, Croatia | MNCN 15.05/88099 | MK323015 | MK322986 | MK323036 |
| *Runcina adriatica* | | Ugljan Island, Croatia | MNCN 15.05/88100 | MK322996 | MK322967 | MK323022 |
| *Runcina adriatica* | | Zut Island, Croatia | MNCN 15.05/88101 | MK322997 | MK322968 | - |
| *Runcina adriatica* | | Otranto, Italy | MNCN 15.05/88102 | MK322993 | MK322966 | - |
| *Runcina adriatica* | | Palamós, Spain | MNCN 15.05/90416 | - | MW172340* | MW182588* |
| *Runcina adriatica* | | Cadaqués, Spain | MNCN 15.05/88103 | MK322994 | MK322987 | MK323020 |
| *Runcina adriatica* | | Cadaqués, Spain | MNCN 15.05/90417 | MW144771* | MW172342* | - |
| *Runcina adriatica* | | Cadaqués, Spain | MNCN 15.05/90418 | MW144772* | MW172343* | MW182590* |
| *Runcina adriatica* | | Otranto, Italy | MNCN 15.05/90666 | MW144775* | MW172345* | MW182592* |
| *Runcina* cf. *adriatica* | | Lecce, Italy | Pending number | MW144778* | MW172348* | MW182595* |
| *Runcina* cf. *adriatica* | | Lecce, Italy | MNCN 15.05/91515 | MW144802* | MW172377* | MW182621* |
| *Runcina* cf. *adriatica* | | La Strea, Italy | MNCN 15.05/91516 | MW144817* | MW172391* | MW182634* |
| *Runcina* cf. *adriatica* | | Ugljan Island, Croatia | MNCN 15.05/91501 | MW144810* | MW172384* | MW182627* |
| *Runcina* cf. *adriatica* | | Ugljan Island, Croatia | MNCN 15.05/91502 | MW144811* | MW172385* | MW182628* |
| *Runcina* cf. *adriatica* | | Ugljan Island, Croatia | MNCN 15.05/91503 | MW144812* | MW172386* | MW182629* |
| *Runcina africana* Pruvot-Fol, 1953 | | Tenerife, Canary Islands, Spain | NHMUK 20030791/1 | KJ022780 | DQ974680 | KJ022942 |
| *Runcina africana* | | Port Lligant, Spain | MNCN 15.05/90651 | MW144776* | - | MW182593* |

**Table 1.** Continued

| Species Preliminary Ids | Revised Ids | Locality | Voucher no. | 16S | *COI* | *H3* |
|---|---|---|---|---|---|---|
| *Runcina* cf. *bahiensis* Cervera *et al.*, 1991 | *R. hornae* | Cadaqués, Spain | MNCN 15.05/88104 | - | MK322988 | MK323005 |
| *Runcina brenkoae* T. Thompson, 1980 | | Split, Croatia | MNCN 15.05/88086 | MK322998 | MK322969 | MK323023 |
| *Runcina brenkoae* | | Nin, Croatia | MNCN 15.05/88089 | MK323017 | MK322972 | MK323038 |
| *Runcina brenkoae* | | Nin, Croatia | MNCN 15.05/88088 | MK323018 | MK322971 | MK323039 |
| *Runcina ferruginea* | | Lecce, Italy | MNCN 15.05/91509 | - | MW172399* | - |
| *Runcina ferruginea* | | Lecce, Italy | MNCN 15.05/91510 | - | MW172400* | MW182641* |
| *Runcina ferruginea* | | Lecce, Italy | MNCN 15.05/91511 | - | MW172401* | - |
| *Runcina ferruginea* | | Lecce, Italy | MNCN 15.05/91512 | - | MW172402* | - |
| *Runcina ferruginea* | | Lecce, Italy | MNCN 15.05/91513 | MW144787* | - | MW182644* |
| *Runcina ferruginea* | | Iz Island, Croatia | MNCN 15.05/90667 | MW144788* | MW172361* | MW182607* |
| *Runcina ferruginea* | | Ugljan Island, Croatia | MNCN 15.05/90668 | MW144786* | MW172359* | MW182605* |
| *Runcina ferruginea* | | Split, Croatia | MNCN 15.05/90669 | - | MW172360* | MW182606* |
| *Runcina ferruginea* | | Banyuls-sur-Mer, France | ZSMMOL 20142074 | - | MW172374* | MW182618* |
| *Runcina hansbechi* Schmekel & Cappellato, 2001 | *R. brenkoae* | Banyuls-sur-Mer, France | ZSMMOL20142071 | MW144789* | MW172362* | MW182608* |
| *Runcina* cf. *hansbechi* | *R. avellana* | Mataró, Spain | MNCN 15.05/88108 | MK323028 | MK322973 | MK32300 |
| *Runcina hornae* Schmekel & Cappellato, 2002 | | Palamós, Spain | MNCN 15.05/88110 | MK323033 | MK322978 | - |
| *Runcina* cf. *hornae* | | Iz Island,Croatia | MNCN 15.05/91504 | MW144802* | - | MW182622* |
| *Runcina lusitanica* Araujo *et al.*, 2019 | | Faro, Portugal | MNCN 15.05/88091 | MK323024 | MK322979 | MK322999 |
| *Runcina lusitanica* | | Faro, Portugal | MNCN 15.05/200065 | - | MK322981 | MK323002 |
| *Runcina lusitanica* | | Faro, Portugal | MNCN 15.05/88093 | - | MK322965 | MK323004 |
| *Runcina brenkoae* | | Roses, Spain | MNCN 15.05/88087 | MK323001 | MK322970 | MK323026 |
| *Runcina brenkoae* | | Roses, Spain | MNCN 15.05/88090 | MK323003 | MK322964 | - |
| *Runcina brenkoae* | | Roses, Spain | MNCN 15.05/90422 | - | MW172349* | MW182596* |
| *Runcina brenkoae* | | Banyuls-sur-Mer, France | ZSMMOL20142072 | MW144777* | MW172347* | - |
| *Runcina coronata* (Quatrefages, 1844) | | Swanage, England | MNCN 15.05/88105<br>MNCN/ADN 118951 | MK323031 | MK322976 | MK323010 |
| *Runcina coronata* | | Swanage, England | MNCN 15.05/90423 | - | MW172356* | MW182602* |
| *Runcina coronata* | *R. aurata* | Cádiz, Spain | MNCN 15.05/88106 | MN057638 | - | MN057641 |
| *Runcina coronata* | *R. aurata* | Cádiz, Spain | MNCN 15.05/88107 | MN057639 | MN057640 | MN057642 |

**Table 1.** Continued

| Species Preliminary Ids | Revised Ids | Locality | Voucher no. | 16S | *COI* | *H3* |
|---|---|---|---|---|---|---|
| *Runcina coronata* | *R. aurata* | Cádiz, Spain | MNCN 15.05/91500 | MW144785* | MW172358* | MW182604* |
| *Runcina coronata* | *R. aurata* | Cádiz, Spain | MNCN/ADN 118948 | MW144780* | MW172351* | MW182597* |
| *Runcina coronata* | *R. aurata* | Cádiz, Spain | MNCN/ADN 118950 | - | MW172353* | MW182599* |
| *Runcina coronata* | *R. caletensis* sp. nov | Cádiz, Spain | MNCN/ADN 118949 | MW144781* | MW172352* | MW182598* |
| *Runcina coronata* | *R. caletensis* sp. nov | Cádiz, Spain | MNCN 15.05/200113 | MW144783* | MW172355* | MW182601* |
| *Runcina coronata* | *R. tingensis* sp. nov | Tangier, Morocco | MNCN 15.05/91514 | MW144782* | MW172354* | MW182600* |
| *Runcina coronata* | *R. tingensis* sp. nov | Tangier, Morocco | MNCN 15.05/200114 | MW144784* | MW172357* | MW182603* |
| *Runcina ferruginea* Kress, 1977 |  | Newlyn, Cornwall, England | MNCN 15.05/88109 | MK323032 | MK322977 | MK323011 |
| *Runcina ferruginea* |  | Roses, Spain | MNCN 15.05/91517 | MW144779* | MW172350* | - |
| *Runcina ferruginea* |  | Lecce, Italy | MNCN 15.05/91508 | - | MW172397* | MW182639* |
| *Runcina lusitanica* |  | Faro, Portugal | MNCN 15.05/88092 | MK323035 | MK322980 | MK323014 |
| *Runcina lusitanica* |  | Faro, Portugal | MNCN 15.05/88094 | MK323027 | MK322989 | - |
| *Runcina lusitanica* |  | Faro, Portugal | MNCN 15.05/90424 | MW144790* | MW172363* | MW182609* |
| *Runcina lusitanica* |  | Faro, Portugal | MNCN 15.05/90425 | MW144791* | MW172364* | MW182610* |
| *Runcina marcosi* Araujo *et al.*, 2019 |  | Roses, Spain | MNCN 15.05/88098 MNCN/ADN 118952 | MK323025 | MK322982 | MK323000 |
| *Runcina marcosi* |  | Mataro, Spain | MNCN 15.05/88095 | MK323037 | MK322983 | MK323016 |
| *Runcina marcosi* |  | Roses, Spain | MNCN 15.05/88097 | - | MK322990 | MK323012 |
| *Runcina marcosi* |  | Roses, Spain | MNCN 15.05/88096 | MK323034 | MK322984 | MK323013 |
| *Runcina marcosi* |  | Cadiz, Spain | MNCN 15.05/200066 | - | MK322985 | MK323006 |
| *Runcina marcosi* |  | Banyuls-sur-Mer, France | ZSM MOL 201442089 | MK323021 | MK322991 | MK322995 |
| *Runcina marcosi* |  | Roses, Spain | MNCN 15.05/90426 | - | MW172393* | - |
| *Runcina marcosi* |  | Roses, Spain | MNCN 15.05/90427 | MW144819* | MW172394* | MW182636* |
| *Runcina ornata* (Quatrefages, 1844) |  | Azores Islands, Portugal | ZMBN87929 | MW144796* | MW172368* | - |
| *Runcina ornata* |  | Azores Islands, Portugal | ZMBN87949 | MW144795* | MW172367* | - |
| *Runcina* sp. 3 | *R. hornae* | Mataro, Spain | MNCN 15.05/90654 | MW144818* | MW172392* | MW182635* |
| *Runcina* sp. 4 | *R. hornae* | Palamos, Spain | MNCN/ADN 118954 | - | MW172395* | MW182637* |
| *Runcina* sp. 8 | *R. hornae* | Roses, Spain | MNCN 15.05/90655 | MW144801* | MW172376* | MW182620* |
| *Runcina* sp. 10 | *R. hornae* | Mataro, Spain | MNCN 15.05/90656 | MW144822* | MW172404* | MW182643* |
| *Runcina* sp. 11 | *R. hornae* | Mataro, Spain | MNCN 15.05/90657 | MW144821* | MW172398* | MW182640* |

**Table 1.** Continued

| Species Preliminary Ids | Revised Ids | Locality | Voucher no. | 16S | *COI* | *H3* |
|---|---|---|---|---|---|---|
| *Runcina* sp. 13 | *R. hornae* | Mataro, Spain | MNCN 15.05/90658 | - | MW172403* | MW182642* |
| *Runcina* sp. 15 | *R. hornae* | Cadaqués, Spain | MNCN 15.05/90659 | - | MW172369* | MW182614* |
| *Runcina* sp. 17 | *R. adriatica* | Cadaqués, Spain | MNCN 15.05/90419 | MW144798* | MW172371* | MW182616* |
| *Runcina* sp. 18 | *R. hornae* | Cadaqués, Spain | MNCN 15.05/90660 | MW144797* | MW172370* | MW182615* |
| *Runcina* sp. 19 | *R. avellana* | Roses, Spain | MNCN/ADN 118953 | MW144805* | MW172379* | - |
| *Runcina* sp. 19 | *R. avellana* | Roses, Spain | MNCN 15.05/90652 | MW144806* | MW172380* | MW182624* |
| *Runcina* sp. 20 | *R. hornae* | Roses, Spain | MNCN 15.05/90661 | MW144813* | MW172387* | MW182630* |
| *Runcina* sp. 20 | *R. hornae* | Roses, Spain | MNCN 15.05/90662 | MW144814* | MW172388* | MW182631* |
| *Runcina* sp. 20 | *R. hornae* | Roses, Spain | MNCN 15.05/90663 | MW144815* | MW172389* | MW182632* |
| *Runcina* sp. 21 | | Zut Island, Croatia | MNCN 15.05/91505 | MW144816* | MW172390* | MW182633* |
| *Runcina* sp. 22 | | Zut Island, Croatia | MNCN 15.05/91506 | MW144807* | MW172381* | - |
| *Runcina* sp. 22 | | Zut Island, Croatia | MNCN 15.05/91507 | MW144809* | MW172383* | MW182626* |
| *Runcina* sp. 24 | *R. hornae* | Roses, Spain | MNCN 15.05/90664 | MW144820* | MW172396* | MW182638* |
| *Runcina* sp. 26 | *R. avellana* | Roses, Spain | MNCN 15.05/90653 | MW144808* | MW172382* | MW182625* |
| *Runcina* sp. 31 | *R. adriatica* | Es Caials (SP) | MNCN 15.05/90420 | MW144792* | MW172365* | MW182611* |
| *Runcina* sp. 33 | *R. hornae* | Port Lligant (SP) | MNCN 15.05/90665 | MW144793* | - | MW182612* |
| *Runcina* sp. 34 | *R. adriatica* | Port Lligant (SP) | MNCN 15.05/90421 | MW144794* | MW172366* | MW182613* |
| *Runcinida marisae* Chernyshev, 1998 | | Vostok Bay, Russia | - | - | KP313667 | - |
| *Runcinida valentinae* Chernyshev, 2006 | | Iturup Island, Russia | - | - | KP313666 | - |
| *Runcinida* sp. 1 | | Cap Ferret, France | MNCN 15.05/90670 | MW144800* | MW172375* | MW182619* |
| Runcinid sp. 1 | | Alagoas, Brazil | MZUSP103407 | MW144799* | MW172373* | - |
| *Lapinura* sp. 1 | | Maui, Hawaii | MNCN 15.05/90671 | MW144804* | MW172378* | MW182623* |
| Runcinid sp. 2 | | Quirimbas, Mozambique | ZMBN105119 | - | MW172372* | MW182617* |
| *Aplysia dactylomela* Rang, 1828 | | Cape Verde (GB) | NHMUK 20030795/20030796 | KJ022921 | KF992168 | KJ022798 |
| *Micromelo undatus* (Bruguière, 1792) | | Tenerife, Canary Island (GB) | NHMUK 20030800 | KJ022944 | DQ974653 | KJ022778 |

and ML trees were visualized in FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Minimum and maximum pairwise uncorrected *p*-distances of *COI* were calculated in MEGA X (Kumar *et al.*, 2018).

### SPECIES DELIMITATION

For species delimitation analyses, the Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al.*, 2012) and Bayesian Poisson Tree Processes (bPTP) (Zhang *et al.*, 2013) methods were used. For the ABGD analyses we used the fast-evolving *COI* gene using the ingroup sequences obtained in this study and those available in GenBank. We used the default setting ($P_{min} = 0.001$, $P_{max} = 0.1$, Steps = 10, $X = 1.2$, Nb bins = 20) under the three models of evolution, namely Jukes–Cantor (JC69), Kimura (K80) and Simple Distance. bPTP analyses were run with default parameters using the *COI* trees at the webserver (https://species.h-its.org/ptp/) (Zhang *et al.*, 2013).

### MORPHOLOGY

The external morphology of the specimens used in our molecular phylogenies were, in most cases, studied based on photographs of live specimens (175 images) and from living animals collected during sampling activities. In the latter case, examination of the shape and coloration was carried out under a stereomicroscope. In order to identify all specimens, we compared these observations with original descriptions of species and specialized literature.

For the internal morphology, the animals were dissected either dorsally or ventrally, and the buccal bulbs and gizzards were extracted and dissolved in a solution of 10% sodium hydroxide to expose and clean them. The radulae and gizzard plates were then immersed in water, dried and mounted for scanning electron microscopy (SEM) with a Nova NanoSEM 450 available at the University of Cadiz (Cadiz, Spain). The reproductive systems were dissected out of the animals and examined and drawn using a stereomicroscope equipped with a *camera lucida*. All specimens previously identified as *R. coronata* used for morphological comparison are listed in the 'Examined material' in the Systematic description.

## RESULTS

### PHYLOGENETIC ANALYSES

The combined dataset yielded a sequence alignment of 1475 positions. We obtained 189 new sequences, 64 for *H3*, 68 for *COI* and 57 for 16S (Table 1). No saturation was observed in the *COI* and *H3* genes, not even in the third-codon positions (not shown). The combined tree (*H3* + *COI* + 16S) provided better resolution than *H3*, *COI* or 16S separately (see Supporting Information, Figs S1–S3). The results of ML and BI trees rendered similar topologies, but bootstrap values were lower than posterior probabilities in some clades (Fig. 1).

Both ML and BI supported the monophyly of the order Runcinida (PP = 1; BS = 99), with the species *Ilbia ilbi* Burn, 1963 (family Ilbiidae) sister to Clade A (family Runcinidae). Clade A (PP = 0.96; BS = -) is divided into two main sister subclades, here called Clade B (PP = 0.98, BS = - [no value was recovered]) and Clade C (PP = 1, BS = 78).

Clade B was subdivided into three well-supported clades: the first, Clade D, including only the specimen labelled as runcinid sp. 2 from Mozambique; the second, Clade E (PP = 1; BS = 96), containing *Metaruncina setoensis* (Baba, 1967) and *Metaruncina nhatrangensis* Chernyshev, 2005; and the third, Clade F (PP = 1; BS = 99), with most of *Runcina* specimens and *Pseudoilbia avellana* (Schmekel & Cappellato, 2001). Within Clade F, *Runcina ornata* appears as sister to the remaining *Runcina* specimens. Within the latter, three clades with maximum support correspond to well-established species, such as *R. lusitanica* Araujo *et al.*, 2019, *R. marcosi* Araujo *et al.*, 2019 and the type species, *R. coronata*. However, specimens attributed to *Runcina adriatica* Thompson, 1980, *Runcina ferruginea* Kress, 1977, *Runcina hansbechi* Schmekel & Cappellato, 2001 and *Runcina africana* Pruvot-Fol, 1953 branched off in different clades. In addition, specimens early identified as *Runcina* cf. *bahensis* Cervera *et al.*, 1991 and *Runcina hornae* Schmekel & Cappellato, 2002 clustered together with several unidentified species from Spain (PP = 1; BS = 100).

Clade C (PP = 1; BS = 78) was subdivided into two clades: Clade G, containing specimens identified as *Runcina ferruginea* (PP = 1; BS = 100); and Clade H (PP = 1; BS = 80) with two subgroups, one including *Lapinura divae* (Marcus & Marcus, 1970), *Lapinura* sp. 1, specimens identified as *R. ferruginea* and runcinid sp. 1 (PP = 1; BS = 82), and the other with *Runcinida marisae* Chernyshev, 1998, *Runcinida valentinae* Chernyshev, 2006 and *Runcinida* sp. 1 (PP = 1; BS = 90).

### SPECIES DELIMITATION

The ABGD analyses identified 31 groups with all three models of evolution implemented (Fig. 1A). Within these groups and for all models, the specimens identified as *R. coronata* (type species of the genus *Runcina*) split in four distinct groups: RC1, RC2, RC3

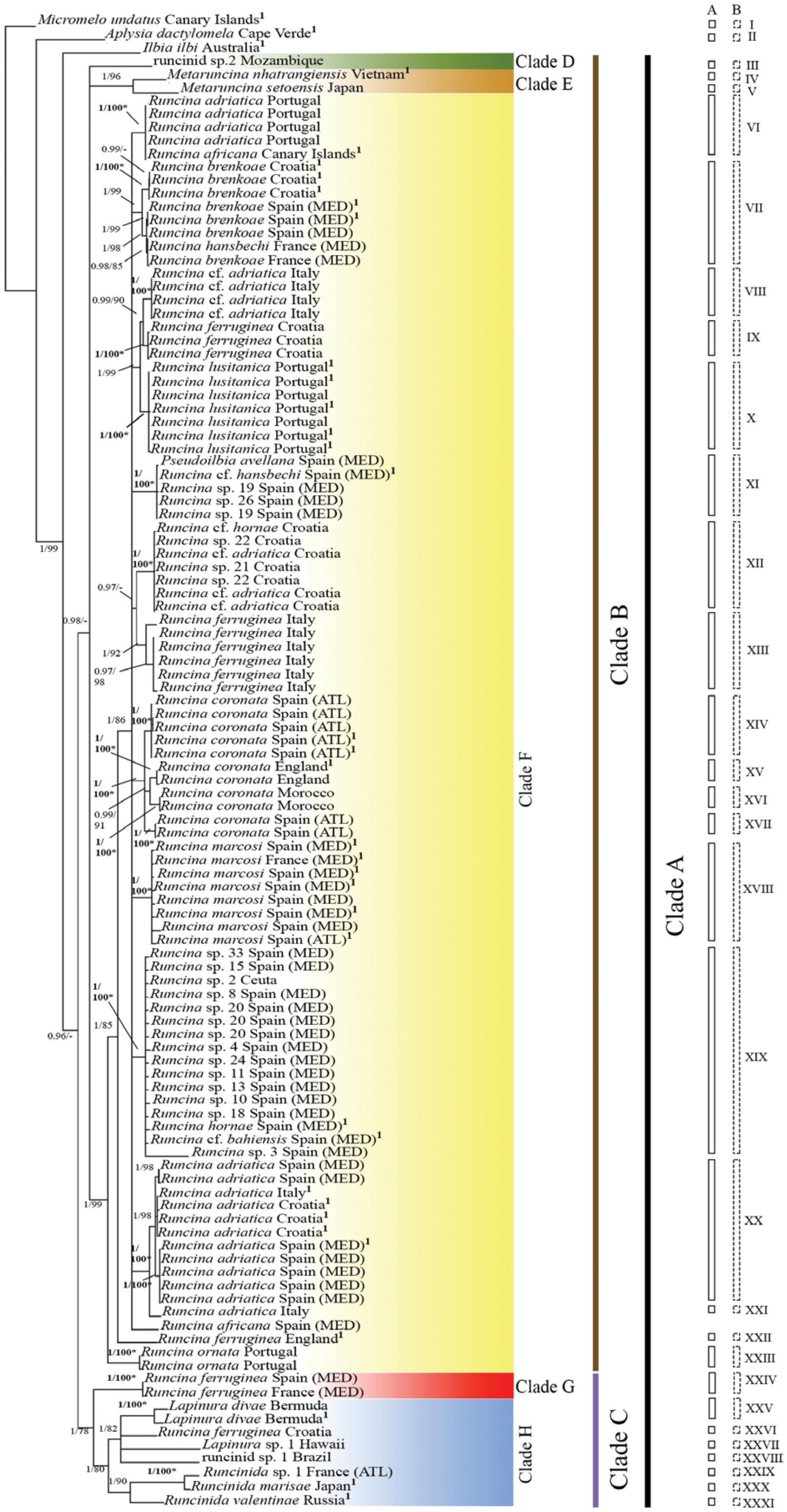

**Figure 1.** Phylogenetic hypothesis of the order Runcinida based on the combined genes *H3*, *COI*, and 16S inferred by Bayesian analysis. Numbers on the left of the slash are posterior probabilities and on the right bootstrap values derived from maximum likelihood analysis. A, ABGD results based on the *COI* dataset. B, bPTP results based on the *COI* dataset. Rectangles in Ilbia ilbi are missing since there is no *COI* sequence available. Abbreviations: ATL, Atlantic Ocean; MED, Mediterranean Sea. *, branches with maximum support. 1, refers to sequences from Genbank.

and RC4 (Fig. 2). The bPTP analysis showed the same result (Figs 1B, 2).

The minimum pairwise uncorrected *p*-distances for *COI* among groups of Runcinida recovered by ABGD are presented in Table 2, and ranged from 5.6% to 23.1%. Within the genus *Runcina*, distances ranged from 6% to 17.7% between species (Table 2, groups VI–XXIII; see Fig. 1). Regarding the specimens originally identified as *R. coronata*, the distances between the four groups (Fig. 2) were considerably higher compared with the distance between specimens within each group (Table 3). Groups RC1 (*Runcina aurata*) and RC4 (*Runcina caletensis* sp. nov.) contain specimens from the same locality (Cádiz, Spain) and the minimum distance between them was 7.8%. Comparing both groups with group RC2 (*Runcina coronata*), which contains specimens from Swanage, England, the minimum distances were 6.3% for RC1 and 8.8% for RC4. Finally, the minimum genetic distances when these three groups are compared with group RC3 (*Runcina tingensis* sp. nov.; specimens from Morocco) were 6.4% (RC1), 7.0% (RC2) and 9.9% (RC4) (Table 3).

Our morphological studies (see theme Systematic description below) and molecular results support four different species among the specimens originally identified as *Runcina coronata*. We present a redescription of *R. coronata* and *R. aurata*, and the description of the two new species.

## SYSTEMATIC DESCRIPTION

### FAMILY RUNCINIDAE H.ADAMS & A.ADAMS, 1854

### (FIGS 3A, B, 4A–C, 5A, 6A)

*Runcina* Forbes in Forbes & Hanley, 1851.
*Runcina coronata* (Quatrefages, 1844).

### *Synonymy*

*Pelta coronata* Quatrefages, 1844: 151, pl.3, fig. IV.
*Runcina hancocki* Forbes (*in* Forbes & Hanley), 1851: 611, pl. C.C.C, fig. 2.
*R. calaritana* Colosi, 1915: 1, figs 1–18.

*Type locality:* Bréhat, Bretagne, France (48º50'59"N; 2º59'47"W).

*Examined material:* MNCN 15.05/88105, Swanage (50º36'28"N; 1º56'45"W), southern England, coll. Ian F. Smith, Apr 2016, 3 mm living animal, found by brushing the bases of stones with encrusting pink coralline algae (dissected and sequenced). MNCN 15.05/90423, Swanage (50º36'28"N; 1º56'45"W), southern England, coll. Ian F. Smith, Apr 2016, 4 mm living animal, found by brushing the bases of stones

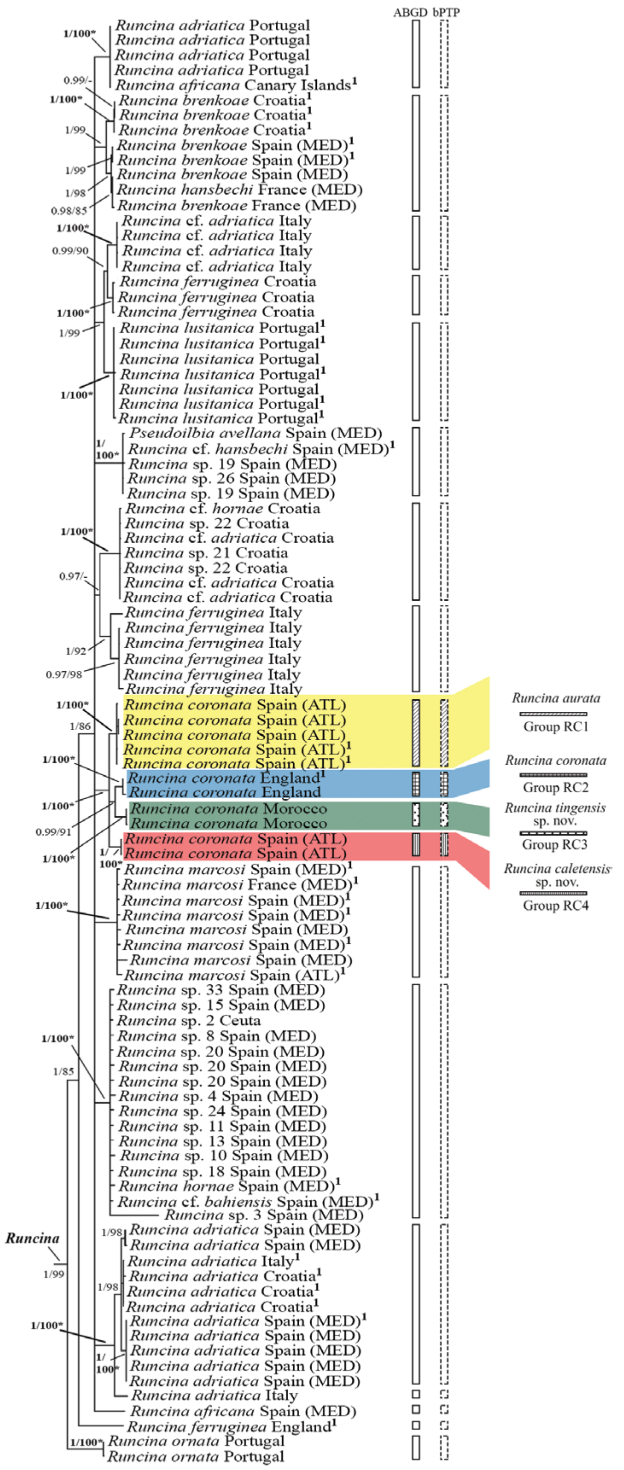

**Figure 2.** *Runcina coronata* species-complex. Detail of Clade F extracted from the analyses illustrated in Figure 1. Continuous rectangles, ABGD analysis based on the *COI* dataset. Dotted rectangles, bPTP analysis based on the *COI* dataset. *, branches with maximum support. 1, refers to sequences from Genbank.

**Table 2.** Uncorrected *p*-distances based on the *COI* gene within the order Runcinida. Roman numbers are based on species delimitation groups represented in Figure 1

| | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IV | 15.5 | | | | | | | | | | | | | |
| V | 18 | 15.8 | | | | | | | | | | | | |
| VI | 12.2–12.7 | 16.7–18.3 | 16.6–16.9 | | | | | | | | | | | |
| VII | 14.9–15.4 | 17.4–18.3 | 17.6–18.5 | 8.2–9 | | | | | | | | | | |
| VIII | 14.5–15.2 | 18.1–19.1 | 16.8–17.6 | 9.8–10.9 | 9.9–12.2 | | | | | | | | | |
| IX | 12.7–13.4 | 16.7–17.1 | 15.8–16.1 | 7.6–8.8 | 7.7–10.7 | 5.6–7 | | | | | | | | |
| X | 15.7–16.9 | 17.5–18.4 | 18–19.5 | 9.1–10.4 | 9.9–12.4 | 7.8–9.8 | 6.1–7.8 | | | | | | | |
| XI | 15.5–15.9 | 17.9–18.7 | 16.7–17.4 | 9.8–11 | 9.8–13 | 11.1–12.7 | 9–10.5 | 11.1–13 | | | | | | |
| XII | 15.9–16.4 | 17.1–17.5 | 16.7–17.5 | 10.6–11.4 | 10.2–12.2 | 11.2–12.6 | 9.4–10.2 | 10.2–12.5 | 10.3–11.3 | | | | | |
| XIII | 14–15 | 16.2–17.1 | 17.8–18.5 | 7.2–9.3 | 7.2–11.1 | 9.6–11.7 | 9–10.5 | 8.8–11.5 | 9.6–11.3 | 8.4–10.4 | | | | |
| XIV | 15.4–15.5 | 18.3–18.7 | 16.9–17 | 10.5–10.9 | 8.9–10.6 | 11.3–13.1 | 9.4–10.5 | 11.8–13.2 | 11.5–12.2 | 11.1–12 | 9.4–12 | | | |
| XV | 15.7 | 18.9–19.7 | 16.7–17.4 | 10.5–11.6 | 9.3–11.1 | 11.2–12.3 | 9.1–10.2 | 9.9–11 | 10.9–11.8 | 10.9–12.4 | 10.4–12.6 | 6.3–7.3 | | |
| XVI | 16 | 19–19.8 | 18.4 | 10.8–12.2 | 9.2–11.4 | 12.7–13.4 | 10.5–11.6 | 11.8–12.9 | 11–12.3 | 12.7–13.7 | 10.8–12.6 | 6.4–7.8 | 7–7.7 | |
| XVII | 17 | 19.7 | 17.8–17.9 | 11.7–12.3 | 10–12.3 | 13.1–13.7 | 9.5–10 | 12.3–13.4 | 13.4–14.5 | 12.8–13.7 | 12.6–13.7 | 7.8–8 | 8.8–9.5 | 9.9–10.2 |
| XVIII | 15.6–17.6 | 17.8–19.4 | 17.2–19.7 | 9–11.3 | 10.2–13.1 | 10.5–13.6 | 9.5–11.8 | 11.6–13.9 | 11.4–13.8 | 10.9–14.4 | 9.4–11.8 | 11.6–13.5 | 12.3–14.6 | 12–13.7 |
| XIX | 15.1–15.7 | 18.2–19 | 16.7–17.8 | 10.4–11.2 | 10.1–12.3 | 10.2–11.7 | 8.4–9.9 | 9.9–12.4 | 10.7–12.4 | 11.1–13.2 | 9.6–12.3 | 12.1–12.9 | 12.2–13.7 | 12.5–13.5 |
| XX | 16.9–18.7 | 17.7–19.2 | 19.4–20.3 | 11.5–13 | 11.6–13.6 | 13.3–15.3 | 11.3–13.1 | 11.3–14.4 | 12–14.9 | 11.5–13.7 | 11.4–13.9 | 12.8–14 | 12.8–14.5 | 12.3–13.8 |
| XXI | 17.9 | 19.4 | 18.4 | 12.8–13.6 | 14.4–14.8 | 14.4–14.8 | 13.6–13.9 | 14.6–15.3 | 14.1–14.9 | 12.9–13.3 | 11.8–13.7 | 13.9–14.1 | 14.1 | 13.4–13.6 |
| XXII | 15.4 | 16.8 | 16.3 | 9.6–10.4 | 10.1–12.1 | 10.7–11.1 | 9.6–10.4 | 12.2–13.9 | 11.6–12.5 | 12.4–13 | 9.8–11.1 | 12.6–13.3 | 12.3–13.1 | 12.7–13 |
| XXIII | 17.5–17.6 | 18.3–18.7 | 18.6–18.7 | 13.5–14.3 | 12.9–14.5 | 15.2–15.9 | 13.5–14.1 | 13.8–14.9 | 14.6–15.6 | 14.7–15.6 | 12.3–13.4 | 13.5–14.1 | 15.3–16.1 | 15.1–15.6 |
| XXIV | 18.3–18.5 | 18.8–19 | 20.8–21 | 14.4–15.2 | 16.7–18.2 | 18.7–19.3 | 16.6–17 | 18.2–19.5 | 16.6–18.1 | 15.5–16.3 | 16.4–17.2 | 16.1–16.6 | 17.2–18.1 | 17–18 |
| XXV | 16.9 | 20.5 | 19.5 | 16.7–17.3 | 16.7–18.8 | 18.3–18.8 | 15–15.9 | 18.1–18.8 | 15.6–16.7 | 17–17.5 | 16.2–17.7 | 16–16.6 | 17.2–17.8 | 16.9–17.8 |
| XXVI | 17.5 | 19.9 | 19.5 | 16.1–16.8 | 15.7–17.1 | 16.9–17.7 | 15.3–16.3 | 17.2–18.6 | 15.7–16.9 | 15.7–16.8 | 15–15.5 | 17.3–17.6 | 17.7–18.7 | 17.9–18 |
| XXVII | 16.9 | 18.3 | 20.1 | 16.6–16.9 | 17.9–18.8 | 18.5–19 | 16.7–17.9 | 17.4–18.6 | 17.5–18.1 | 17.2–17.6 | 16.7–17.9 | 17.6–17.8 | 18.5–19.2 | 18–18.2 |
| XXVIII | 21 | 21.4 | 20.5 | 17.8–19.1 | 20.1–21.1 | 20.7–21.5 | 19.6–19.9 | 20.1–21.1 | 20.1–21 | 21.3–21.5 | 19.6–20.5 | 19.2–19.3 | 20.6–21.3 | 20.8–21 |
| XXIX | 20.7 | 20.7 | 18.1 | 16.9–17.3 | 17.1–18.9 | 18.4–18.7 | 16.7–17 | 18.3–19.5 | 18.2–19.6 | 18.2–18.7 | 17.4–18.8 | 16.9–17.1 | 17.3–17.9 | 17.6–18.2 |
| XXX | 20.2 | 18.3 | 17.1 | 17.7–18.9 | 18.5–19 | 16.7–17.5 | 16.1–16.6 | 17.5–18.9 | 18–19 | 18.8–19.8 | 18–19.1 | 17.2–17.5 | 17.1–17.8 | 18.5–18.7 |
| XXXI | 19.3 | 20.5 | 19.3 | 17.4–17.7 | 17.5–19.8 | 18.5–19 | 16.7–17.4 | 18.5–20 | 17.9–19.6 | 18.2–18.6 | 17.8–19.2 | 18.2–18.5 | 18.4–19 | 19–19.7 |

| | XVII | XVIII | XIX | XX | XXI | XXII | XXIII | XXIV | XXV | XXVI | XXVII | XXVIII | XXIX | XXX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III | 17 | 15.6–17.6 | 15–15.7 | 16.9–18.7 | 17.9 | 15.4 | 17.5–17.6 | 18.3–18.5 | 16.9 | 17.5 | 16.9 | 21 | 20.7 | 19.3 |
| IV | 19.7 | 17.8–19.4 | 18.2–19 | 17.7–19.2 | 19.4 | 16.8 | 18.3–18.7 | 18.8–19 | 20.5 | 19.9 | 18.3 | 21.4 | 20.7 | 18.3 |
| V | 17.8–17.9 | 17.2–19.7 | 16.7–17.8 | 19.4–20.3 | 18.4 | 16.3 | 18.6–18.7 | 20.8–21 | 19.5 | 19.5 | 20.1 | 20.5 | 18.1 | 17.1 |
| VI | 11.7–12.3 | 9–11.3 | 10.4–11.2 | 11.5–13 | 12.8–13.6 | 9.6–10.4 | 13.5–14.3 | 14.4–15.2 | 16.7–17.3 | 16.1–16.8 | 16.6–16.9 | 17.8–19.1 | 16.9–17.3 | 17.7–18.9 |
| VII | 10–12.3 | 10.2–13.1 | 10.1–12.3 | 11.6–13.6 | 14.4–14.8 | 10.1–12.1 | 12.9–14.5 | 16.7–18.2 | 16.7–18.8 | 15.7–17.1 | 17.9–18.8 | 20.1–21.1 | 17.1–18.9 | 18.5–19 |
| VIII | 13.1–13.7 | 10.5–13.6 | 10.2–11.7 | 13.3–15.3 | 14.4–14.8 | 10.7–11.1 | 15.2–15.9 | 18.7–19.3 | 18.3–18.8 | 16.9–17.7 | 18.5–19 | 20.7–21.5 | 18.4–18.7 | 16.1–16.6 |
| IX | 9.5–10 | 9.5–11.8 | 8.4–9.9 | 11.3–13.1 | 13.6–13.9 | 9.6–10.4 | 13.5–14.1 | 16.6–17 | 15–15.9 | 15.3–16.3 | 16.7–17.9 | 19.6–19.9 | 16.7–17 | 16.7–17.5 |
| X | 12.3–13.4 | 11.6–13.9 | 9.9–12.4 | 11.3–14.4 | 14.6–15.3 | 12.2–13.9 | 13.8–14.9 | 18.2–19.5 | 18.1–18.8 | 17.2–18.6 | 17.4–18.6 | 20.1–21.1 | 18.3–19.5 | 17.5–18.7 |
| XI | 13.4–14.5 | 11.4–13.8 | 10.7–12.4 | 12–14.9 | 14.1–14.9 | 11.6–12.5 | 14.6–15.6 | 16.6–18.1 | 15.6–16.7 | 15.7–16.9 | 17.5–18.1 | 20.1–21 | 18.2–19.6 | 18–19 |
| XII | 12.8–13.7 | 10.9–14.4 | 11.1–13.2 | 11.5–13.7 | 12.9–13.3 | 12.4–13 | 14.7–15.6 | 15.5–16.3 | 17–17.5 | 15.7–16.8 | 17.2–17.6 | 21.3–21.5 | 18.2–18.7 | 18.8–19.8 |

**Table 2.** Continued

| | XVII | XVIII | XIX | XX | XXI | XXII | XXIII | XXIV | XXV | XXVI | XXVII | XXVIII | XXIX | XXX |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XIII | 12.6–13.7 | 9.4–11.8 | 9.6–12.3 | 11.4–13.9 | 11.8–13.7 | 9.8–11.1 | 12.3–13.4 | 16.4–17.2 | 16.2–17.7 | 15–15.5 | 16.7–17.9 | 19.6–20.5 | 17.4–18.8 | 18–18.8 |
| XIV | 7.8–8 | 11.6–13.5 | 12.1–12.9 | 12.8–14 | 13.9–14.1 | 12.6–13.3 | 13.5–14.1 | 16.1–16.6 | 16–16.6 | 17.3–17.6 | 17.6–17.8 | 19.2–19.3 | 16.9–17.1 | 17.2–17.5 |
| XV | 8.8–9.5 | 12.3–14.6 | 12.2–13.7 | 12.8–14.5 | 14.1 | 12.3–13.1 | 15.3–16.1 | 17.2–18.1 | 17.2–17.8 | 17.7–18.7 | 18.5–19.2 | 20.6–21.3 | 17.3–17.9 | 17.1–17.8 |
| XVI | 9.9–10.2 | 12–13.7 | 12.5–13.5 | 12.3–13.8 | 13.4–13.6 | 12.7–13 | 15.1–15.6 | 17–18 | 16.9–17.8 | 17.9–18 | 18–18.2 | 20.8–21 | 17.6–18.2 | 18.5–18.7 |
| XVII | | | | | | | | | | | | | | |
| XVIII | 12.7–14.9 | | | | | | | | | | | | | |
| XIX | 12.7–13.5 | 10.8–13.9 | | | | | | | | | | | | |
| XX | 13.6–14.5 | 11.5–14.8 | 9.9–11.6 | | | | | | | | | | | |
| XXI | 16.5 | 12.4–13.9 | 13.2–14.1 | 9.1–9.8 | | | | | | | | | | |
| XXII | 12.2–12.4 | 12.9–14.9 | 12.4–13.3 | 13.9–15.5 | 16.5 | | | | | | | | | |
| XXIII | 15–15.4 | 13.4–15.7 | 13.8–15.1 | 16.3–17.7 | 16.1–16.4 | 13.3–13.7 | | | | | | | | |
| XXIV | 16.6–16.9 | 17.8–19.2 | 17.8–18.7 | 16.3–18.2 | 17.9–18 | 18.3–18.5 | 19.1–19.9 | | | | | | | |
| XXV | 17.8 | 19.4–21.1 | 17.6–18.3 | 19.3–20.7 | 20.3 | 17.3 | 18.9–19.1 | 17.7–17.8 | | | | | | |
| XXVI | 18.6–18.7 | 17.9–20.2 | 16.1–17.3 | 18.3–19 | 19.6 | 16.6 | 16.7–16.2 | 15.3–15.5 | 13.1 | | | | | |
| XXVII | 18.8 | 18.6–20.9 | 17.2–17.7 | 18.6–19.5 | 18.9 | 17.9 | 20.1–20.4 | 16.3–16.4 | 13 | 15.7 | | | | |
| XXVIII | 20.5 | 20.2–22.1 | 19.8–20.5 | 22.3–23.1 | 22 | 21.1 | 20.1–20.9 | 20.7–20.9 | 18.8 | 18.1 | 19.6 | | | |
| XXIX | 16 | 18–20.2 | 16.9–17.3 | 17.2–19.6 | 20.3 | 17.4 | 19.3–19.5 | 17.3–17.5 | 16.7 | 18.1 | 18.5 | 19.3 | | |
| XXX | 17.1 | 19.6–22 | 17.5–18 | 18.9–20.5 | 21.2 | 17.9 | 19.8–19.9 | 17.4–17.5 | 16.2 | 18.6 | 18.5 | 19.8 | 6.5 | |
| XXXI | 17.4–17.7 | 18–20.2 | 16.7–17.8 | 18.5–19.8 | 20.1 | 18.1 | 19.9–20.1 | 16.9–17.1 | 17.4 | 19 | 19.3 | 19.1 | 16.6 | 16.4 |

**Table 3.** Uncorrected *p*-distances based on *COI* sequences for specimens previously identified as *Runcina coronata*

| | Distance between groups (%) | | | Distance within groups (%) |
|---|---|---|---|---|
| | RC1 | RC2 | RC3 | |
| RC1 | | | | 0.2–0.5 |
| RC2 | 6.3–7.3 | | | 1.0 |
| RC3 | 6.4–7.8 | 7.0–7.7 | | 0.7 |
| RC4 | 7.8–8.0 | 8.8–9.5 | 9.9–10.2 | 0.0 |

with encrusting pink coralline algae (dissected and sequenced).

*External morphology (Fig. 3A, B):* Living specimens 3–4 mm in length. Body elongated and moderately broad. Lateral grooves on both sides between notum and foot. Notum smooth. Posterior part of the notum rounded. Foot slightly wider than the notum. Propodium rounded and wide, metapodium pointed. General ground colour of body dark brown. Front part of the notum dark brown on central zone between the eyes and pale fawn on the sides. White and yellowish spots all over the body, concentrated as semicircle bands behind the eyes and anterior to the notum end, and on the surface of the foot. Eyes small and visible. Three rounded gill laminae present on right of anus; the largest in the middle divided into two arcs. Gills yellowish with slightly brown margins. Anus located in median line of body, beneath the end of notum.

*Internal anatomy (Figs 4A–C, 5A, 6A):* Radular formula 19 × 1.1.1 (MNCN 15.05/88105). Rachidian tooth bilobed with long and smooth lateral wings on each side. Central part of rachidian tooth contains a pair of pads, each possessing 9–11 denticles. Size of denticles variable, with smaller and more developed denticles randomly distributed along pads. Small depression present between pads, with minute denticle present in some rows (Fig. 4A). Lateral teeth denticulate, elongate, hooked shape with 30–34 relatively long denticles (Fig. 4B). Triangular jaws present. Four gizzard plates with ten crests (Fig. 4C). Shell absent. Reproductive system monaulic. Female gland mass placed on right side and behind the digestive gland divided into two lobes. Female gland opens to exterior through median size common genital duct (Fig. 5A). Male pore opens next to mouth, on the right side. Male copulatory organ elongated and cylindrical. Penial papilla absents. Prostate gland cylindrical, posterior to a narrow atrium. Slender seminal vesicle half size of prostate gland (Fig. 6A).

*Distribution:* Bréhat, Bretagne, France (Atlantic) (Quatrefages, 1844); Torbay, England. (Alder & Hancock, 1846, Forbes, 1853); Swanage, England (present study).

*Remarks:* A discussion of this species is included together with *R. aurata* in the 'Remarks' of the latter species.

### *RUNCINA AURATA* GARCÍA *ET AL.*, 1986 (FIGS 3C–F, 4D–F, 5B, 6B)

*Type locality:* Club La Hacienda, Cádiz, Spain (36°14'18"N; 5°18'36"W)

*Examined material:* MNCN 15.05/91500, La Caleta (Cádiz) (36°31'59"N; 6°18'31"W), Andalusia, south-western Spain, 8 April 2019, 3.5 mm living animal, depth 0.5–1.0 m (dissected and sequenced). MNCN 15.05/88106, La Caleta (Cádiz) (36°31'59"N; 6°18'31"W), Andalusia, south-western Spain, coll. Josep Romà, 18 April 2015, 2 mm in length preserved, depth 0.5–1.0 m. (dissected and sequenced). MNCN 15.05/88107, La Caleta (Cádiz) (36°31'59"N; 6°18'31"W), Andalusia south-western, Spain, coll. Josep Romà, 18 April 2015, 2 mm in length preserved, depth 0.5–1.0 m (dissected and sequenced). MNCN:ADN 118948, La Caleta (Cádiz) (36°31'59"N; 6°18'31"W), Andalusia south-western, Spain, coll. Josep Romà, 17 May 2015, 1.5 mm in length preserved, depth 0.5–1.0 m (dissected and sequenced). MNCN:ADN 118950, El Chato (Cádiz) (36°28'39"N; 6°15'49"W), Andalusia south-western, Spain, coll. Ana Bartual, 13 April 2015, 1 mm in length preserved, depth 0.5–1.0 m (dissected and sequenced).

*External morphology (Fig. 3C–F):* Living specimen 3.5 mm length and preserved specimens 1–2 mm length. Body elongated and moderately broad. Lateral grooves on both sides between notum and foot. Anterior part of notum ('head') slightly bilobed. Posterior part of notum rounded. Propodium rounded, metapodium pointed. Foot as wide as notum. Foot extended beyond notum on rear part. Ground colour of body translucent pale fawn or yellowish. Digestive system visible as a broad brownish blotch. White spots on central zone of notum, behind eyes forming

**Figure 3.** Living animals of *Runcina coronata* (A, B) and *Runcina aurata* (C–F). A, MNCN 15.05/88105, 3 mm in length, Swanage (southern England). B, MNCN 15.05/90423, 4 mm in length, Swanage (southern England) (photos Ian F. Smith). C, MNCN/ADN 118948, 1.5mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). D, MNCN/ADN 118950, 1mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). E, MNCN 15.05/88106, 2 mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). F, MNCN 15.05/88107, 2mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). Photos A and B, courtesy of Ian F. Smith; photos C–F by Ana Karla Araujo.

triangular patches and anterior to notum end. White spots maybe also absent. Black dots dispersed on notum and more concentrated on head zone. Eyes inconspicuous. Dark band on middle of dorsal surface of foot. Black dots may be present on ventral surface of foot. Four rounded and relatively large gills laminae to the right of anus. Gills yellowish with slightly brown margins. Anus located in median line of body, beneath the end of notum.

*Internal anatomy (Figs 4D–F, 5B, 6B):* Radular formulae 12 × 1.1.1 (MNCN 15.05/88106) and 13 × 1.1.1 (MNCN 15.05/91500). Rachidian tooth bilobed with long and smooth lateral wings on each side. Central

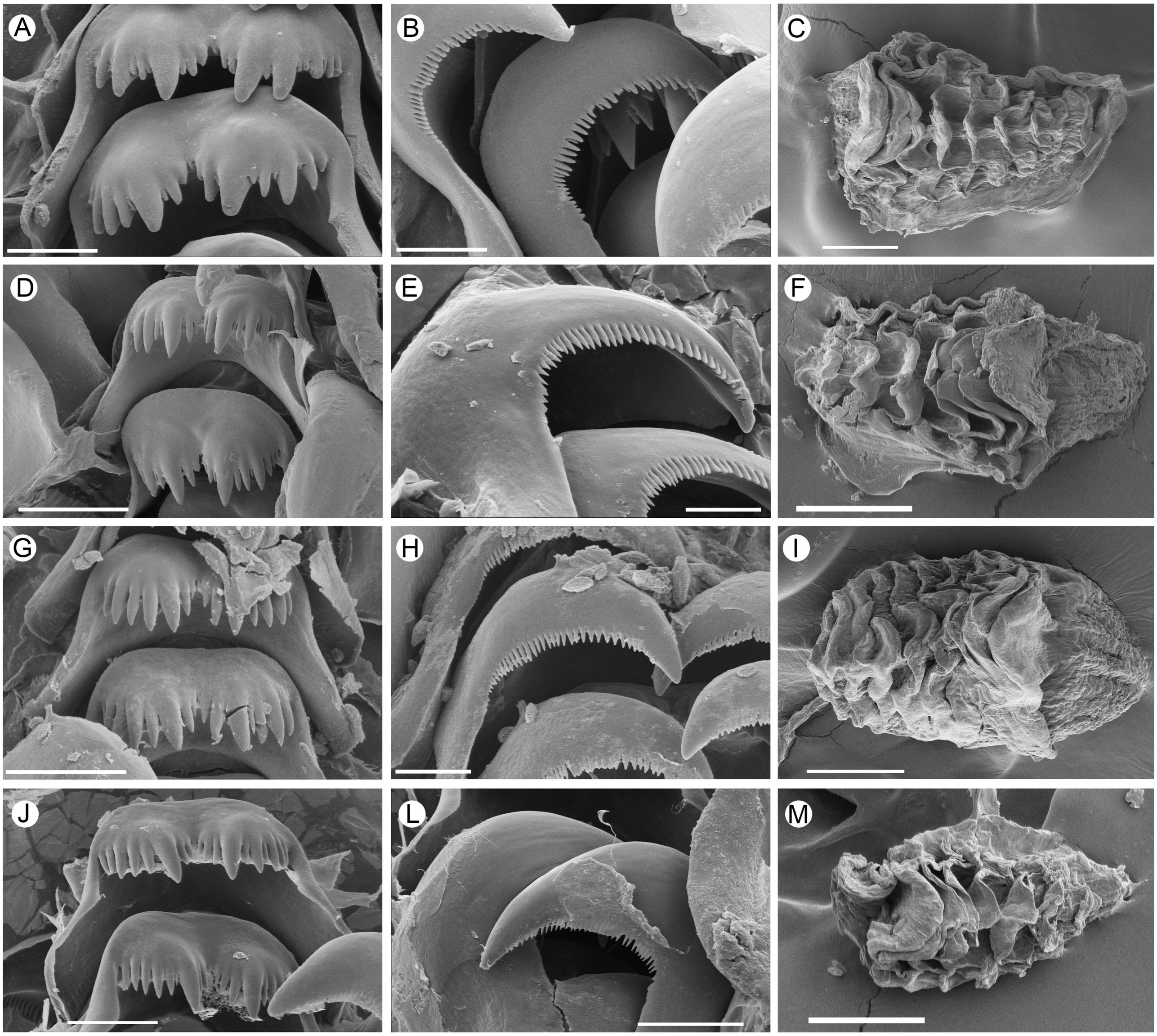

**Figure 4.** Scanning electron micrographs of radula and gizzard plates. A–C, *Runcina coronata*. A, rachidian teeth (MNCN 15.05/88105). B, lateral teeth (MNCN 15.05/88105). C, gizzard plate (MNCN 15.05/90423). D–F, *Runcina aurata*. D, rachidian teeth (MNCN 15.05/91500). E, lateral teeth (MNCN 15.05/88106). F, gizzard plate (MNCN 15.05/88106). G–I, *Runcina caletensis* (MNCN 15.05/200113) G, rachidian teeth. H, lateral teeth. I, gizzard plate. J–M, *Runcina tingensis*. J, rachidian teeth (MNCN 15.05/200114). L, lateral teeth (MNCN 15.05/200114). M, gizzard plate (MNCN 15.05/91514). Scale bars: A, B, E, J, L = 10 µm; C, F, I, M = 50 µm; D, G = 20 µm; H = 5 µm.

part contains pair of pads, each possessing 10–11 long, slender, pointed denticles. Size of denticles variable. Small denticles between large denticles. Small depression present between pads, with minute denticle present (Fig. 4D). Lateral teeth denticulate, elongate, hooked shape with 35–36 long, pointed and same-size denticles (Fig. 4E). Triangular jaws present. Four gizzard plates with seven to nine crests (Fig. 4F). Shell absent. Reproductive system monaulic. Female gland mass placed on right side and behind digestive gland, opening to exterior through small size common genital duct (Fig. 5B). Male pore opens next to mouth, on the right side. Elongated and cylindrical male copulatory organ. Penial papilla not observed. Cylindrical and long prostate gland ends in slender and small seminal vesicle with black pigmentation (Fig. 6B).

*Distribution:* Cádiz, Strait of Gibraltar, Malaga and Murcia (southern Spain) (Templado, 1984; Garcia *et al.*, 1986) and Azores Islands (Portugal) (Gosliner, 1990).

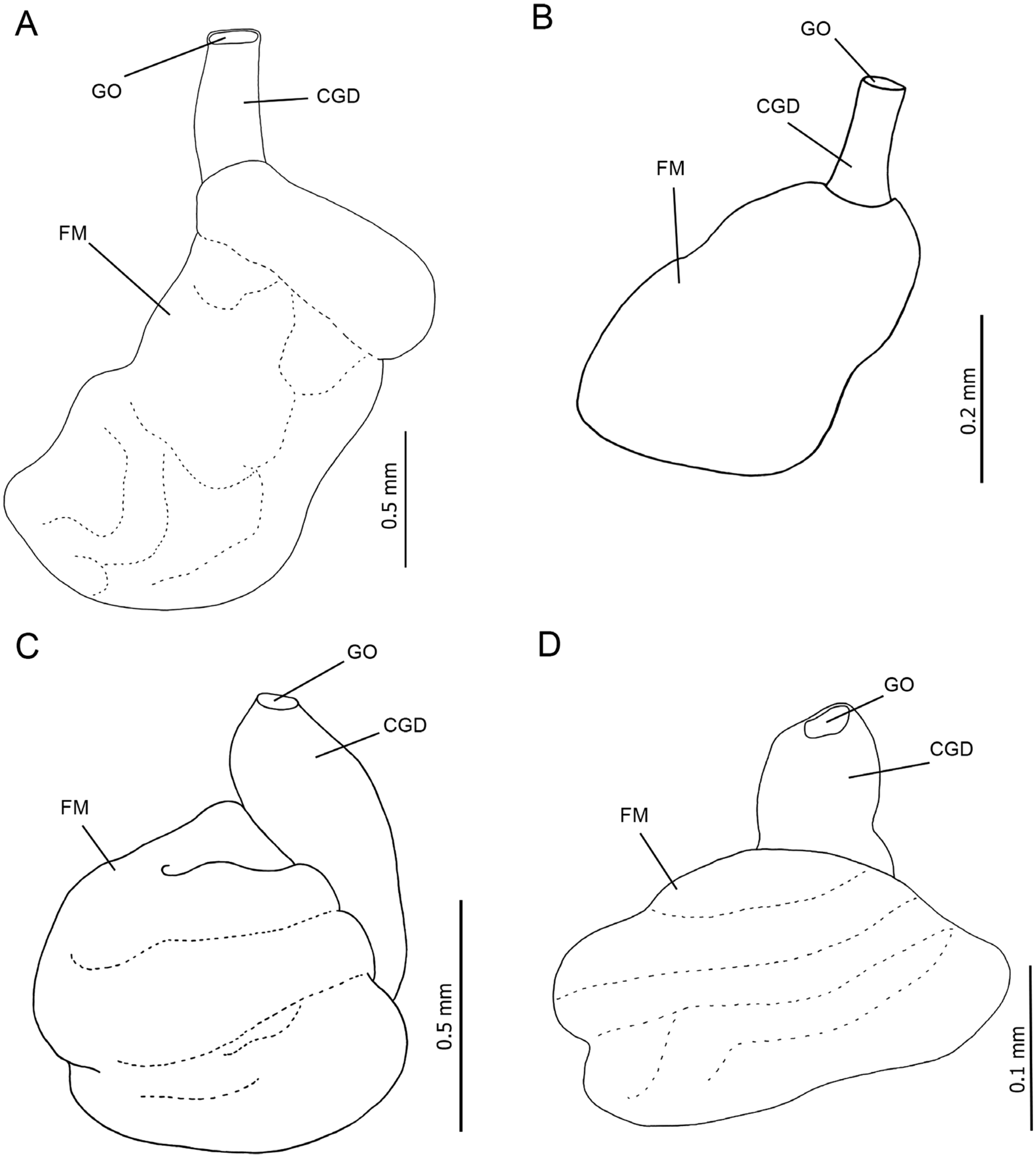

**Figure 5.** Female reproductive system. A. *R. coronata*, southern England (MNCN 15.05/90423). B. *R. aurata*, La Caleta, Cádiz, south-western Spain, Atlantic Ocean (MNCN 15.05/88106). C. *R. caletensis*, La Caleta, Cádiz, south-western Spain, Atlantic Ocean (MNCN 15.05/200113). D. *R. tingensis*, Tangier, north-western Morocco, Atlantic Ocean (MNCN 15.05/91514). Abbreviations: FM, female mass; CGD, common genital duct; GO, gonopore.

*Remarks: Runcina coronata* has been considered a taxonomically difficult species (see Introduction). Originally described from Brehat (Atlantic coast of France), this species was first reported in the Mediterranean Sea by Vayssière (1883) who identified specimens from Marseille (Mediterranean coast of France) as *R. coronata*. However, Burn (1963), based on morphological differences, especially the shape of the body and the colour pattern, suggested the specimens identified by Vayssière (1883) could be *R. calaritana*. We cannot confidently attribute those specimens to a specific species, but we agree with Burn (1963) that they probably do not correspond to *R. coronata*. Pruvot-Fol (1954), and Cervera *et al.* (1991) regarded *R. calaritana* (Gulf of Cagliari, Sardinia, Italy) and *R. aurata* (from around the Strait of Gibraltar) conspecific with *R. coronata*. This problematic has ultimately created the perception

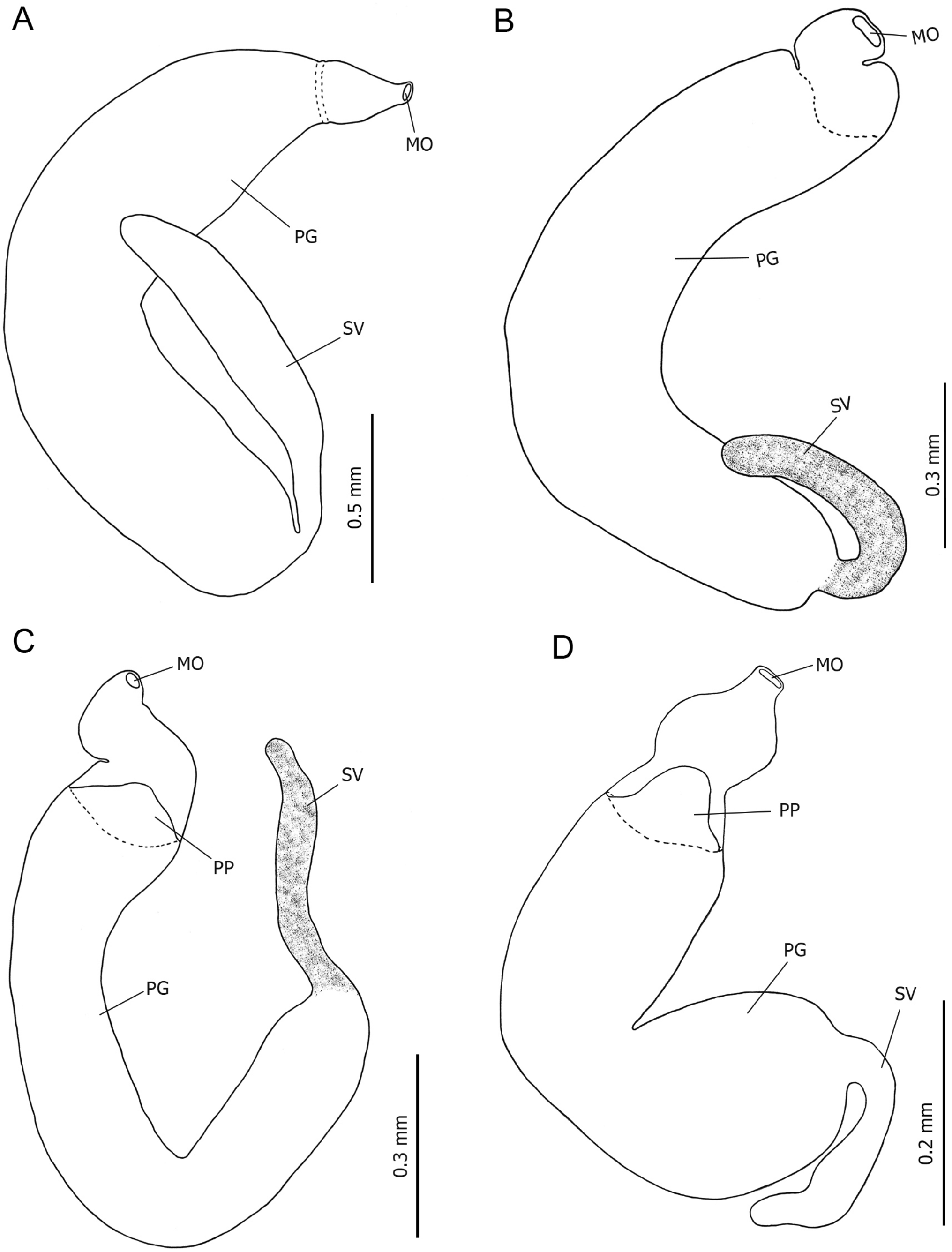

**Figure 6.** Male reproductive system. A, *Runcina coronata*, southern England (MNCN 15.05/90423). B, *Runcina aurata*, La Caleta, Cádiz, south-western Spain, Atlantic Ocean (MNCN 15.05/88106). C, *Runcina caletensis*, La Caleta, Cádiz, south-western Spain, Atlantic Ocean (MNCN 15.05/200113). D, *Runcina tingensis*, Tangier, north-western Morocco, Atlantic Ocean (MNCN 15.05/91514). Shaded area indicates the presence of sperm. Abbreviations: MO, male opening; PP, penial papilla; PG, prostate gland; SV, seminal vesicle.

that *R. coronata* was present in the Mediterranean Sea (Schmekel & Cappellato, 2002; Cervera *et al.*, 2004; Ballesteros *et al.*, 2016).

In general, the external and internal morphology of our specimens of *R. coronata* from Swanage (England) are consistent with the original description of the species (Quatrefages, 1844), and with the description provided by Schmekel & Cappellato (2002) based on specimens from Roscoff (Atlantic coast of France) and Plymouth (south of

England). However, compared with the description provided by García *et al.* (1986; specimens from the Strait of Gibraltar), our animals from England exhibit several differences, mainly in the shape of the body and colour pattern. The anterior and posterior ends of the notum are rounded, while in Spanish specimens it is pointed (García *et al.*, 1986). The colour pattern of our specimens (Fig. 3A, B) differs drastically from those from the Strait of Gibraltar, which have a uniformly dark colour pattern, two whitish bands on both sides of the head and one white small band on the posterior right side of the notum (García *et al.*, 1986). This suggests that likely specimens attributed to *R. coronata* by García *et al.* (1986) belong to a distinct species.

The original description of *R. coronata* describes briefly the male copulatory organ as 'a rather short testicular bag in the shape of a "club", with a seminal vesicle sometimes absent (Quatrefages, 1844).' Kress (1977) studied specimens from Plymouth (England) and provided additional anatomical data on the reproductive system. Comparatively, our specimens from Swanage (England) exhibit a similarly long and cylindrical prostate, but a slightly different seminal vesicle and common genital duct. Kress (1977) referred to a seminal vesicle 'considerably shorter than prostate' and a common genital duct forming a long loop, whereas in our material the seminal vesicle was approximately half the size of the prostate and the common genital duct was short (Fig. 6A).

The species *R. aurata* was described by García *et al.* (1986) from the southern coast of Spain (Cádiz, Strait of Gibraltar and Malaga). Gosliner (1990) reported the species from the Azores and suggested that a specimen illustrated and depicted by Thompson & Brodie (1988: fig. 1E) from Plymouth as *R. coronata* was most likely *R. aurata*. Despite the fact that the description and illustration provided by Thompson & Brodie (1988) are vague and lacking important information, the reference to the presence of a light area surrounding the eyes, suggests their identification as *R. coronata* to be correct.

The features of our specimens collected in Cádiz (Spain) are consistent with the original description of the species *R. aurata* (García *et al.*, 1986). Externally, they differ from *R. coronata* by having a translucent yellow colour with black spots on the notum and on the ventral surface of the foot (Fig. 3; Table 4). Also, the number of gills is distinct: three gills in *R. coronate*, while our animals of *R. aurata* from Cádiz have four gills (Table 4). The original description of *R. aurata* refers, in fact, to three gills only, but the authors did not seem to have thoroughly looked at this character, which is difficult if not examined properly and across several specimens (García *et al.*, 1986).

Concerning the radula, we observed some subtle differences between *R. aurata* and *R. coronata*, namely in the number of radular rows and shape of the denticles in the pads of the rachidian teeth (Table 4). However, in runcinids, these features can vary, even within species (Schmekel & Cappellato, 2001; 2002; Araujo *et al.* 2019), and are, therefore, difficult to use

**Table 4.** Summary of diagnostic characters between *Runcina coronata*, *Runcina aurata, Runcina caletensis* and *Runcina tingensis*. Data based on Quatrefages (1844), Vayssière (1883), García *et al.* (1986), Cervera *et al.* (1991), Schmekel & Cappellato (2002) and present study

| | *Runcina coronata* | *Runcina aurata* | *Runcina caletensis* | *Runcina tingensis* |
|---|---|---|---|---|
| Colour pattern | Ground colour dark brown. White semi-circles bands behind eyes and anterior to notum end. | Ground colour translucent pale fawn or yellowish. White spots behind eyes and anterior to notum end. | Ground colour translucent pale fawn or yellowish. Black dots on notum end. Two black longitudinal lines on the head. | Ground colour light brown. Triangular white patches behind eyes and anterior to notum end. |
| Gills | Three rounded | Four rounded and relatively large. | Three rounded | Two rounded |
| Shell | Absent | Absent | Absent | Absent |
| Radular formulae | 19 × 1.1.1 | 12–13 × 1.1.1 | 13 × 1.1.1 | 12–14 × 1.1.1 |
| Rachidian teeth | 9–11 short denticles on each pad | 10–11 long, slender, pointed denticles | 7–8 denticles stalactite-shaped. Depression between pads absent. | 7–10 short triangular denticles. |
| Lateral teeth | 30–34 denticles | 35–36 denticles | 33–36 denticles | 34–37 denticles |
| Gizzard plate | 10 crests | 7–9 crests | 7–10 crests | 8 crests |

in species identification. Regarding the gizzard plates, our specimens of *R. coronata* show ten crests in each plate, while in our animals of *R. aurata* it ranges from seven to nine, which is consistent with its original description (García, *et al.*, 1986).

The male reproductive system in our specimens of *R. aurata* resembles the description of this organ by Gosliner (1990) for specimens from the Azores, but we could not observe a penial papilla. The seminal vesicle is shorter than in *R. coronata* and the atrium and male opening are broader (Fig. 6A, B). The female gland mass of *R. aurata* was never studied before and in our specimens of consists of one lobe, while in the studied specimens of *R. coronata* it is divided into two lobes (Fig. 5B).

The minimum uncorrected *p*-distance for the *COI* gene between *R. coronata* and *R. aurata* is 6.3% (Table 3) and, in addition to the phylogenetic tree, the species delimitation analyses suggested both species as valid (Fig. 2).

### *RUNCINA CALETENSIS* ARAUJO, POLA, MALAQUIAS & CERVERA, *SP. NOV.*

(FIGS 4G–I, 5C, 6C, 7A, B)

*Zoobank registration:* urn:lsid:zoobank.org:act:EC216698-6700-4607-9E35-515AB69BD17C

*Examined material:* Holotype: MNCN 15.05/200113, La Caleta (Cádiz) (36º31’59”N; 6º18’31”W), Andalusia, south-western Spain, coll. Josep Romà, 17 May 2015, 3 mm living animal, depth 0.5–1 m (dissected and sequenced). Paratype: MNCN:ADN 118949, La Caleta (Cádiz) (36º31’59”N; 6º18’31”W), Andalusia, south-western Spain, coll. Josep Romà, 16 June 2015, 1.5 mm in length preserved, depth 0.5–1.0 m. (dissected and sequenced).

*Etymology:* Named after the type locality: the beach of La Caleta in Cádiz, Spain.

*External morphology (Fig. 7A, B):* Living and preserved specimens 3.0 mm and 1.5 mm in length, respectively. Body elongated and broad. Lateral grooves on both sides between notum and foot. Anterior part of notum (‘head’) slightly bilobed. Posterior part of notum rounded. Foot as wide as notum. Foot extends posteriorly beyond notum. Ground colour translucent pale fawn or yellowish. Digestive system visible as broad brownish blotch in juvenile specimens. Tiny white, black and yellow spots all over the body. White spots on the lobes of the head and on the anterior ventral surface of the foot. Triangular white patches behind the eyes. White semicircle anterior to the notum end. Very few white spots in juvenile. Small black dots forming two longitudinal lines on head region. Black spots concentrated on the notum end posteriorly to white semicircle. In juveniles, only a few larger black spots are present along the margin of the notum. Eyes inconspicuous. Dark band on middle of posterior region of foot. Three rounded gill laminae located on right side of anus. Gills yellowish with slightly brown margins. Anus located in the midline of the body beneath the notal edge.

*Internal anatomy (Figs 4G–I, 5C, 6C):* Radular formula 13 × 1.1.1 (15.05/200113). Rachidian tooth slightly bilobed with long and smooth lateral wings on each side. Central part of rachidian tooth contains a pair of pads, each possessing seven to eight developed denticles. Central depression between pads absent. Denticles long and pointed, decreasing in size towards middle of the tooth (Fig. 4G). Lateral teeth denticulate, elongate, hooked shaped with 33–36 long and pointed denticles (Fig. 4H). Triangular jaws present. Four gizzard plates with seven to ten crests (Fig. 4I). Shell absent. Reproductive system monaulic. Female gland mass placed on right side and behind digestive gland. Female gland opens to exterior through common genital duct (Fig. 5C). Male pore opens next to mouth, on right side. Elongated and cylindrical male copulatory organ. Short, conical and unarmed penial papilla projects into large atrium. Cylindrical prostate gland strongly curved. Long and slender seminal vesicle with black pigmentation (Fig. 6C).

*Distribution:* Cádiz, southern Spain (present study).

*Remarks:* The species *R. caletensis* shares with *R. coronata* the presence of white bands and spots on the notum, and is overall externally similar to *R. aurata*. However, in *R. caletensis* the yellow ground colour is opaque, while in *R. aurata* the colour is translucent (Figs 3E, F, 7B). In addition, *R. caletensis* lacks black spots on the ventral surface of the foot, a feature present in *R. aurata*. Based on available data, *R. caletensis* is the only one among these species with rachidian teeth lacking the depression between the pads and with well-developed denticles of similar length along the masticatory edge of pads (Fig. 4G). The female gland mass of *R. caletensis* has a rounded shape and the common genital duct is larger than in the other two species (Fig. 5A–C). The seminal vesicle in *R. caletensis* is thinner than in *R. coronata* and more elongated than in *R. aurata*. Unlike for *R. coronata* and *R. aurata*, a penial papilla was observed in *R. caletensis*.

The minimum uncorrected *p*-distances for the *COI* gene is 7.8% between *R. caletensis* and *R. coronata*,

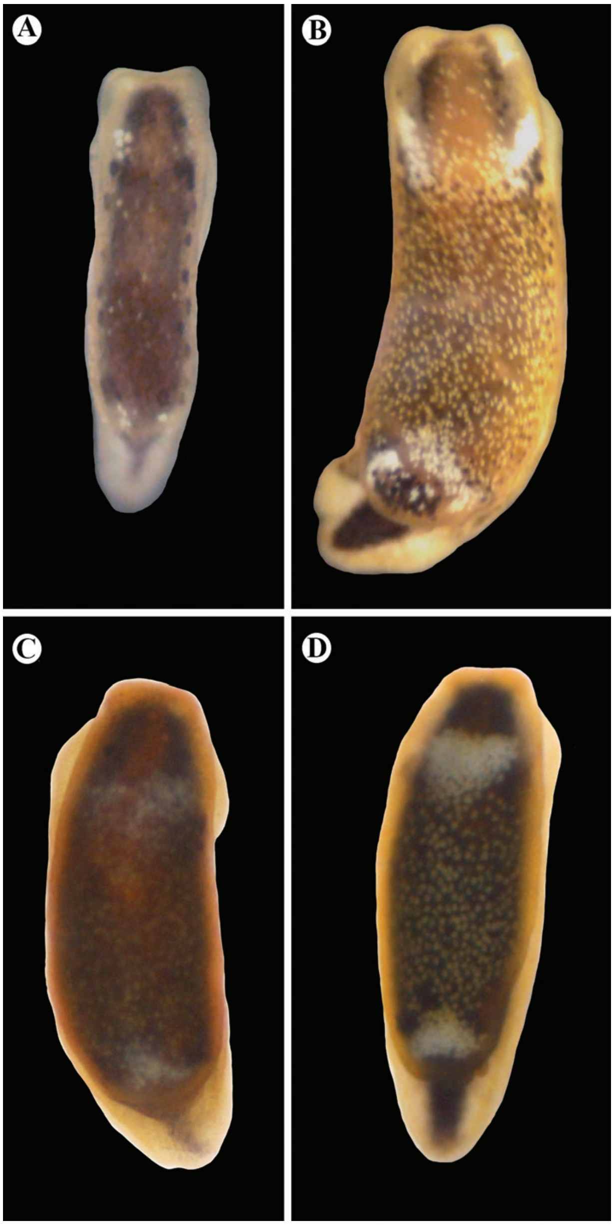

**Figure 7.** Living animals of *Runcina caletensis* (A, B) and *Runcina tingensis* (C, D). A, MNCN/AND 118949, 1.5 mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). B, MNCN 15.05/200113, 3 mm in length, La Caleta, Cádiz (south-western Spain; Atlantic Ocean). C, MNCN 15.05/91514, 1.5mm in length, Tangier (north-western Morocco; Atlantic Ocean). D, MNCN 15.05/200114, 2mm in length, Tangier (north-western Morocco; Atlantic Ocean). A, B images by Ana Karla Araujo; C, D images courtesy of Naoufal Tamsouri.

and 8.8% between *R. caletensis* and *R. aurata* (Table 3). Species delimitation analyses recognized *R. caletensis* as a valid species (Fig. 2).

### *RUNCINA TINGENSIS* ARAUJO, POLA, MALAQUIAS & CERVERA, *SP. NOV.*

(FIGS 4J–M, 5D, 6D, 7C, D)

*Zoobank registration:* urn:lsid:zoobank.org:act:84C29263-8B36-4FA7-A84A-94F4191CE491

*Examined material:* Holotype: MNCN 15.05/200114, Tangier (35°47'32"N; 5°49'04"W), Morocco, coll. Naoufal Tamsouri, 22 March 2018, 2 mm living animal, depth 0.5–1.0 m (dissected and sequenced). Paratype: MNCN 15.05/91514, Tangier (35°47'32"N; 5°49'04"W), Morocco, coll. Naoufal Tamsouri, 22 March 2018, 1.5 mm fixed animal, depth 0.5–1.0 m (dissected and sequenced).

*Etymology:* The name *tingensis* refers to Tingi, the Greek name of Tangier (Morocco), the type locality of the species.

*External morphology (Fig. 7C, D):* Living and preserved specimens 2.0 mm and 1.5 mm in length, respectively. Body elongated. Lateral grooves on both sides between notum and foot present. Anterior part of notum ('head') straight, rounded on posterior end. Foot as wide as notum, with propodium and metapodium rounded; metapodium extends beyond notum. Ground colour of body light brown. Broad continuous light orange line on edge of notum. Digestive system visible as a broad brownish blotch. White spots concentrated behind eyes and on anterior end of notum forming a triangle. Yellowish dots dispersed on middle of notum. Dark small spots can be present behind anterior white spots on head region and rear part of notum. Eyes inconspicuous. Longitudinal mid-dorsal dark band on foot. Two rounded gills laminae on right side of anus. Gills light brown. Anus located on right lateral side beneath edge of notum, approximately in mid-region of body length.

*Internal anatomy (Figs 4J–M, 5D, 6D):* Radular formulae 14 × 1.1.1 (MNCN 15.05/91514) and 12 × 1.1.1 (MNCN 15.05/200114). Rachidian tooth bilobed with smooth lateral wings on each side. Central part of rachidian tooth contains pair of pads, each possessing seven to ten denticles. Denticles short, pointed. One inner denticle on each pad conspicuously more developed. Central small depression present between pads; small denticle in-between pads absent (Fig. 4J). Lateral teeth denticulate, elongate and hooked shaped with 34–37 long, thin, pointed denticles (Fig 4L). Triangular jaws present. Four gizzard plates with eight crests (Fig. 4M). Shell absent. Reproductive system monaulic. Female gland mass placed on right side and behind digestive gland. Opens to exterior through short and wide common genital duct (Fig. 5D). Male pore opens next to mouth, on right side. Male copulatory organ cylindrical. Short and unarmed penial papilla projects into round atrium. Prostate gland cylindrical and strongly curved with posterior part rounded. Seminal vesicle slender with middle part wider (Fig. 6D).

*Distribution:* Tangier, Morocco (present study).

*Remarks: Runcina tingensis* resembles *R. coronata* by its dark colour and presence of small, white and yellow spots on the notum. However, *R. tingensis* has a broad, continuous, light orange line along the edge of the notum, while *R. coronata* has lighter regions only on the head and on the back of the notum. Compared with *R. aurata* and *R. caletensis*, the dark colour of *R. tingensis* contrasts with the translucent yellowish colour of these two species. The distribution of white spots is also distinctive among these species. In *R. tingensis* they form a triangular pattern on the notum (Fig. 7C, D), while in the other three species they form a semicircle (Figs 3, 7B). According to our observations, the radula of *R. tingensis* has, compared with *R. aurata*, *R. coronata* and *R. caletensis*, a prominently more developed denticle in each pad of the rachidian teeth (Fig. 4J). The female mass of *R. tingensis* differs from *R. coronata* and *R. aurata* by its elliptical shape and a larger common genital duct (Fig. 5), and its prostate is notably curved and the seminal vesicle enlarged in its central region (Fig. 6). A penial papilla was present as observed for *R. caletensis*.

The minimum uncorrected *p*-distances for the *COI* gene is 7% between *R. tingensis* and *R. coronata,* 6.4% between *R. tingensis* and *R. aurata*, and 9.9% between *R. tingensis* and *R. caletensis* (Table 3).

## DISCUSSION

Burn (1963) presented a systematic classification of the order Runcinida (as Runcinacea) based on morpho-anatomical characters: in particular, shell, gills and radula. According to his classification, the rare *Ildica nana* Bergh, 1889 was the most primitive species followed by the genera *Runcina*, *Runcinida* and *Runcinella*, while the genus *Ilbia* was considered the most derived. Our results, suggest a sister-relationship between the genus *Ilbia* and the remaining members of Runcinida with genera *Runcina*, *Runcinida*, *Metaruncina* and *Lapinura* (PP = 1; BS = 99). Unfortunately, samples of *Ildica* and *Runcinella* were not available to us and, therefore, we could not thoroughly test Burn's (1963) hypothesis, but despite the limitations of our dataset, the results suggest the validity of the families Ilbiidae (with *Ilbia*) and Runcinidae (with *Runcina*, *Runcinida*, *Metaruncina* and *Lapinura*).

### *RUNCINA DIVAE OR LAPINURA DIVAE?*

The genus *Lapinura* (type species: *Ildica divae* Marcus & Marcus, 1963), introduced by Marcus & Marcus (1970) for specimens collected in Curaçao, Bonaire and Florida, is characterized by an external cup-shaped larval shell. This genus was synonymized with *Runcina* by Clark (1984) after examining populations from Bermuda of supposed *Lapinura divae* in which some specimens lacked the external shell. Because of this 'variability', Clark disregarded the external shell and others characteristics that define the genus, emphasizing only the radular formula ($N \times 1.1.1$) and the presence of gizzard plates – common for most runcinids – to assign *Lapinura divae* to the genus *Runcina*.

Representatives of the Caribbean species *Lapinura*/*Runcina divae* from Bermuda branched off outside Clade F (the one with the type species of the genus *Runcina*) with other runcinids in Clade H. Therefore, we suggest that the genus *Lapinura* should be reinstated as valid and at least applied for the Caribbean species described by Marcus & Marcus (1963). Moreover, we agree with Ortea *et al.* (2017) about a possible existence of more than one species being referred under the name *Lapinura divae*, due to the discrepancies (number of rows of the radula, presence/absence of the shell and number of crests of the gizzard plates) among specimens collected in the Caribbean Sea and Brazil (Marcus & Marcus, 1963; Marcus & Marcus, 1970; Thompson, 1977; Clark, 1984).

### WHAT ABOUT EUROPEAN RUNCINIDS?

Three genera have been referred in European waters: *Runcina* (31 species)*, Runcinella* (one species) and *Pseudoilbia* (one species). We have studied specimens of *Runcina* and *Pseudoilbia* but, unfortunately, specimens of *Runcinella condio* Moro & Ortea, 2015, described from the Canary Islands, were not available for study.

#### Runcinida

Here we add a fourth genus to the European fauna, namely *Runcinida*. To date, the genus *Runcinida* was restricted to the western Pacific Ocean and included three species, *R. elioti* (Baba, 1937), *R. valentinae* and *R. marisae*. However, according to our results, a specimen identified as *Runcinida* sp. 1, collected in

Cap Ferret, France (Atlantic coast), clustered together with *R. marisae* and *R. valentinae* (PP = 1, BS = 90). Externally, all species of *Runcinida* can be distinguished by their unique colour pattern, with a dark-brown notum, yellowish or orange edge of notum and foot, and gills arranged in a semicircle above the anus. Our specimen (*Runcinida* sp. 1; Fig. 8) fits this colour pattern and arrangement of the gills, but differs from the other species in the genus by having small, black dots spread on the dorsal and ventral surfaces of the foot and several larger white spots on the notum (Fig. 8). *Runcinida elioti* (Baba, 1937) has fewer white spots too, but lacks small, black dots on the foot (Baba, 1937). *Runcinida marisae* has small, black dots on the ventral surface of the foot but white dots are absent (Chernyshev, 1998). Finally, *Runcinida valentinae* lacks black and white dots, but has a triangular orange patch on the anterior part of the notum (Chernyshev, 2006). The ABDG and bPTP recognized *Runcinida* sp. 1, *R. marisae* and *R. valentinae* as distinct species with *COI* uncorrected *p*-distances ranging between 6.5% to 16.6% (Table 2).

### Pseudoilbia avellana *or* Runcina avellana?

The species *Runcina avellana* was originally described by Schmekel & Cappellato (2001) from Banyuls-sur-Mer, French Mediterranean. These authors found an unusual radula (3 × 1.0.1) and no gizzard plates in the single specimen examined, which they pointed out could be a juvenile. Ortea (2013), because of the absence of raquidian teeth and gizzard plates assigned the species to the genus *Pseudoilbia*, proposing the new combination name *Pseudoilbia avellana*.

Our specimens characterized by features consistent with the original description of *R. avellana* (*sensu* Schmekel & Cappellato, 2001; Fig. 9), like the shape of the body, presence of a round brownish mark on the centre of tail, general colour pattern, absence of gizzard plates and presence of gills, collected at Roses, Spain about 45 km from the type locality, clustered in the phylogenetic analyses among species of the genus *Runcina* (Clade F; Fig. 1), questioning the assignment by Ortea (2013) of this species to the genus *Pseudoilbia*. The latter genus is characterized by animals lacking gills, gizzard plates, shell and with radular formula 2.0.2 (Miller & Rudman, 1968). *Runcina avellana* shares some of these features, but not all, and our phylogenetic results support its inclusion in *Runcina*. Moreover, the lack of rachidian teeth in *R. avellana*, reported by Schmekel & Cappellato (2001), might be an artefact since only one apparently juvenile specimen was studied by these authors. Unfortunately, our effort in preparing the radula of this species was not successful and, thus, we could not study this structure. Additional specimens of *R. avellana* are necessary to permit a detailed study of its anatomy and comparison with other species of *Runcina* and *Pseudoilbia*.

### Runcina

The genus *Runcina* has been traditionally defined by the presence of up to four separated gills on the right side of the anus and a triseriate (1.1.1) radula with bilobed rachidian teeth and smooth or denticulated lateral teeth (Burn, 1963; Gosliner, 1991; Schmekel

**Figure 8.** Living animal of *Runcinida* sp. (MNCN 15.05/90670, 3 mm in length, Cap Ferret, north of France, Atlantic Ocean). Image courtesy of Marina Poddubetskaia.

**Figure 9.** *Runcina avellana*. A, schematic illustration taken from Schmekel & Cappellato, 2001 (original description). B, living animal from Catalonia, north-eastern Spain (Mediterranean Sea) (MNCN 15.05/88108, 1,5 mm in length). Image B by Ana Karla Araujo.

& Cappellato, 2001). Our analysis supports the monophyly of the genus *Runcina* (PP = 1; BS = 99) but with *R. avellana*, which lacks rachidian tooth and, in addition, specimens resembling *R. ferruginea* from the Mediterranean coasts of Spain and France, and from Croatia clustered elsewhere in the tree together with other runcinids from Bermuda, Hawaii, Brazil, Japan, Russia and also from the Atlantic coast of France (PP = 1; BS = 78).

We have included in our phylogenetic analysis about 39% of the nominal species of European *Runcina* (MolluscaBase, 2021) and, in addition, several unidentified specimens from Spain, Italy and Croatia (Fig. 1). Within *Runcina*, we retrieved a clade (PP = 1; BS = 100) containing one specimen that we provisionally have identified as *R.* cf. *bahiensis*, one specimen provisionally identified as *R. hornae* and several unidentified specimens. Most specimens in this clade were collected in Catalonia (north-eastern Mediterranean Spanish coast) and, despite the remarkable variation in colour pattern (Fig. 10), species delimitation analyses suggest they all belong to the same species (*COI* uncorrected *p*-distances varied between 0.0–2.0%).

The species *R. bahiensis* was originally described from the Bay of Algeciras (Strait of Gibraltar, Spain) (Cervera *et al.*, 1991) and *R. hornae* from Banyuls-sur-Mer (Mediterranean coast of France) (Schmekel & Cappellato, 2002). Both species have been reported in several localities in Catalonia (Sánchez-Moyano *et al.*, 2000; Ballesteros *et al.*, 2016), and are regarded as differing in colour pattern, number of rows of radular teeth, shape of body and presence of small protuberances (Cervera *et al.*, 1991; Schmekel & Cappellato, 2002). Several of our specimens (Fig. 10B–D) bear a colour pattern and body shape consistent with *R. bahiensis*, but none of them have the small protuberances characteristic of this species. The study of a radula of one specimen from Catalonia (*Runcina* sp. 3) matched the original description of the radula of *R. hornae* (Schmekel & Cappellato, 2002; Fig. 11). Unfortunately, specimens of *R. bahiensis* from the type locality or nearby were not available for this study, but considering the radular similarities and the proximity to the type locality of *R. hornae* (*c.* 25.5 km), we identify specimens is this clade as *R. hornae*, which is here demonstrated to be a species with remarkable colour variability (Fig. 10).

### Runcina coronata *species complex*

In the last few years, several complexes of species have been detected among heterobranch sea slugs (see, among others: Jörger & Schrödl, 2013; Padula *et al.*, 2014; Carmona *et al.*, 2015; Krug *et al.*, 2016; Korshunova *et al.*, 2017; Austin *et al.*, 2018). In Runcinida, the first complex of species was unravelled by Araujo *et al.* (2019) for the species *Runcina brenkoae* with the description of two new species, namely *R. marcosi* and *R. lusitanica*.

In the current study, molecular and morphological data have showed that *R. coronata* hides a complex of at least four species, including two new to science and the previously described species *R. aurata*. Due to similarities with the original description by Quatrefages (1844), and the descriptions provided by Forbes (1851) and Schmekel & Cappellato (2002), we regard our specimens from Swanage (England) conspecific with *R. coronata*.

The geographical distribution of *R. coronata* has been reported to extend from England to the French Mediterranean coast (Vayssière, 1883; Cervera *et al.*, 2004). However, our results questioned the presence of *R. coronata* in the Iberian Peninsula, where most likely the records to this species belong to *R. aurata* (see in Results, 'Remarks' section of *R. aurata*). The morphological differences between specimens identified as *R. coronata* in the Mediterranean Sea and those from England and the Atlantic coast of France suggest, as previously stressed by Burn (1963), that animals studied by Vayssière (1883) from the Mediterranean are a distinct species and also that *R. calaritana* (Colosi, 1915) could be a valid name.

Therefore, we here restrict the distribution of *R. coronata* to southern England and the Atlantic coast of France, a limited geographical span supported by the direct development of the species (Schmekel & Cappellato, 2001). The species *R. aurata* and *R. caletensis*, despite subtle differences, are externally difficult to distinguish and coexist in the same geographical area (Cádiz, south of Spain), whereas the species *R. tingensis* is so far only known from the north-western coast of Morocco.

This work has revealed several additional putative cases of hidden diversity among runcinids in Europe (e. g. *R. adriatica* and *R. ferruginea*), and our detailed study of the *R. coronata* species-complex has made it possible to redefine the type species of the genus, to clarify the taxonomic status of *R. aurata* and to describe two new species to science. In addition, we have provided the first modern approach to understanding relationships in the order Runcinida and a provisional framework to discuss the familial and generic classification of the group.

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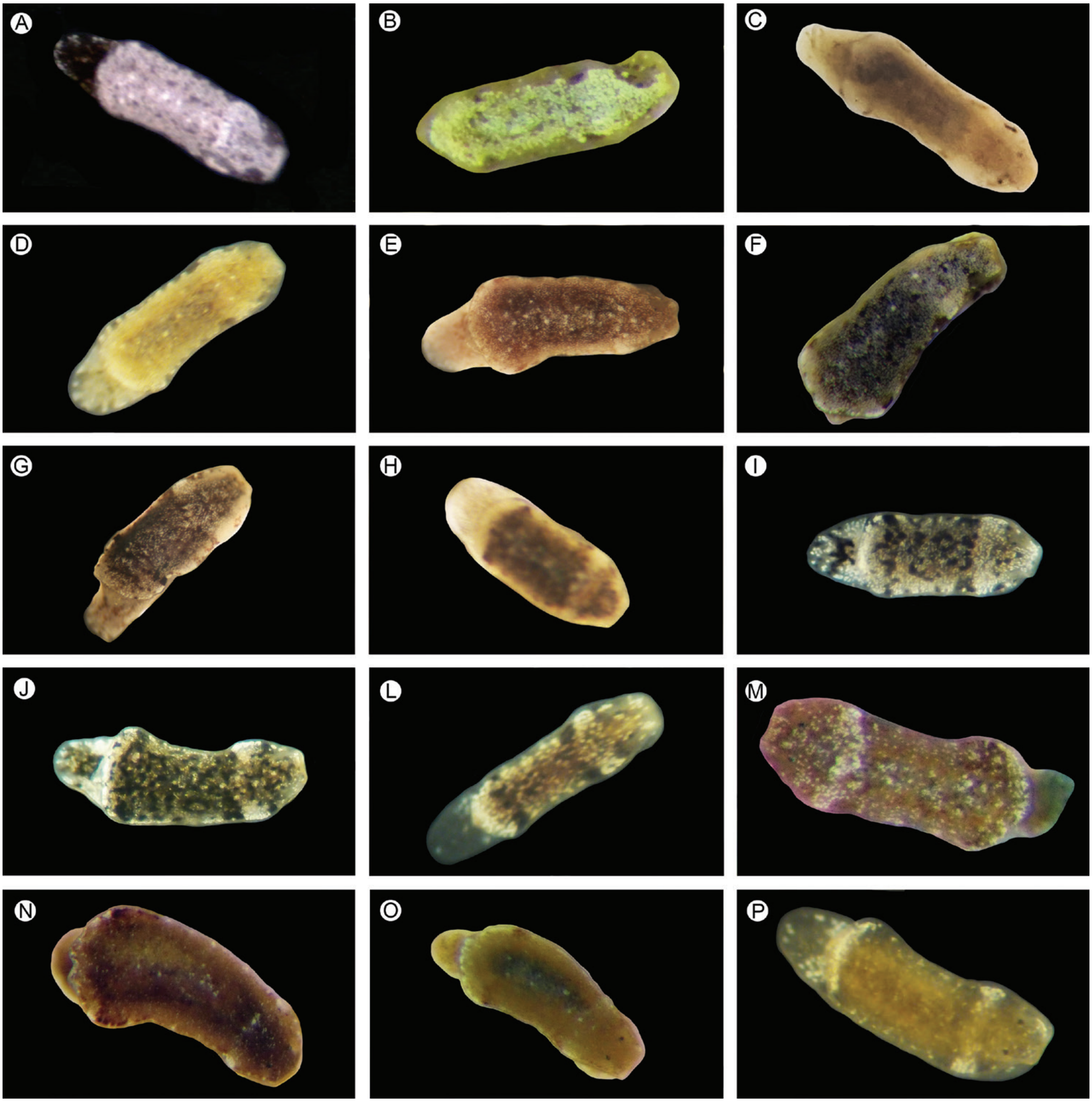

**Figure 10.** Living animals of *Runcina hornae*. Specimens from Catalonia, north-eastern Spain (Mediterranean Sea). A, MNCN 15.05/88104, 1 mm in length. B, MNCN 15.05/90661, 1 mm in length. C, MNCN/AND 118954, 2 mm in length. D, MNCN 15.05/90656, 1.5 mm in length. E, MNCN 15.05/90660, 3 mm in length. F, MNCN 15.05/90655, 3 mm in length. G, MNCN 15.05/90659, 2.5 mm in length. H, MNCN 15.05/90665, 1.5 mm in length. I, MNCN 15.05/90658, 1.5 mm in length. J, MNCN 15.05/90654, 2 mm in length. L, MNCN 15.05/90657, 1 mm in length. M, MNCN 15.05/90662, 2 mm in length. N, MNCN 15.05/90664, 3 mm in length. O, MNCN 15.05/90663, 1 mm in length. P, MNCN 15.05/88110, 2 mm in length. Images A, E, G, H courtesy of Carlés Galià; images B, F, M, N, O courtesy of Marina Poddubetskaia; images C, D, I, J, L, P by Ana Karla Araujo.

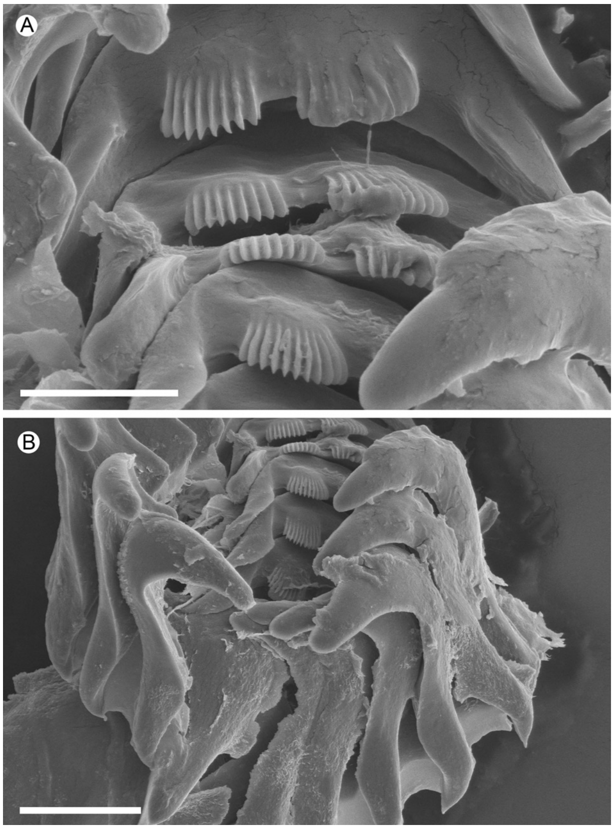

**Figure 11.** Scanning electron micrographs of radula of *Runcina hornae* (MNCN 15.05/90654, 1 mm in length, Mataro, Spain). A, rachidian teeth. B, lateral teeth. Scale bars: A = 5 μm; B = 10 μm.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** Phylogenetic hypothesis based on BI of the 16S gene. Numbers on the left of the slash are posterior probabilities and on the right bootstrap values derived from maximum likelihood. Unsupported branches not labelled.
**Figure S2.** Phylogenetic hypothesis based on BI of the *COI* gene. Numbers on the left of the slash are posterior probabilities and on the right bootstrap values derived from maximum likelihood. Unsupported branches not labelled.
**Figure S3.** Phylogenetic hypothesis based on BI of the *H3* gene. Numbers on the left of the slash are posterior probabilities and on the right bootstrap values derived from maximum likelihood. Unsupported branches not labelled.