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Revision of the *Ocinebrina aciculata* species complex (Mollusca: Gastropoda: Muricidae) in the northeastern Atlantic Ocean and Mediterranean Sea

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ABSTRACT

We used a molecular phylogenetic approach to investigate species delimitation and diversification in the northeastern Atlantic and Mediterranean musseldrills of the *Ocinebrina aciculata* complex, based on molecular data from topotypical material of many of the nominal taxa. The complex is shown to consist of at least five species: *Ocinebrina aciculata* (Lamarck, 1822) from the Atlantic and western Mediterranean; *O.* cf. *corallina* (Scacchi, 1836) from the central Mediterranean Sea; *O. reinai* Bonomolo & Crocetta, 2012 from the Tyrrhenian Sea; *O. corallinoides* Pallary, 1912 from the Gulf of Gabès; and *O. aegeensis* n. sp. currently known from the Aegean Sea only. The new species is differentiated from the other taxa by very subtle morphological diagnostic features, although it is clearly identified by genetic distance and apomorphic DNA-sequence characters. The identity of *Murex corallinus* Scacchi, 1836 (type species of *Ocinebrina* Jousseaume, 1880) could not be defined with certainty, pending genetic comparison of specimens of the 'large Tyrrhenian morphotype' (corresponding to the neotype, but not assayed herein) with the assayed 'small morphotype'.

INTRODUCTION

Muricid gastropods (Neogastropoda: Muricidae) play key roles in shaping the structure of marine benthic communities (Morton, 1999, 2004; Peharda & Morton, 2006; Harding *et al.*, 2007). They include predators of special interest owing to their potential impact on marine resources (oysterdrills, musseldrills and rock-shells). The family Muricidae is among the most diverse groups of marine gastropods, with over 1,600 named extant species and a very rich fossil record. The genus *Ocinebrina* Jousseaume, 1880, as was traditionally conceived, comprised small musseldrills with a fusiform shell up to 26 mm long, paucispiral protoconch, 5–6.5 teleoconch whorls in the adult, short and ventrally sealed siphonal canal and a varicose outer lip with usually 5–6 internal denticles (Houart, 2001). Until recently, the northeastern Atlantic and Mediterranean species were traditionally divided into two groups with morphological affinities: the *Ocinebrina edwardsii* (Payraudeau, 1826) complex and the *O. aciculata* (Lamarck, 1822) complex. However, Barco *et al.* (2017) recently demonstrated that the *O. edwardsii* complex (comprising at least eight molecular operational taxonomic units, MOTUs; Barco *et al.*, 2013) actually belongs to the genus *Ocenebra* Gray, 1857 (type species *Murex erinaceus* Linnaeus, 1758). The *O. aciculata* complex has been partly revised by Crocetta *et al.* (2012), although a wider geographic and taxonomic sampling remains necessary to define the taxonomy of this group more accurately. It is noteworthy that the type species of *Ocinebrina*, *Murex corallinus* Scacchi, 1836, originally described from Italy (Mediterranean Sea), is currently considered (based on a morphological assessment) to be a junior synonym of *O. aciculata*, originally described from Brittany (Atlantic Ocean) (Houart, 2001; Crocetta *et al.*, 2012).

In the present paper we extend the geographic sampling of the *Ocinebrina aciculata* complex with respect to that of Crocetta *et al.* (2012), by sequencing samples from as close as possible to the type localities of: *Murex aciculatus* Lamarck, 1822 (near Vannes, Atlantic), *Murex corallinus* Scacchi, 1836 (Scacchetiello, Baia, Mediterranean), *Ocinebrina corallinoides* Pallary, 1912 (near Sfax, Gulf of Gabès, Mediterranean), *Ocinebrina aciculata exilis* Houart, 2001 (NW of Bou Grara Sea, Mediterranean), *Ocinebrina buzzurroi* Cecalupo & Mariani, 2008 (Borj el Hissar, Kerkennah, Mediterranean), and not far from the area of the type localities of *Murex inconspicuus* Sowerby, 1841 (Jersey, Atlantic) and *Murex subaciculatus* Locard, 1886 (Toulon, Mediterranean). Using an integrative taxonomic approach we have

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thus addressed the identity of the nominal species involved, testing whether DNA sequence-based clustering corresponds to prior morphological taxonomic assignments and exploring whether previously unrecognized species are represented in our dataset.

MATERIAL AND METHODS

Sampling

Collecting data, voucher details and GenBank accession numbers are given in Table 1 (see also Fig. 1). Samples were collected by SCUBA diving with hand-towed net on meadows of *Posidonia oceanica* (Linnaeus) Delile, by direct hand collecting and using an air-lift suction sampler, and were fixed in 100% ethanol upon collection.

Molecular methodology

Total genomic DNA was extracted using a standard proteinase K phenol-chloroform method with ethanol precipitation as used by Oliverio & Mariottini (2001). The DNA-barcode fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified by PCR using the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). PCR conditions involved 30 amplification cycles (30 s at 94 °C, 30 s at 60 °C, 1 min and 30 s at 72 °C). PCR products were purified using Exosap-IT (USB Corporation) and sequenced by Macrogen Inc. (Seoul). Sequences were readily aligned by hand. Pairwise distances were calculated with MEGA v. 6.0 (Tamura *et al.*, 2013) using a Kimura-2-parameters (K2P) substitution model, as in previous COI-based studies.

Phylogenetic analysis and species delimitation

We used an integrative taxonomic approach to delimitate species. Primary species hypotheses (PSH) were made based on a morphological approach. PSH were then tested with DNA data, analysed using a distance-based and a tree-based approach. The former method was used to define the boundary between intraspecific and interspecific distances based on our prior morphological assignments. In the distance-based approach we defined two distance thresholds using the 'threshold optimization' and 'local minima' functions in SPIDER (Brown *et al.*, 2012), as described by Barco *et al.* (2016). We used these thresholds to test our prior taxonomic assignments by verifying that intraspecific distances were all of lower value.

The phylogenetic tree-based method was used to define MOTUs for comparison with both prior morphological and distance-threshold assignments. In the tree-based approach we used the generalized mixed Yule-coalescent (GMYC), as described by Barco *et al.* (2013). GMYC (Pons *et al.*, 2006) uses the differences in the branching rates in an ultrametric phylogenetic tree to distinguish between inter- and intraspecific branching events, and thus to determine species boundaries. An ultrametric tree was obtained in BEAST (Drummond & Rambaut, 2007) using the K2P substitution model, a relaxed

Table 1. List of sequenced specimens, with collection localities, ID codes for vouchers (see text for abbreviations) and GenBank accession numbers.

Prior morphological identification	Final species hypothesis	Voucher codes	*N*	Sampling site	Accession number
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU00778	1	Noreste, São Miguel I., Azores, Portugal	KX534764
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU00964.1, 3-5, 7-9	2	Vigo, Spain	KX534755, KX534757, KX534758, KX534762, KX534763, KX534765, KX534766
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU01060.1-8	3	Baiona, Spain	KF199911-KF199917, KX534759
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU01658.1-3	4	Brittany, France	KX534751, KX534752, KX534769
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU02691.2-3, BAU02440.1,3	5	Tarifa, Spain	MF622957, MF622958, MF622959, MF622960
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU02437, BAU02438	6	Alboràn, Spain	MF622961, MF622962
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU02439.1-3	7	Malaga, Spain	MF622963-MF622965
Ocinebrina aciculata	*Ocinebrina aciculata*	BAU02694	8	Cabo de Gata, Spain	MF622966
Ocinebrina aciculata	*Ocinebrina* cf. *corallina*	BAU01037	9	Secche di Tor Paterno, Italy	FR851899
Ocinebrina aciculata	*Ocinebrina* cf. *corallina*	BAU01042.1	9	Secche di Tor Paterno, Italy	FR851901
Ocinebrina aciculata	*Ocinebrina* cf. *corallina*	MZB-R2.01, MZB-R2.03, MZB-SP4.13, MZB-SP4.15, MZB-SP4.19, MZB-SP5.03	9	Secche di Tor Paterno, Italy	FR85190, FR851902-FR850906
Ocinebrina aciculata	*Ocinebrina* cf. *corallina*	BAU01304.1-3	10	Giannutri I., Italy	KX534754, KX534756, KX534760
Ocinebrina corallinoides	*Ocinebrina corallinoides*	MNHN-IM-2013-31799 BAU02376	11	Gulf of Gabès, Tunisia	KX534768-KX534761
Ocinebrina reinai	*Ocinebrina reinai*	BAU01038, BAU01039, MZB-R2.02, MZB-SP2.04, MZB-SP3.02, MZB-SP3.04, MZB-SP4.17, MZB-SP4.18	9	Secche di Tor Paterno, Italy	FR851907-FR851914
Ocinebrina reinai	*Ocinebrina reinai*	BAU02316	10	Giannutri I., Italy	MF622967
Ocinebrina aciculata	*Ocinebrina aegeensis*	MNHN-IM-2013-32780, MNHN-IM-2013-32784, MNHN-IM-2013-32787	12	Néa Péramos, Greece	KX534753, KX534770, KX534767

N indicates the collection locality number in Figure 1.

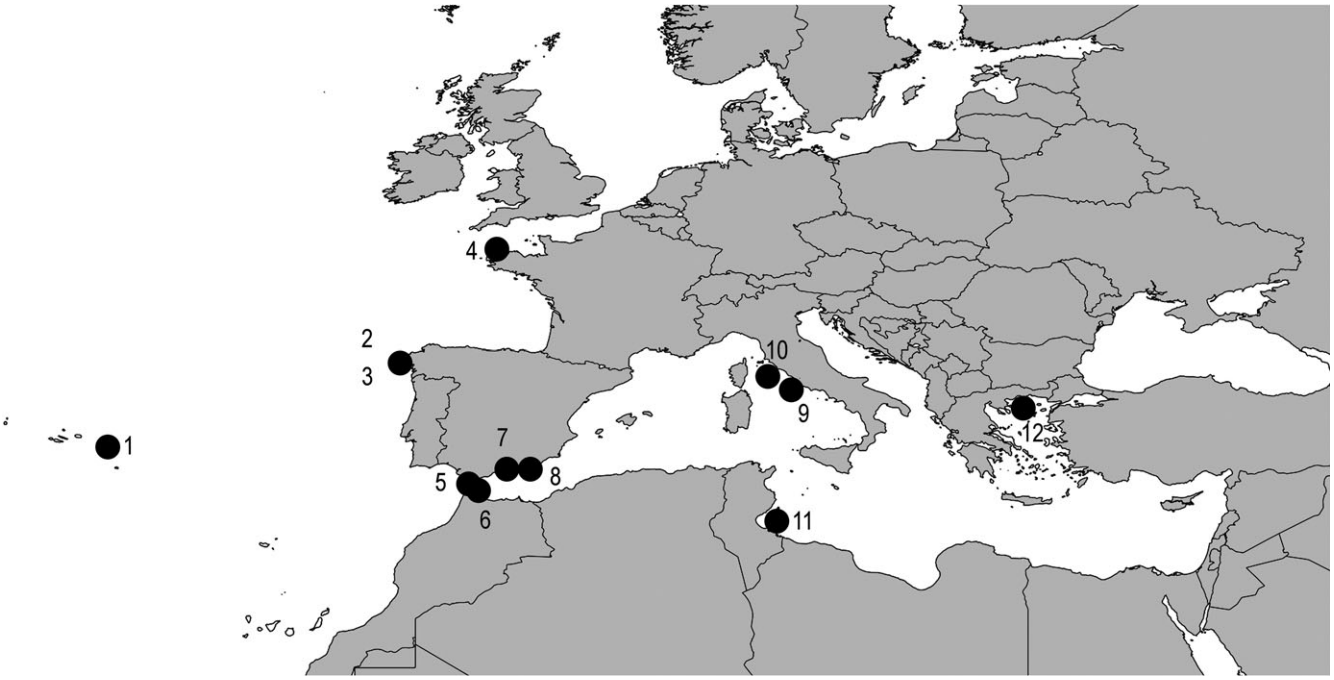

Figure 1. Map showing collecting sites (numbered as in Table 1).

lognormal clock, a substitution rate fixed at 1 and a constant coalescent prior. Two BEAST runs with 10^8 generations each were performed; a tree was sampled every 10^4 generations to obtain two samples of 10^4 trees. Convergence was evaluated with Tracer (Rambaut & Drummond, 2003) for effective sample size (ESS) values >200. Trees retained after a 50% 'burn-in' were combined using LogCombiner (Rambaut & Drummond, 2003) and then summarized in a maximum-credibility tree using TreeAnnotator (Rambaut & Drummond, 2003). The consensus tree was analysed, applying the multiple-threshold GMYC function from the SPLITS package (Ezard *et al.*, 2009) in R (http://www.R-project.org). A Bayesian phylogenetic tree was also built using MrBayes v. 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Two analyses with four chains each were run for 2×10^8 generations and sampled every 2×10^4 generations. Chain convergence was evaluated by plotting the standard deviation of average split frequencies and the potential scale reduction factor (Gelman & Rubin, 1992). Trees sampled during the 50% burn-in were discarded and the remainder were summarized in a consensus tree. Clades recovered with a posterior probability (PP) between 0.95 and 1 were considered well supported.

Diagnostic (autapomorphic) nucleotides among MOTUs were identified in our alignment using the 'nucDiag' function in SPIDER (Brown *et al.*, 2012).

Morphological methodology

Radulae were extracted from the buccal mass after tissues had been partly dissolved in a 10% solution of sodium hydrochloride, then rinsed in distilled water, air dried, mounted on SEM stubs, gold-palladium coated and examined with SEM. Protoconchs were similarly mounted and coated.

The material examined for each species description includes that examined by Crocetta *et al.* (2012), reidentified for *O.* cf. *corallina* and *O. aciculata* according to the present results.

The abbreviations for the terms used to describe the spiral cords and the apertural denticles (after Merle, 2001, 2005; Fig. 4) are as follows. For the convex part of teleoconch whorl and siphonal canal: ab, abapical (or abapertural); abis, abapical infrasutural secondary cord (on subsutural ramp); ABP, abapertural primary cord on the siphonal canal; ad, adapical (or adapertural); adis, adapical infrasutural secondary cord (on subsutural ramp); ADP, adapertural primary cord on the siphonal canal; IP, infrasutural primary cord (primary cord on subsutural ramp); MP, median primary cord on the siphonal canal; P, primary cord; P1, shoulder cord; P2–P6, primary cords of the convex part of the teleoconch whorl; s, secondary cord; s1–s6, secondary cords of the convex part of the teleoconch whorl (e.g. s1 = secondary cord between P1 and P2; s2 = secondary cord between P2 and P3, etc.). For the aperture: D1 to D5, abapical denticles; ID, infrasutural denticle.

Institutional abbreviations

BAU	Dipartimento di Biologia e Biotecnologie "Charles Darwin", Sapienza University (Rome, Italy)
CAS	Chicago Academy of Science (Chicago, Illinois)
FCP	Fabio Crocetta private collection (Naples, Italy)
FDP	Fabio Daga private collection (Carbonia, Italy)
GBP	Giuseppe Bonomolo private collection (Vallefoglia, Italy)
GFP	Giuseppe Fasulo private collection (Naples, Italy)
MHNG	Muséum d'Histoire Naturelle de Genève (Geneva, Switzerland)
MNCN	Museo Nacional de Ciencias Naturales (Madrid, Spain)
MNHM	Museo Civico di Storia Naturale (Milano, Italy)
MNHN	Muséum national d'Histoire naturelle (Paris, France)
MOP	Marco Oliverio private collection (Rome, Italy)
MRP	Michele Reina private collection (Palermo, Italy)
MSNT	Museo di Storia Naturale (Trieste, Italy)
MSNVE	Museo di Storia Naturale (Venice, Italy)
MZN	Museo Zoologico, Centro Museale Università degli Studi di Napoli Federico II (Naples, Italy)
NHMUK	Natural History Museum (London, UK)
PAP	Paolo Giulio Albano private collection (Bologna, Italy)
PSP	Paolo Sordino private collection (Naples, Italy)
RHP	Roland Houart private collection (Landen, Belgium)
SDP	Sergio Duraccio private collection (Naples, Italy)
SZN	Stazione Zoologica Anton Dohrn di Napoli (Naples, Italy)
WHP	Winfried Engl private collection (Düsseldorf, Germany).

Table 2. Genetic distances (K2P) within and between *Ocenebrina* species.

	Mean intraspecific distance	Mean interspecific distance			
		O. aciculata	*O.* cf. *corallina*	*O. corallinoides*	*O. reinai*
O. aciculata	0.006				
O. cf. *corallina*	0.002	0.048			
O. corallinoides	0.002	0.047	0.056		
O. reinai	0.002	0.063	0.074	0.070	
O. aegeensis	0.019	0.062	0.069	0.075	0.043

RESULTS

Partial sequences of the mtDNA COI gene were obtained from 44 specimens morphologically ascribed to four species of the *Ocenebrina aciculata* complex (Table 1). A total of 658 bp were unambiguously aligned, without gaps, with 98 variable positions and 78 parsimony-informative sites. Mean genetic distances within and across MOTUs (as defined below) are given in Table 2.

Both runs in BEAST reached ESS values higher than 200. A final sample of 10^4 trees after burn-in was used to obtain the maximum clade credibility tree shown in Figure 2. The same topology was retrieved in the Bayesian analysis with MrBayes (on a final sample of 10^4 trees). In the phylogenetic analysis one major clade was supported (clade B), including sequences of *O. corallinoides* from the Gulf of Gabès (B1) and of specimens morphologically identified as *O. aciculata* from several localities in the Tyrrhenian Sea (B2) and from the northeastern Atlantic (B3). The remaining sequences formed an unsupported group (A), including sequences of specimens from the Aegean Sea (clade A1: morphologically ascribed to *O. aciculata*) and sequences of *O. reinai* from the Tyrrhenian Sea (clade A2).

All prior morphological identifications except that of *O. aciculata* were confirmed by highly supported monophyly of the corresponding groups. The Aegean specimens morphologically ascribed to *O. aciculata* were monophyletic, but the relationships of this clade (clade A1) were not resolved. A specimen from the Azores (BAU00778), morphologically identified as *O. aciculata*, was separate from all other subclades in clade B, but its relationships within clade B were not supported. The rest of the specimens morphologically ascribed to *O. aciculata* were split into two clades (B2 and B3).

The threshold optimization method calculated that a distance range of 0.027–0.031 defines the best limit between intraspecific and interspecific distances, and the local minima threshold gave a comparable value of 0.023. Intraspecific distances for *O. reinai* and *O. corallinoides* were below this threshold, but several distances within the PSH *O. aciculata* were above this threshold, in agreement with the phylogenetic relationships described above. The maximum intraspecific distance among specimens morphologically ascribed to *O. aciculata* was 0.077, considerably above the threshold.

GMYC species delimitations are shown on the tree resulting from our BEAST analysis (Fig. 2). The estimated threshold delimited eight MOTUs, with a broad confidence interval (2 to 29). The polyphyly of *O. aciculata* as morphologically conceived was confirmed, giving three to four distinct MOTUs. Clades A1 and B3 were divided in two MOTUs each but, in both cases, the first node above each clade was well within the confidence interval; the third MOTU was represented by the single specimen (BAU00778) from the Azores.

Diagnostic nucleotides were found for all six MOTUs and are reported under each species in the Systematic Descriptions below.

DISCUSSION

Both the molecular-based approaches highlighted discrepancies with prior morphological attributions (i.e. PSH). In particular, specimens morphologically ascribed to *Ocinebrina aciculata* were shown to represent genetically divergent and phylogenetically distinct MOTUs. As is well known, GMYC tends to overestimate the number of species, especially when the splits identified correspond to allopatric subclades (Tanzler *et al.*, 2012). In addition, the multiple thresholds method shows a general tendency to overestimate the number of clusters (Monaghan *et al.*, 2009; Puillandre *et al.*, 2012; Fujisawa & Barraclough, 2013). Therefore, we retained as final species hypotheses those MOTUs that were suggested by both methods, distance- and tree-based. These were as follows (Fig. 2):

Clade A1: this clade was divided into two subclades by GMYC, but the node above the single divergent specimen was well within the confidence interval and therefore we retain this clade as a single MOTU. It comprised three specimens from the Aegean Sea, corresponding to an undescribed species (Fig. 3).

Clade A2: this clade corresponded (congruently with morphology) to *O. reinai*.

Clade B1: this clade corresponded (congruently with morphology) to *O. corallinoides*.

Clade B2: specimens of this clade were morphologically identified as *O. aciculata*, but corresponded to a different species from the central Mediterranean Sea, for which the first available name would be *Murex corallinus* Scacchi, 1836. However, the specimens assayed molecularly (a small morphotype) do not perfectly agree with the morphology of the neotype of *M. corallinus* Scacchi (a large morphotype, similar to *O. aciculata*) as designated by Crocetta *et al.* (2012) (see Systematic Descriptions for further details). Therefore, although a species distinct from *O. aciculata* is certainly present at least in the central Mediterranean, it is not clear if (1) it represents *O. corallina* (comprising large and small morphotypes), the type species of the genus that replaces *O. aciculata* in the central Mediterranean; (2) it represents a species (small morphotype) distinct from *O. corallina* (large morphotype), which in turn replaces *O. aciculata* in the central Mediterranean; or finally (3) the central Mediterranean small morphotype is a species distinct from the large morphotype, which in turn is conspecific with *O. aciculata*. However, the actual taxonomic significance of size in this group is not clear, since in the *O. aciculata* clade (see B3 below) small and large morphotypes proved to be conspecific. We have, therefore, conservatively included all central Mediterranean specimens under *O.* cf. *corallina*, pending the availability of DNA sequence data from large specimens from the central Mediterranean.

Clade B3: broadly topotypic specimens of *M. aciculatus* from Brittany were clustered in the same MOTU as other specimens from the Atlantic coast of Spain and from the westernmost Mediterranean Sea. The Mediterranean samples included both large (Malaga, Alboràn) and small (Cabo de Gata from −1 m on *Posidonia*, BAU2694) morphotypes (analogous to those found in the central Mediterranean discussed above). This may suggest that size may be environmentally related, as we have found for several morphological traits in the *Ocenebra edwardsii* complex (Barco *et al.*, 2013).

Finally, a single specimen (BAU00778) from the Azores was remarkably divergent from all the other species.

These results on genetic variation in the *O. aciculata* complex indicate a pattern of taxonomic diversity paralleling that in the *Ocenebra edwardsii* complex (Barco *et al.*, 2013), i.e. several cryptic

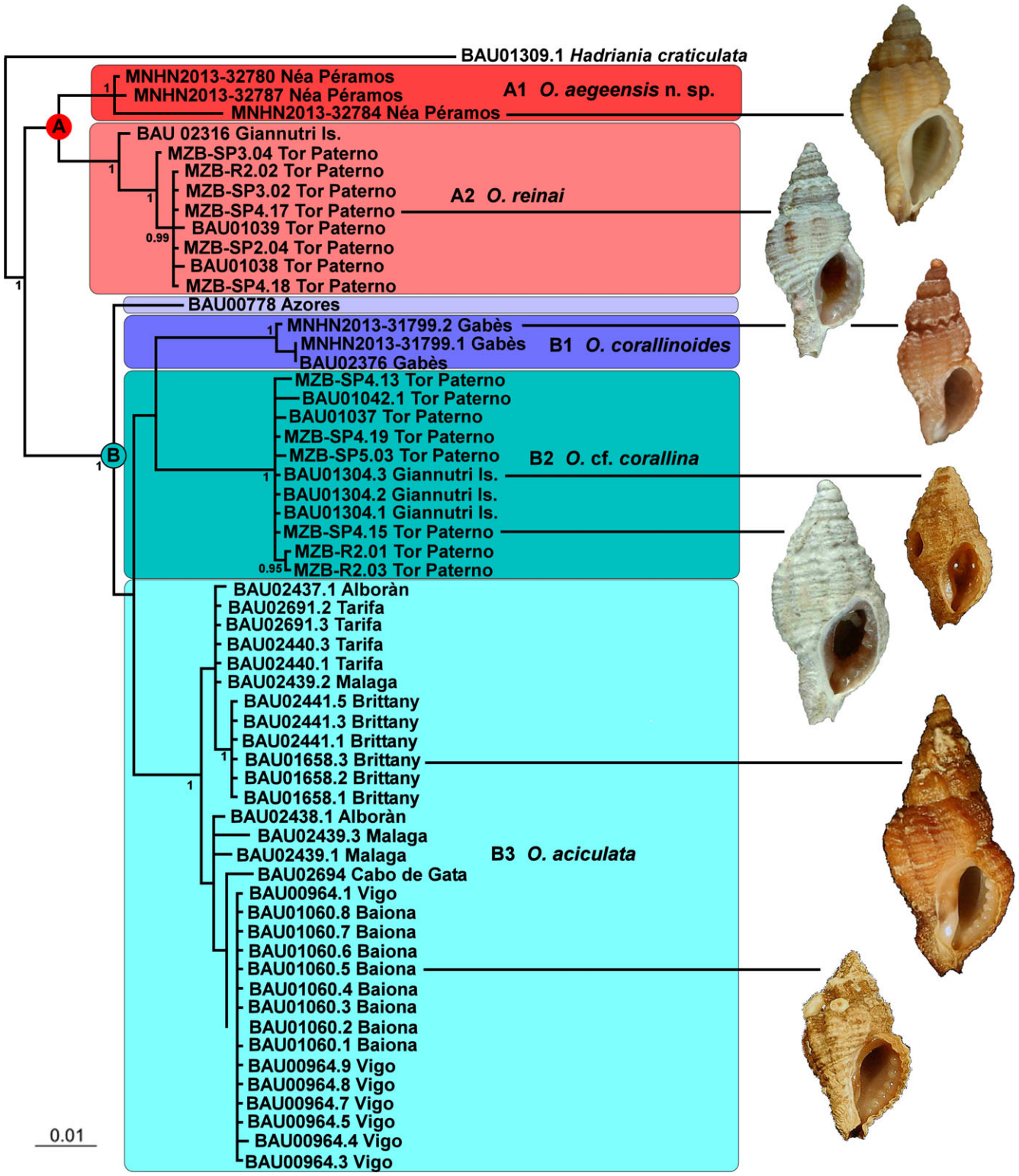

Figure 2. Phylogenetic relationships among the sequenced specimens. Consensus tree obtained after a Bayesian analysis by BEAST (same topology retrieved by MrBayes), showing only PP > 0.95. MOTUs consistently identified by distance- and tree-based methods (A1–B3) are enclosed in squares. Representative shells of vouchers are not to scale.

species genetically identifiable, but not completely fitting the observed morphological variation, and probably with a geographical pattern consistent with their nonplanktotrophic development. The presence of a new species in the Aegean Sea, the specimen from the Azores belonging to a distinct species and the two morphotypes (large and small) in the Mediterranean Sea (conspecific in *O. aciculata*, but with a still unresolved taxonomic status in *O.* cf. *corallina* in the Tyrrhenian Sea), all indicate that denser and wider geographic coverage of molecular sampling is required to reveal the full biodiversity of this group in the eastern Atlantic Ocean.

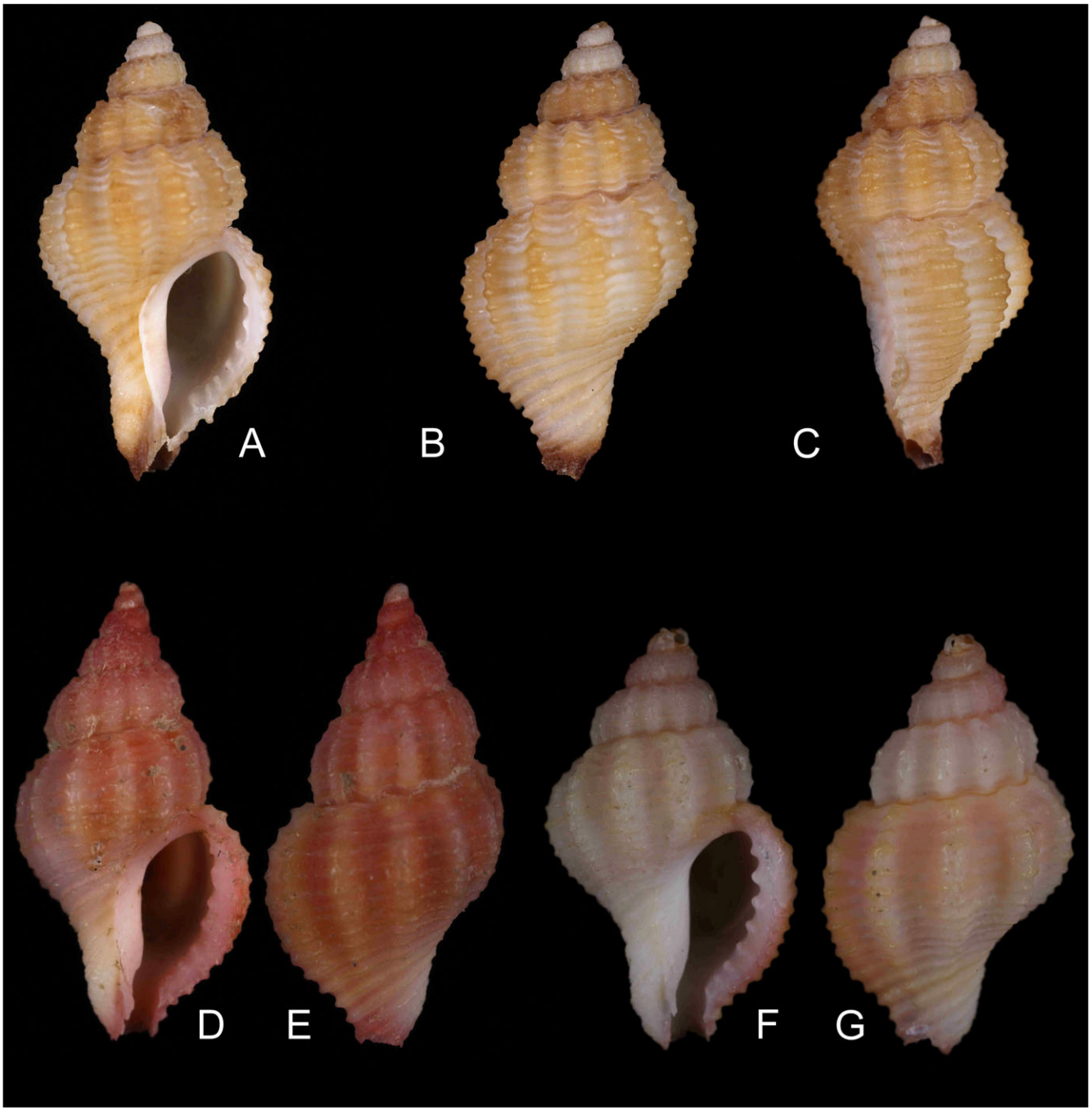

Figure 3. *Ocinebrina aegeensis* n. sp., Néa Péramos (Greece). **A–C.** Holotype (MNHN-IM-2013-32780, 11.8 mm). **D, E.** Paratype (MNHN-IM-2013-32784, 8 mm). **F, G.** Paratype (MNHN-IM-2013-32787, 7.5 mm).

SYSTEMATIC DESCRIPTIONS

Family MURICIDAE Rafinesque, 1815
Subfamily OCENEBRINAE Cossman, 1903

Genus *Ocinebrina* Jousseaume, 1880

Ocinebrina Jousseaume, 1880: 335 (type species *Murex corallinus* Scacchi, 1836 by original designation [misspelled *Fusus coralinus* by Jousseaume, 1880]).

Corallinia Bucquoy & Dautzenberg, in Bucquoy, Dautzenberg & Dollfus, 1882: 24 (type species *Murex aciculatus* Lamarck, 1822 by original designation).

Remarks: Distinction of *Ocenebra* and *Ocinebrina* has largely been based on the work of Mclean (1996), who characterized the former by the repeated formation of an apertural varix (a thick lip with labial denticles) and the simultaneous sealing of the siphonal canal. Although Houart (2001) raised doubts on the validity of these morphological characters, he still retained *Ocinebrina* as a separate genus. Recently, Barco *et al.* (2017) have shown that the two genera should be maintained, although with different scopes than in traditional usage. Within this framework, the true identities of the type species of the nominal taxa in ocenebrines is of particular relevance. Jousseaume (1880) introduced the new genus *Ocinebrina* with type species '*Fusus coralinus* Scacchi', a chrysonym and incorrect spelling for *Murex corallinus* Scacchi, 1836. According to the present data, Mediterranean

specimens corresponding to *M. corallinus* Scacchi may be distinct from the North Atlantic ones ascribed to *M. aciculatus* Lamarck, 1822 and may thus represent the actual type species of the genus. On the other hand, *Corallinia* Bucquoy & Dautzenberg, 1882 has *M. aciculatus* as type species by original designation. Here, the authors Bucquoy & Dautzenberg (in Bucquoy, Dautzenberg & Dollfus, 1882) may have confused two species, if an exclusively Mediterranean *O. corallina* is proved to be distinct from a mainly Atlantic and western Mediterranean *O. aciculata* (see above). If this is the case, it would be wise to designate (under ICZN, 1999: art. 70.3) *M. corallinus* as type species (making *Corallinia* an objective synonym of *Ocinebrina*).

Dentocenebra Monterosato, 1917 must be removed from the synonymy of *Ocinebrina* and moved into that of *Ocenebra*, since its type species is *Purpura edwardsii* (as *Murex edwardsii*) (Monterosato, 1917; by original designation).

Consistent diagnostic features of the two genera include: *Ocinebrina* develops a labral tooth that is lacking in *Ocenebra*; *Ocinebrina* lacks the 3–5 nodose/spinose varices of *Ocenebra*; the animal is reddish in *Ocinebrina*, but whitish in *Ocenebra*.

***Ocinebrina* cf. *corallina* (Scacchi, 1836)**

(Fig. 4J, K)

Murex corallinus Scacchi, 1836: 12 (original type locality Cuma, Italy; locality of neotype MZN Z7010 (designated by Crocetta *et al.*, 2012) Scacchietello, Baia, Naples, Italy).

Fusus acrisius Nardo, 1847: 59–60 (Gulf of Venice, Italy; 13 syntypes MSNVE 21980).

Fusus titii Stossich, 1865: 11 (northern Adriatic Sea; no type material present in MSNT: A. Colla, personal communication).

Murex subaciculatus Locard, 1886: 164 (Toulon, France; 2 syntypes MNHN-IM-2000-26502 (http://coldb.mnhn.fr/catalognumber/mnhn/im/2000-26502); the taxon is based on Locard's original material from Toulon, but also on *O. aciculata* as figured by Hidalgo, 1870, pl. 13, figs 7, 8, which is probably in MNCN).

Material examined: The available type material and samples from Morocco, Spain, Tunisia, Corsica, Italy, Croatia and Slovenia (see Supplementary Material).

Distribution: Mediterranean Sea.

Remarks: We have not sequenced specimens from the Adriatic; therefore, the actual identity of *Fusus acrisius* and *F. titii* as synonyms of *O. corallina* is hypothesized, pending confirmation by genetic data. The same holds for samples from the westernmost Mediterranean (putatively all ascribed to *O. aciculata*) until the precise ranges of *O. corallina* and *O. aciculata* are identified. The neotype of *M.corallinus* was designated by Crocetta *et al.* (2012) as a shell of 11.9 × 6.5 mm from Naples, thus representing the large central Mediterranean morphotype (whereas small morphotype shells attain a maximum height of 7 mm). Morphological features distinguishing *O. corallina* (large morphotype) and *O. aciculata* are hardly to be found, with the possible exceptions of *O. corallina* (large morphotype) being frequently smaller, occasionally broader and more cylindrical and with pinkish protoconch and first teleoconch whorl (Houart, 2001).

Position of diagnostic nucleotides in alignment: 13 (C), 100 (G), 187 (C), 208 (G), 214 (G), 271 (G), 292 (C), 318 (C), 544 (A), 589 (C).

***Ocinebrina aciculata* (Lamarck, 1822)**

(Fig. 4E, F, I)

Murex aciculatus Lamarck, 1822: 176 (near Vannes, France, Atlantic Ocean; no type material present in MNHN, or in MHNG (Y. Finet, personal communication)).

Murex inconspicuus Sowerby G.B. II, 1841: 5 (67), fig. 81 (Jersey, English Channel; no type material present in NHMUK (R. Houart, unpublished data)).

Murex pistacia Reeve, 1845: pl. 34, sp. 174 (3 syntypes NHMUK 1972021; 2 syntypes figured by Houart, 2001: 70, figs 78–79; unknown locality).

Ocinebra wardiana Baker, 1891: 134, pl. 11, fig. 5 (holotype CAS 20698; figured by Vokes, 1994: 120, fig. 11; original locality 'Australia', here corrected to North Atlantic).

Material examined: Available type material and shells and specimens from Channel Is, Portugal, France, Madeira, Azores, Canary Is, Morocco and Spain (see Supplementary Material).

Distribution: Northern Atlantic European coasts up to Scilly and Channel Is to north and at least to southern Spain and Alboràn Sea to south.

Remarks: Specimens morphologically similar to *O. aciculata* and *O. corallina* are found further south to Mauritania and northern Senegal and also in all the islands of Macaronesia; however, as suggested by the extreme divergence of the specimen BAU00778 from the Azores that represents a distinct evolutionary lineage (see Results and Discussion), their identity cannot be ascertained without genetic data. The lack of a clear locality for *Murex pistacia* and *Ocinebra wardiana* make it difficult to identify them with confidence. We correct the evidently wrong locality of *Ocinebra wardiana* (see synonymy above and discussion by Vokes, 1994) to North Atlantic (ICZN, 1999: Art 76.A.2), which stabilizes its synonymy with *O. aciculata*.

Position of diagnostic nucleotides in alignment: 79 (C), 109 (A), 130 (G), 641 (C).

***Ocinebrina corallinoides* Pallary, 1912**

(Figs 4G, 5B)

Ocinebrina corallinoides Pallary, 1912: 221, unnumbered pl., fig. 48 (near Sfax, Gulf of Gabès, Tunisia; types not found).

Ocinebrina aciculata exilis Houart, 2001: 62–63, figs 8, 9, 167, 168, 324, 325 (NW of Bou Grara Sea, Gulf of Gabès, Tunisia; holotype MNHN IM-2000-362).

Ocinebrina buzzurroi Cecalupo & Mariani, 2008 in Cecalupo *et al.*, 2008: 90–91, pl. 43: figs 1–7 (Borj el Hissar, Kerkennah Is, Gulf of Gabès, Tunisia; holotype MNHM 33489).

Material examined: Available type material and shells and specimens from Gulf of Gabès, Tunisia (see Supplementary Material).

Distribution: Currently known only from Gulf of Gabès, Tunisia (Pallary, 1912; Houart, 2001; Cecalupo *et al.*, 2008).

Remarks: Although this has sometimes been considered as a subspecies of *O. aciculata* (e.g. Nordsieck, 1968: 120, pl. 19, fig. 71.11), our genetic data unequivocally indicate that it is a distinct species.

Position of diagnostic nucleotides in alignment: 1 (G), 14 (C), 22 (A), 79 (G), 212 (C), 220 (C), 268 (G), 313 (C), 355 (G), 370 (C), 484 (G), 541 (G), 580 (A).

***Ocinebrina reinai* Bonomolo & Crocetta, 2012**

(Fig. 4D)

Ocinebrina reinai Bonomolo & Crocetta, in Crocetta *et al.*, 2012: 182, figs 1K, L; 2A–F, I–L (Procida I., Italy, 40 m depth, 40°46'N, 014°01'E; holotype MNHN-IM-2000-24566 (http://coldb.mnhn.fr/catalognumber/mnhn/im/2000-24566); paratypes: A MZN-Z7008 with radula and operculum mounted and coated, from type locality; B-C SZN-MOL 677 (2 shells), D-F SZN-MOL 686 (3 shells), all Gulf of Naples, Italy).

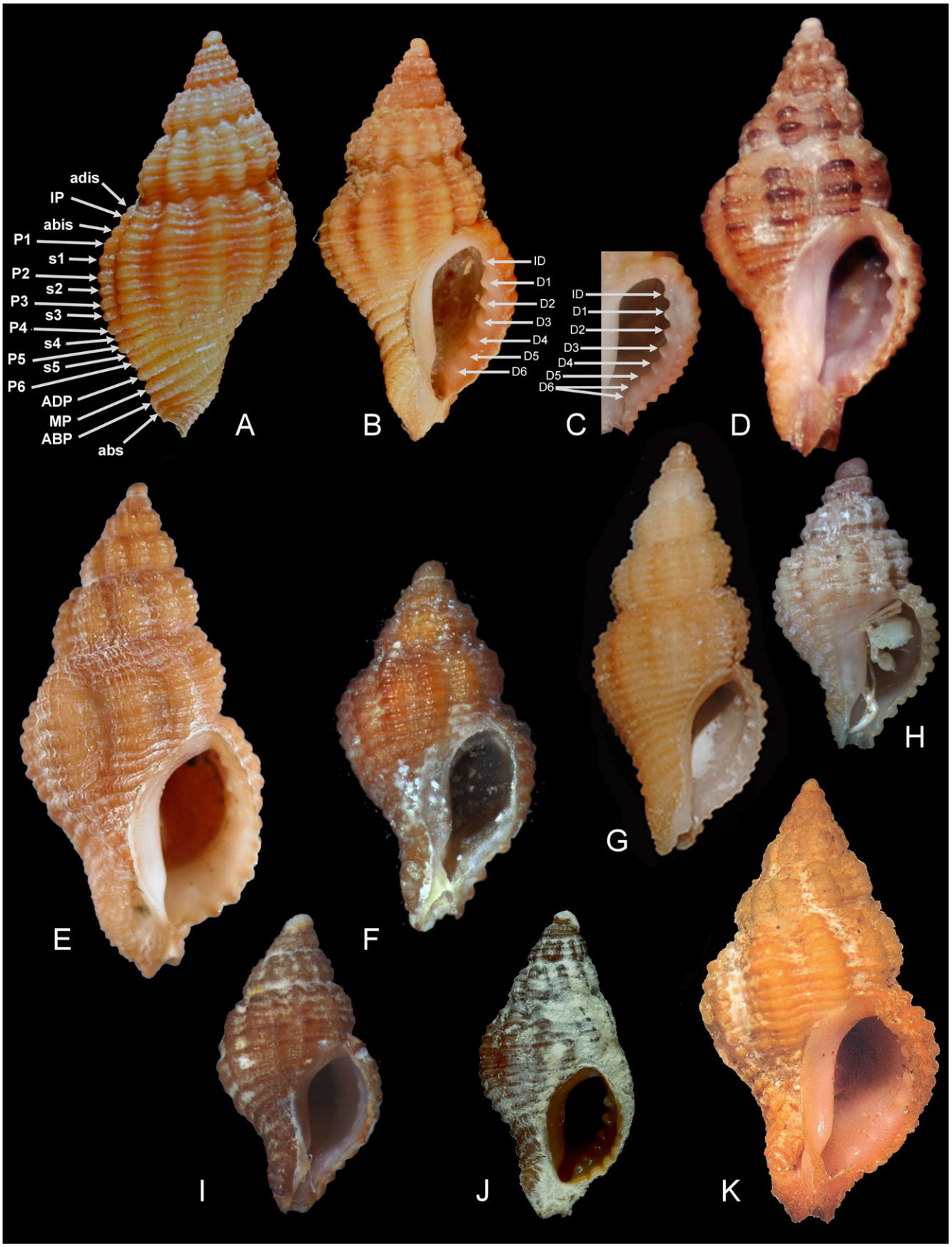

Figure 4. Morphological variation in *Ocinebrina* species. **A–C.** *O. aegeensis* n. sp., Néa Péramos, Greece. For abbreviations for sculptural terminology, see text. **A, B.** Paratype (MNHN-IM-2013-32781,9.5 mm). **C.** Aperture of paratype (MNHN-IM-2013-32787, 7.5 mm). **D.** *O. reinai* Bonomolo & Crocetta,

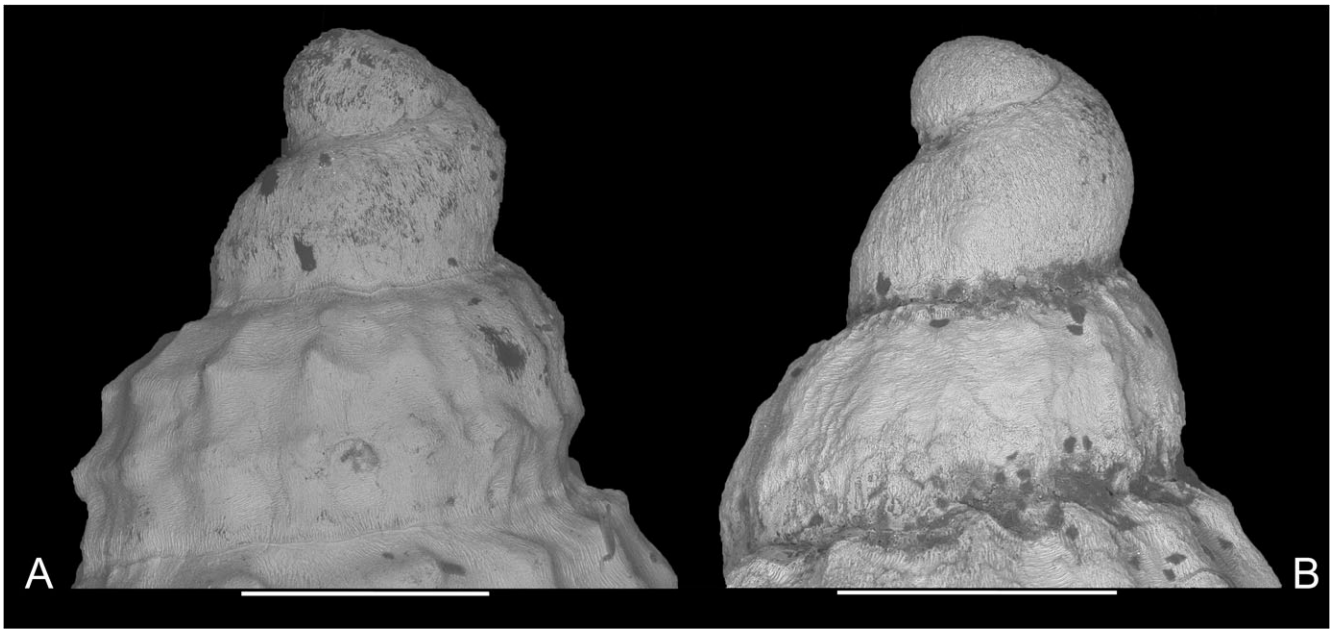

Figure 5. Protoconchs of *Ocinebrina* species. **A.** *O. aegeensis* n. sp., Néa Péramos, Greece (paratype, MNHN-IM-2013-32781). **B.** *O. corallinoides*, Djerba, Tunisia. Scale bars = 500 μm.

Material examined: Type material and shells and specimens from Tuscany, Latium, Campania and Sardinia (Italy) (see Supplementary Material).

Distribution: Known with certainty from central Mediterranean Sea, from 43°33′N to 40°34′N and from 010°13′E to 014°12′E.

Remarks: It differs from *O. corallina*, *O. aciculata* and *O. corallinoides* mainly in the dark spots on the ribs, and in the differently sized primary and secondary cords and smaller threads.

Position of diagnostic nucleotides in alignment: 40 (T), 172 (G), 190 (G), 206 (C), 223 (G), 562 (A/G), 577 (G).

***Ocinebrina aegeensis* Aissaoui, Barco & Oliverio new species**

(Figs 3A–G, 4A–C, 5A, 6)

Type material: Holotype (MNHN-IM-2013-32780, 11.8 mm) and two paratypes assayed genetically (MNHN-IM-2013-32784, 8 mm, MNHN-IM-2013-32787, 7.5 mm); 6 paratypes not sequenced (MNHN-IM-2013-32779, 5 mm; MNHN-IM-2013-32781, 9.5 mm; MNHN-IM-2013-32782, 8.5 mm; MNHN-IM-2013-32783, 7 mm; MNHN-IM-2013-32785, 9 mm; MNHN-IM-2013-32786, 7.5 mm) all from type locality Nea Peramos, Greece, Aegean Sea, 40 m (leg. K. Papavasileiou).

ZooBank registration: urn:lsid:zoobank.org:pub:F4957494-7575-49E9-972B-34999A117A50

Material examined: Type material and putative additional samples from Greece and Turkey (see Supplementary Material).

Diagnosis: shell of medium size, fusiform, not slender; protoconch paucispiral, slender. Teleoconch sculpture with equally sized primary and secondary cords. Diagnostic nucleotides in COI alignment: 70 (G), 263 (T), 539 (C).

Distribution: Only known with certainty from type locality. We assume that the eastern Mediterranean population of *O. corallina*-like small mussel drills may belong to the new species (see Material Examined), but this needs to be tested genetically.

Description (data of holotype in parentheses): Shell of medium size for genus, length 7.5–11.8 mm, fusiform, solid. Protoconch (MNHN-IM-2013-32781) paucispiral, 1.25 whorls, barely keeled, slender, corroded in type material with only traces of microsculpture of small granules. Teleoconch uniformly pinkish or pale orange; 4–5 (5) convex whorls, slightly elongate, rounded, last whorl about ¾ of total length; suture impressed. Spiral sculpture of 6 low, weakly squamous, rounded primary cords and approximately equally sized secondary cords. P1 and P2 starting in early teleoconch, IP at half of first teleoconch whorl; s1, s2 and abis at half of second teleoconch whorl. Last teleoconch whorl with adis, IP, abis, P1, s1, P2, s2, P3, s3, P4, s4, P5, s5, P6, ADP, MP, ABP and abs. Primary and secondary cords approximately of same strength, except s4 and s5 slightly narrower. Axial sculpture of 11–13 (11) low, weak, broad, rounded ribs on all whorls. Outer lip weakly crenulated, with 7–8 (8) internal denticles, usually ID, D1–D6 (D6 usually split); labral tooth not observed in type material (but present in other material examined). Labral varix nodose, narrow, rounded. Columellar lip expanded ventrally, smooth, adherent adapically, erect abapically. Aperture relatively narrow, elongate-ovate, pinkish to whitish within. Siphonal canal short, narrow, straight, ventrally sealed. Animal reddish. Operculum corneous, pale to dark brown, D-shaped/ovate, with subterminal nucleus surrounded by many concentric ridges. Radula (MNHN-IM-2013-32786): sickle-shaped lateral teeth with slightly broader base; rachidian bearing short, thick central cusp and longer, thicker

2012, Procida I., Italy; holotype (MNHN-IM-2000-24566, 9.9 mm). **E, F, I.** *O. aciculata* (Lamarck, 1822). **E.** Erquy, Brittany, France (RHP, 15.8 mm). **F.** Alboràn, Spain (BAU02438.1, 7.2 mm). **I.** Cabo de Gata, Spain (BAU02694, 4.9 mm). **G.** *O. corallinoides* Pallary, 1912, Djerba, Tunisia (RHP, 9.9 mm). **H.** *O.* sp., Fajal I., Azores (MOP, 5.2 mm). **J.** *O.* cf. *corallina* (Scacchi, 1836), Secche di Tor Paterno, Italy (MZB-R2.01, 5.2 mm). **K.** *O. corallina* (Scacchi, 1836), Scacchetiello, Baia, Italy (neotype of *Murex corallinus* Scacchi, MZN Z7010, 11.9 mm).

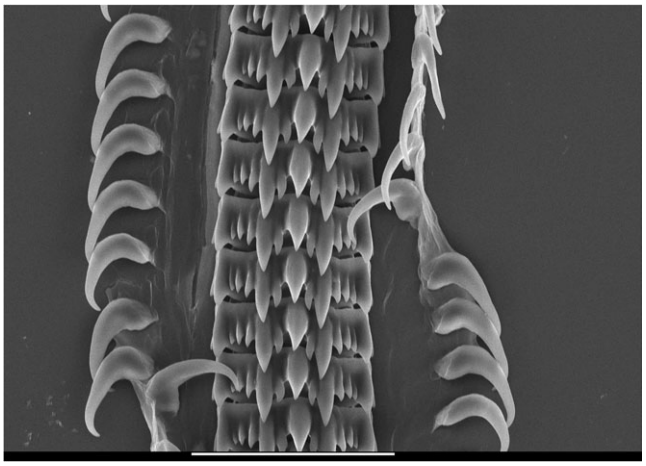

Figure 6. Radula of *Ocinebrina aegeensis* n. sp., Néa Péramos, Greece (paratype, MNHN-IM-2013-32786). Scale bar = 100 μm.

lateral cups, with short, thick inner lateral denticle on each side of base; marginal area with short denticles and thick, short marginal cusp.

Remarks: *Ocinebrina aegeensis* is very similar to the other species of the complex. A labral tooth has not been observed in the type material, but is present in samples from the type locality and from Turkey (both in FCP). Very subtle differences can be found in comparisons with other species of the complex: *O. aciculata* and *O. corallina* differ in their more bulbous, dome-shaped protoconch (cf. slender in *O. aegeensis*); *O. reinai* also has a more bulbous protoconch and a teleoconch spiral sculpture with secondary cordlets of smaller size (cf. all spirals of same size in *O. aegeensis*); *O. corallinoides* has a more slender shell.

Position of diagnostic nucleotides in alignment: see Diagnosis.

***Ocinebrina* sp.**

(Fig. 4H)

Material examined: specimen used for sequencing (BAU00778), from Azores and additional shells and specimens from Azores, Madeira and Canary Is (see Supplementary Material).

Remarks: Only one specimen from the Azores has been assayed genetically and this proved to represent a distinct species. Therefore, material from the other Macaronesian archipelagos needs to be tested to ascertain its taxonomic status. We have been unable to find morphological diagnostic features between the few Macaronesian specimens and the other Atlantic specimens ascribed to *O. aciculata* s. s.

Position of diagnostic nucleotides in alignment: 94 (G), 97 (G), 197 (C), 244 (A), 337 (C), 547 (A).

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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