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Molecular data reveal cryptic lineages within the northeastern Atlantic and Mediterranean small mussel drills of the *Ocinebrina edwardsii* complex (Mollusca: Gastropoda: Muricidae)

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We used a molecular phylogenetic approach to investigate species delimitations and diversification in the mussel drills of the *Ocinebrina edwardsii* complex by means of a combination of nuclear (internal transcribed spacer 2, ITS2) and mitochondrial [cytochrome oxidase subunit I (COI) and 16S] sequences. Our sample included 243 specimens ascribed to seven currently accepted species from 51 sites. Five of the samples were from either the type locality of a nominal species or a close nearby locality (*O. edwardsii* from Corsica, *O. carmelae* and *O. piantonii* from the Kerkennah Islands, *O. hispidula* from the Gulf of Gabès and *O. leukos* from the Canary Islands), one from the inferred original locality (*O. ingloria* from Venice Lagoon), and specimens assigned in the recent literature to *O. nicolai*. We used a combination of distance- and tree-based species delimitation methods to identify Molecular Operational Taxonomic Units (MOTUs) to compare with the *a priori* species identifications. The consensus tree obtained by BEAST on the COI alignment allows the recognition of several distinct clades supported by the three species delimitation methods employed. The eight-MOTUs scenario, shared by the Automatic Barcode Gap Discovery (ABGD) and Generalized Mixed Yule-Coalescent (GMYC) methods, comprises the following major clades: clade A contains the south Tunisian species *Ocinebrina piantonii* Cecalupo, Buzzurro & Mariani from which the sympatric taxon *O. carmelae* Cecalupo, Buzzurro & Mariani (new synonym) cannot be separated; clades B and C bring together all populations from the Aegean Sea and some from the Ionian Sea, respectively; clade D groups, on the one hand, the south Tunisian samples morphologically assigned to *O. hispidula* Pallary and, on the other, Atlantic and Alboran Sea samples (including the Canarian taxon *O. leukos* Houart); clade E includes a sample from the type locality of *O. edwardsii* and several samples from the Tyrrhenian Sea; clades F and G correspond to a few samples from the Venice Lagoon and the Tyrrhenian Sea, respectively; clade H groups the bulk of samples from the Adriatic Sea, including samples from the Venice Lagoon morphologically identified as *Ocinebrina ingloria* (Crosse), and some from the Ionian Sea. No final conclusions could be reached to reconcile the currently recognized morphological taxa with the clades suggested by the COI data. The geographical structure proposed by the mitochondrial markers is similar to that found in other marine invertebrates and partially corresponds to the species defined by shell characters. We propose here a framework for the revision of the *Ocinebrina edwardsii* species complex, suggesting a geographical pattern for the diversification of this group in the studied area.

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INTRODUCTION

The existence of sibling species among marine invertebrates is a widely recognized phenomenon (Knowlton, 1993), emphasized by the increasing use of DNA-based methods for species delimitation (Vogler & Monaghan, 2007). These techniques are contributing to the steady number of new marine species described (Costello, Wilson & Houlding, 2012), allowing the recognition of unknown diversity (Caron *et al*., 2012) and testing the status of known species (Templeton, 2001).

Cryptic diversity, however, does not surface exclusively in poorly known lineages (Richards *et al*., 2012) or in organisms from relatively unexplored areas (Goetze, 2003). Even within the well-known fauna of the Mediterranean Sea, with its long-lasting tradition of taxonomic studies, many species have turned out to be complexes of sibling lineages (Boisselier-Dubayle & Gofas, 1999; Carreras-Carbonell, Macpherson & Pascual, 2005; Calvo *et al*., 2009; Sá-Pinto *et al*., 2010; Xavier *et al*., 2011). Molluscan taxonomy, in particular, historically has been almost entirely based on shell characters, which are still commonly used even though morphological variation caused by adaptation to environmental pressures has been documented (Vermeij, 1978). Consequently, traditional shell-based taxonomic practice has proved to be problematic for molluscs, and particularly for morphologically diverse groups. Therefore, in several of the most recent studies on marine molluscs, the combination of molecular and traditional approaches has proven to be successful to resolve taxonomically doubtful groups (e.g. Puillandre *et al*., 2009; Claremont *et al*., 2011; Zou, Li & Kong, 2012).

The genus *Ocinebrina* Jousseaume, 1880 is widely distributed in the Mediterranean Sea, northeastern Atlantic and northeastern Pacific, with species commonly called small mussel drills. It includes 23 currently accepted living species: 16 in the northeastern Atlantic and Mediterranean and seven in the northeastern Pacific (WoRMS: Appeltans *et al*., 2012, at http://www.marinespecies.org – accessed on 13 March 2013). The Pacific species are often attributed to other genera, such as *Ocenebra* Gray, 1847 and *Urosalpinx* Stimpson, 1865 (two of the *c*. 29 genera in the subfamily Ocenebrinae Cossmann, 1903: see, for example, Radwin & D'Attilio, 1976; Vermeij & Vokes, 1997; but see WoRMS – accessed on 13 March 2013 – for an updated listing), and their relationships will be dealt with in an ongoing study on the phylogenetics of the Ocenebrinae Cossmann, 1903. The northeastern Atlantic and Mediterranean species are traditionally divided into two groups of morphological affinities: the *O. aciculata* complex and the *O. edwardsii* complex. The *O. aciculata* complex, recently revised by Crocetta *et al*. (2012), includes *O. aciculata* (Lamarck, 1822), *O. corallinoides* Pallary, 1912 and *O. reinai* Bonomolo and Crocetta, 2012. The *O. edwardsii* complex includes the following species: *O. edwardsii* (Payraudeau, 1826), *O. carmelae* Cecalupo, Buzzurro & Mariani, 2008, *O. helleri* (Brusina, 1865), *O. hispidula* Pallary, 1904, *O. hybrida* (Aradas & Benoît, 1876), *O. ingloria* (Crosse, 1865), *O. inordinata* (Houart & Abreu, 1994), *O. leukos* Houart, 2000, *O. miscowichae* Pallary, 1920, *O. nicolai* Monterosato 1884, *O. paddeui* Bonomolo & Buzzurro, 2006, *O. piantonii* Cecalupo, Buzzurro & Mariani, 2008 and *O. purpuroidea* Pallary, 1920 (see Table 2 for a state of the art nomenclature). *Ocinebrina edwardsii* is traditionally considered to be a common species and one of the most variable European muricids, as witnessed also by the existence of numerous names established for the various morphotypes (see Table 2), whose status has been debated extensively (see, for example, Houart, 2001). Its distribution spans the entire Mediterranean Sea and, in the Atlantic Ocean, it is recorded up to the Bay of Biscay and to the Canary Islands. The shell reaches (and sometimes exceeds) 20 mm in length with up to 5–5.5 teleoconch whorls, has a sealed siphonal canal, five to six small denticles on the internal part of the outer lip and variable coloration. The protoconch is paucispiral (1.25–1.75 whorls) and its morphology is similar to that of *O. aciculata*. Therefore, a development similar to that described for *O. aciculata* by Franc (1940) can be assumed, i.e. entirely intracapsular or with a very short pelagic phase.

The combination of the high morphological variability of the shell among and within species over a wide geographical range (see Supporting Information Figs S4-S6) has contributed to the confused taxonomy in the *O. edwardsii* complex. Here, we have applied DNA-based methods to a sample including 243 specimens from the northeastern Atlantic and the Mediterranean Sea (including the area of the type locality of *Purpura edwardsii*, Corsica) in order to define the limits of this species and check for the presence of cryptic lineages. We also included specimens morphologically ascribed to some of the nominal species of the same complex to investigate their relationships with *O. edwardsii*: *O. ingloria* from the Venice Lagoon, *O. hispidula* from the Gulf of Gabès, *O. carmelae* and *O. piantonii* from the Kerkennah Islands, *O. leukos* from the Canary Islands and *O. miscowichae* from southern Morocco. In addition, we also processed specimens assigned in the recent literature to *O.* cf. *nicolai* and *O. nicolai* from Spain and Portugal (Rolán, 1983; Afonso *et al*., 2011; Gofas, 2011), although their morphology was not perfectly matching that of the nominal taxon. Using three

markers [mitochondrial cytochrome oxidase subunit I (COI) and 16S, and the nuclear internal transcribed spacer 2 (ITS2)], we applied several methods of species delimitation and used the results to discuss the status of the *O. edwardsii* complex. We used the ocinebrine *Nucella lapillus* (Linné, 1758) as an outgroup in the phylogenetic analyses, and also included specimens of *Ocinebrina aciculata* and *O. reinai* as recently defined by Crocetta *et al*. (2012).

MATERIAL AND METHODS

Sample data

The specimens used in this work were collected by hand, snorkelling or scuba diving. Sample localities are summarized in Table 1 (see also Figs 1 and 4) with their tissue-processing codes. Each specimen was identified on collection and fixed in 96–100% ethanol. A piece of tissue was later dissected from the foot for DNA extraction.

DNA sequencing and alignment

DNA extraction was performed after tissue digestion in proteinase K using a phenol–chloroform protocol (Hillis, Moritz & Mable, 1996) with slight modifications as described by Oliverio & Mariottini (2001b). A fragment of the mitochondrial COI was amplified using the universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994), part of the mitochondrial 16S was obtained with the primers 16SA (Palumbi *et al*., 2002) and CGLeu^{UUR}R (Hayashi, 2005), and the entire nuclear ITS2 rRNA gene was amplified using the primers ITS-3d and ITS-4r (Oliverio & Mariottini, 2001a). Polymerase chain reaction (PCR) amplifications were performed in 25 μL containing 2.5 μL of BIOLINE 10× buffer, 0.5 μL of 10 mM deoxynucleoside triphosphates (dNTPs) mix, 0.4 μL of each primer (10 mM), 2.5–3 μL of 50 mM $MgCl_2$, 1 U of BIOLINE TaqPolymerase and 0.5–1 μL of genomic DNA.

The DNA fragments were amplified with an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C (COI and 16S) and 62 °C (ITS2) for 40 s and extension at 72 °C for 50 s. These cycles were followed by an extension at 72 °C for 10 min. PCR products were purified with the ExoSAP protocol. A mix of 2 μL containing 20 U μL^{-1} Exonuclease I (New England Biolabs, Ipswich, MA, USA) and 1 U μL^{-1} Shrimp Alkaline Phosphatase (Roche, Basel, Switzerland) was used to purify 5 μL of PCR product. Fragments were sequenced by Macrogen Inc. (Seoul, South Korea) using the same PCR primers in BigDye terminator cycling conditions (Applied Biosystems, Carlsbad, CA, USA). Reacted products were purified using ethanol precipitation and run using an automatic sequencer AB3730XL (Applied Biosystems).

Forward and reverse sequences were assembled and reciprocally edited with Sequencher (v. 4.1.4; Gene Codes Corporation, Ann Arbor, MI, USA). The COI dataset was aligned manually and translated into amino acids to check for stop codons. The 16S and ITS2 dataset was aligned with MAFFT (Katoh *et al*., 2002) using the Q-INS-i algorithm (Katoh & Toh, 2008), which accounts for secondary structures in the sequences. The nucleotide substitution models were selected by jModelTest (Posada, 2008) using the Bayesian Information Criterion (Schwarz, 1978). The likelihood scores for 88 possible substitution models were calculated with PhyML (Guindon & Gascuel, 2003) using a tree obtained separately for each model with BIONJ (Gascuel, 1997) employing a JC model (Jukes & Cantor, 1969).

Species delimitation

Three methods of species delimitation were applied to identify Molecular Operational Taxonomic Units (MOTUs) (Blaxter, 2004) in the COI dataset: the Automatic Barcode Gap Discovery (ABGD) method (Puillandre *et al*., 2012a), the Generalized Mixed Yule-Coalescent (GMYC) technique (Pons *et al*., 2006) and statistical parsimony network analysis (Posada & Crandall, 2001; Templeton, 2001).

ABGD is a distance-based method designed to detect the so-called 'barcode gap' in the distribution of pairwise distances calculated in a COI alignment (Puillandre *et al*., 2012a, b). A distance value corresponding to the most reliable gap was used to group the sequences in MOTUs. The web-based ABGD program (available at http://wwwabi.snv.jussieu.fr/public/abgd/) was employed to generate a preliminary partition of sequences, using a distance matrix calculated from the COI dataset. The COI alignment was submitted and processed in ABGD (excluding the outgroup *O. miscowichae*) using the Kimura two-parameter (K2P) model (Kimura, 1980) and the following settings: a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 25 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 0.1.

The GMYC method identifies the species–population boundary in a sample of multiple species and populations using a time-calibrated (ultrametric) tree and a likelihood statistic (Pons *et al*., 2006). The boundary represents the shift of the tree branching rates from a Yule (interspecific) to a coalescent (intraspecific) model, and is estimated as the likelihood peak of the transition along the branches. An ultrametric tree based on the COI alignment (excluding the outgroup *O. miscowichae*) was generated with

Table 1. Collection sites with relevant BAU voucher codes, number of assayed specimens (*N*), their preliminary identification on collection and the Molecular Operational Taxonomic Unit (MOTU) in which they were included, according to the four-MOTUs (I–IV) and eight-MOTUs (A–H) hypotheses (see text). Vouchers from each sampling site are figured in Supporting Information Figs S4–S6.

BAU voucher code	*N*	Sampling locality, environmental data	Coordinates	*A priori* morphological identification	MOTU
902	5	Italy, Venice Lagoon, low tide on/under stones	45°17′18″N, 012°16′18″E	*O. ingloria*	IV H
903	5	Tunisia, Djerba Island, south-east of Adjim, 0.5 m depth, amidst *Posidonia* rhizomes	33°41′49″N, 010°47′25″E	*O. hispidula*	III D
904	5	Italy, Augusta, Megara harbour, 0–1 m depth, on/under stones	37°13′50″N, 015°13′27″E	*O. edwardsii*	II C
905, 923	3, 4	Italy, Marzamemi, harbour, 0–1 m depth, on/under stones	36°44′30″N, 015°07′03″E	*O. edwardsii*	II C
907	11	Italy, Siracusa, Punta Asparano, 1 m depth, cave, amidst calcareous algae	36°59′32″N, 015°15′41″E	*O. edwardsii*	II C
909	16	Italy, Lampedusa Island, Cala Francese, low depth, in cave	35°29′43″N, 012°37′26″E	*O. edwardsii*	II C
920, 1299	3, 7	Italy, Pozzuoli, 'o' valjone' harbour, 0.5 m depth, on/under rocks amidst algae	40°49′18″N, 014°07′08″E	*O. edwardsii*	III E, G
921	2	Italy, Bacoli, Punta Pennata, 0.5 m depth, amidst mussels	40°47′35″N, 014°05′11″E	*O. edwardsii*	III E
922	7	Italy, Marina di Schiavonea, 0–1 m depth, on/under rocks and amidst mussels	39°38′29″N, 016°34′00″E	*O. edwardsii*	IV H
924	4	Italy, Marsala, 0–1 m depth, under stones	37°47′50″N, 012°25′45″E	*O. edwardsii*	III E
927	6	Greece, Limnos Island, 0–1 m depth, on/under stones	39°49′58″N, 025°15′55″E	*O. edwardsii*	II B
936	3	Greece, Heraklion, Lygaria, Crete, 0–1 m depth, on/under stones	35°23′56″N, 025°01′41″E	*O. edwardsii*	II B
965, 1058, 1059, 1061, 1062	1, 1, 10, 3, 2	Spain, Bayona, Cabo Silleiro, low tide, on rocks	42°06′40″N, 008°53′57″W	*O. edwardsii* (partim) *O. nicolai* (partim) *sensu* Rolán (1983)	III D
959	4	Croatia, Črvar, 0–1 m depth, on/under stones	45°27′14″N, 013°31′03″E	*O. edwardsii*	IV H
960	6	Croatia, Katoro, 0–1 m depth, on/under stones	45°27′56″N, 013°30′31″E	*O. edwardsii*	IV H
961	4	Italy, Gallipoli, Punta Pizzo, 0–1 m depth, on/under stones	40°00′01″N, 017°59′37″E	*O. edwardsii*	IV H
962	3	Italy, Muggia, Punta Sottile, 0–1 m depth, on/under stones	45°36′20″N, 013°43′10″E	*O. edwardsii*	IV H
963, 1033	1, 5	Spain, Vigo, low tide, on rocks	42°13′52″N, 008°42′44″W	*O. edwardsii* (partim) *O. nicolai* (partim) *sensu* Rolán (1983)	III D
969	2	Italy, Marettimo Island, Cammello shoal, 10 m depth, under stones	37°59′21″N, 012°13′55″E	*O. edwardsii*	III E
1026	4	Italy, Campomarino, 0–1 m depth, on/under stones	40°18′03″N, 017°30′13″E	*O. edwardsii*	II C
1029	2	Croatia, Umag, Sol Stella Maris, 0–1 m depth, on/under stones	45°27′02″N, 013°30′45″E	*O. edwardsii*	IV H
1044	5	Tunisia, Sfax, fishing nets residuals, from *Posidonia* meadows	34°42′N, 010°42′E	*O. hispidula*	III D
1045	5	Italy, Pugnochiuso, 0–1 m depth, on/under stones	41°47′14″N, 016°11′22″E	*O. edwardsii*	IV H
1046, 1293	5, 7	Italy, Pellestrina, Venice Lagoon, low tide under stones	45°15′13″N, 012°17′57″E	*O. edwardsii*	III F IV H
1048	5	Tunisia, Djerba Island, Borj Kastil, 0.5 m depth, amidst *Posidonia* rhizomes	33°41′00″N, 010°51′27″E	*O. hispidula*	III D
1049	6	Italy, Siracusa, harbour, 0.5 m depth, amidst mussels	33°04′N, 015°17′E	*O. edwardsii*	II C
1050	5	Croatia, Murter Island, 1–10 m depth, on/under rocks	43°47′48″N, 015°36′34″E	*O. edwardsii*	IV H
1051	1	Tunisia, Kerkennah Islands, Ra's Bu Numah, 0.5–0.8 m depth, amidst *Posidonia* rhizomes	34°47′00″N, 011°12′56″E	*O. piantonii*	I A
1052	1	Tunisia, Kerkennah Islands, Borj el-Hissar, −0.5 m, under stones	34°42′42″N, 011°09′06″E	*O. carmelae*	I A
1053	1	Tunisia, Kerkennah Islands, Ra's 'Āmir, −0.5 m, near a small stone	34°40′60″N, 011°07′00″E	*O. hispidula*	III D
1054	1	Tunisia, Kerkennah Islands, near Kraten, low tide in very quiet waters, sandy beach	34°43′N, 011°11′E	*O. hispidula*	III D
1055	7	Greece, Milos Island, low depth, on/under rocks	36°39′43″N, 024°25′41″E	*O. edwardsii*	III D
1063	4	Spain, O Grove, low depth, on/under rocks	42°30′N, 008°52′W	*O. edwardsii* (partim) *O. nicolai* (partim) *sensu* Rolán (1983)	III D
1064	1	Croatia, Cres Island, Punta Križa, low depth, on/under rocks	44°38′29″N, 014°30′20″E	*O. edwardsii*	IV H
1065	5	Portugal, Peniche, Peniche de Cima beach, very low tide, rocky bottom, under and on rocks	39°22′15″N, 009°22′18″W	*O. edwardsii*	III D
1106	4	Italy, Sardina, Santa Maria Navarrese, 3 m depth, on rocks	39°59′15″N, 009°41′26″E	*O. edwardsii*	III E
1107	3	Italy, Zannone Island, 10 m depth, on rocks	40°57′54″N, 013°03′26″E	*O. edwardsii*	III E
1109	3	Italy, Marettimo Island, Punta Mugnone, 18 m depth, under stones	37°59′15″N, 012°01′28″E	*O. edwardsii*	III E
1280	5	Spain, Benalmádena, 1–5 m depth, on rocks	36°35′03″N, 004°31′07″W	*O. edwardsii*	III D
1281	1	Spain, Calahonda, low tide, amidst rocks and algae	36°29′04″N, 004°41′08″W	*O. edwardsii*	III D
1282	2	Spain, Barbate, low tide, amidst rocks	36°11′00″N, 005°56′09″W	*O. nicolai sensu* Gofas (2011)	III D
1290	2	Spain, Tenerife Islands, La Caleta, low depth, on/under rocks,	22°28′00″N, 016°47′00″W	*O. leukos*	III D
1291	3	Spain, Tenerife Islands, Punta de Teno, −15 m	28°20′31″N, 016°55′23″W	*O. edwardsii*	III D
1294	1	Portugal, off Ponta da Piedade, low depth, on/under rocks	37°03′32″N, 008°38′54″W	*O. nicolai sensu* Afonso *et al.* (2011)	III D
1295	6	Italy, Cefalù, low tide on rocks	38°00′57″N, 014°06′55″E	*O. edwardsii*	III E
1296	3	France, Corsica, Porto Vecchio, Saint-Cyprien beach, 0-0.5 m depth, on granitic rocks	41°36′N, 009°17′E	*O. edwardsii*	III E
1298	6	Greece, Kavala, −0.5 m, under rocks	40°54′49″N, 024°22′42″E	*O. edwardsii*	II B
1300	4	Italy, Palinuro, tide level amidst algae	40°02′07″N, 015°17′07″E	*O. edwardsii*	III G
1308	3	Italy, Sardinia, Cagliari, Marina Piccola, harbour, −2 m, on rocks	39°11′28″N, 009°09′53″E	*O. edwardsii*	III E
1133	5	Morocco, Dakhla, low water, beach, on rocks	23°41′N, 015°55′W	*O. miscowichae*	–
1060	6	Spain, Bayona, Cabo Silleiro, low tide, on rocks	42°06′40″N, 008°53′ 57″W	*O. aciculata*	–
1037, 1042	1, 1	Italy, Tor Paterno MPA, −25 m	41°36′18″N, 012°20′30″E	*O. aciculata*	–
1038	1	Italy, Tor Paterno MPA, −25 m	41°36′21″N, 012°20′28″E	*O. reinai*	–
1039	1	Italy, Tor Paterno MPA, −20 m	41°36′13″N, 012°20′30″E	*O. reinai*	–
187	1	UK, Portobello, intertidal	55°57′N, 030°6′W	*Nucella lapillus*	–

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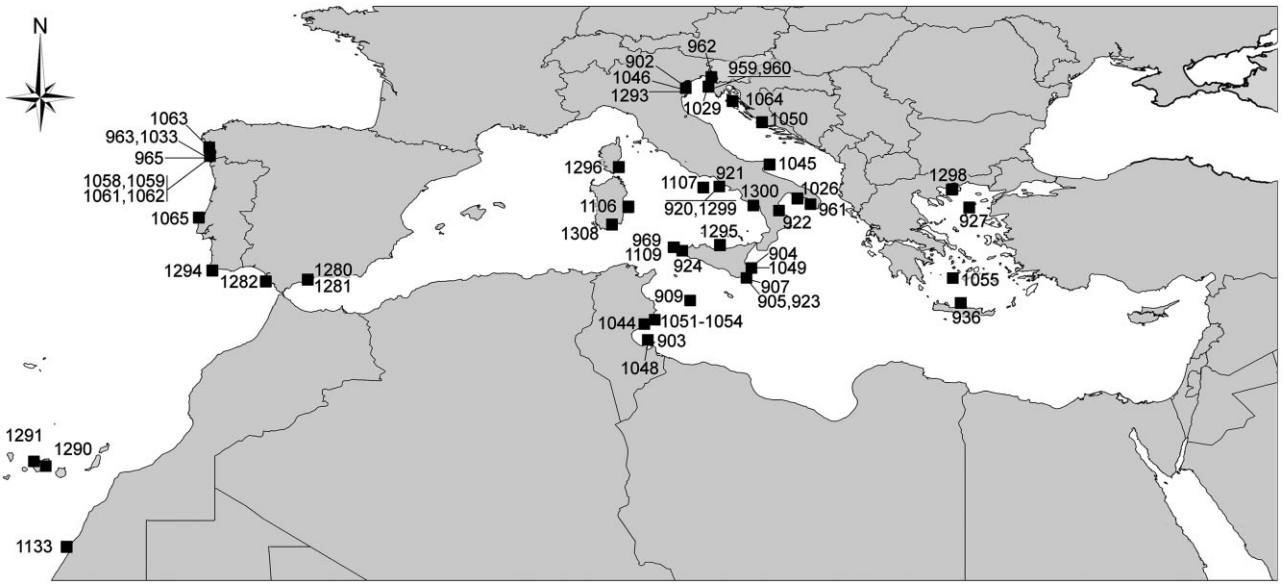

Figure 1. Location map of the sampling sites. Numbers of the sites as in Table 1.

BEAST (Drummond & Rambaut, 2007), using the substitution model proposed by jModelTest. A relaxed lognormal clock, a substitution rate fixed to one (no calibrations were used as our aim was to estimate the branching rates only) and a constant coalescent prior (which is thought to be more conservative than a Yule prior for species delimitation: see Monaghan *et al*., 2009) were used. Four BEAST runs were performed, each with 10^8 generations sampling every 10^4. Two samples of 10^4 trees were obtained, and convergence was evaluated, reading the log files with Tracer (Rambaut & Drummond, 2003), to verify that the effective sample size (ESS) values were > 200. Trees from all the runs were combined with LogCombiner and then summarized in a maximum credibility tree with TreeAnnotator (both included in the BEAST package). The consensus tree was analysed, applying the single-threshold GMYC function from the SPLITS package (Ezard, Fujisawa & Barraclough, 2009) in R (http://www.R-project.org). Having found no incongruence between ITS2 and COI single-gene analyses (see below), we also analysed with the same settings the combined COI + ITS2 dataset, where the clock and substitution model were unlinked for each gene, and the substitution models suggested in jModelTest were used.

The statistical parsimony network analysis calculates the maximum number of mutational steps constituting a parsimonious connection between two haplotypes (Posada & Crandall, 2001; Templeton, 2001). The haplotypes are then joined into networks following the algorithms proposed by Templeton, Crandall & Sing (1992), and those separated by more mutational steps (i.e. with probability of secondary mutations exceeding 5%) remain disconnected. This method has been used previously to differentiate species in a mixed sample (Pons *et al*., 2006; Hart & Sunday, 2007). It was applied to our COI dataset (excluding the outgroup *O. miscowichae*) using TCS (Clement, Posada & Crandall, 2000) with a 95% limit of tolerance.

We also analysed the ITS sequences for fixed sites between MOTUs defined in the species delimitation tests using DNAsp (Librado & Rozas, 2009)

Phylogenetic analysis

Bayesian phylogenetic trees for each single gene and for the combined COI + ITS2 and COI + ITS2 + 16S datasets were estimated using MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Sequences of *Nucella lapillus* (Linnaeus, 1758) were used to root the trees and, where available, sequences of *O. aciculata*, *O. reinai* and *O. miscowichae* were included to test their relationships with the species included in this study. Two Metropolis-coupled Markov chain Monte Carlo (MC^3) algorithms, with four chains each, were run for 8×10^7 generations using the substitution models selected by jModelTest, and sampling every 8×10^3. Convergences of the chains were evaluated by plotting values of the standard deviation of average split frequencies, and with the Potential Scale of Reduction Factor (PSRF) (Gelman & Rubin, 1992). All the analyses in MrBayes were computationally intensive, and thus run on the University of Oslo Bioportal (Kumar *et al*., 2009).

To test for incongruence among trees obtained with single-gene analyses, we compared clades recovered with high support in each topology. Conflict among clades with high support can be seen as evidence for divergent evolutionary histories, and can be used as an argument against multiple-gene phylogenetic analyses. However, we found no conflict among single-gene trees, and combined the datasets to estimate Bayesian topologies using COI + ITS2 and COI + ITS2 + 16S.

RESULTS

Sequence analysis

We obtained 208 original sequences of COI, 58 of 16S and 155 of ITS2, and included in our analyses 38 COI sequences of *O.* cf. *edwardsii* and *O. hispidula* from a previous study (Barco, Corso & Oliverio, 2013). Ten COI sequences of *O. aciculata* and *O. reinai* from Crocetta *et al*. (2012) were included in the phylogenetic analyses, together with six new COI sequences of *O. aciluata* from Spain (BAU 1060). The COI alignment spanned 658 bp (no stop codon detected), 167 of which were variable and 133 were parsimony informative; the 16S alignment comprised 763 bp, 83 of which were variable and 58 were parsimony informative; the ITS2 alignment comprised 559 sites, 157 of which were variable and 66 were parsimony informative. Accession numbers for the sequences are provided in Supporting Information Table S1. The substitution models selected by jModelTest were the TPM2uf + I + Γ (Kimura, 1981) for COI, HKY + Γ (Hasegawa, Kishino & Yano, 1985) for ITS2 and TIM3 + I + Γ (Posada, 2003) for 16S. The distances for the ABGD analysis on the COI dataset were obtained using the K2P model (Kimura, 1980) implemented in the online software.

Species delimitation

The ABGD method on the 236 COI sequences proposed nine distinct groupings ranging from 67 MOTUs to a single one depending on the value of the maximum intraspecific divergence used as a boundary between inter- and intraspecific distance values. The results obtained for K2P values lower than 0.012 were discarded for the unrealistic assumption of more than 10 species. The histogram of the pairwise distances (Fig. 2) shows a multimodal distribution for the frequencies, with a relevant minimum for values of K2P distance between 0.020 and 0.025, corresponding approximately to the partitions 15–17 (K2P distance = 0.014–0.021, eight MOTUs) and 18 (K2P distance = 0.026, four MOTUs).

All of the four BEAST runs reached ESS values of > 200, and the 'burnin' was set for one-half of the generations. A final sample of 10^4 trees was used to obtain the maximum clade credibility tree shown in Figure 3. The single-threshold model in GMYC was significantly better than the null model of no shift in branching rates ($P < 0.001$). The GMYC method proposed eight MOTUs (confidence interval, 2–18). In addition, in the combined dataset (COI + ITS2), the GMYC method performed better than the null model, but the dataset was divided into only three MOTUs (confidence interval, 1–36; Supporting Information Fig. S1).

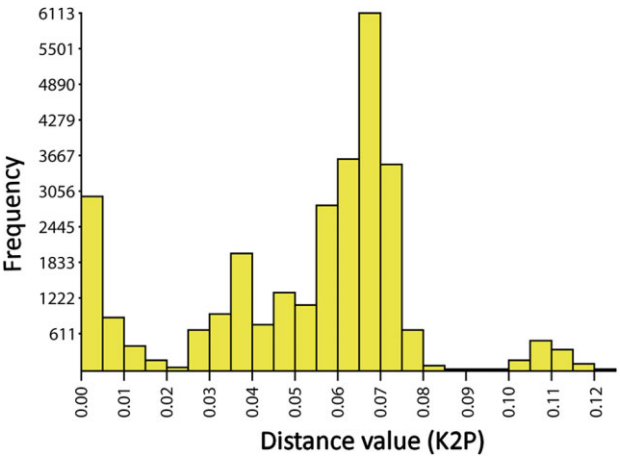

Figure 2. Histogram of the frequency of Kimura two-parameter (K2P) genetic divergence in all pairwise comparisons of cytochrome oxidase subunit I (COI) sequences.

The 238 COI sequences comprised 70 distinct haplotypes, 37 of which were unique. Using a 95% cut-off, the haplotypes were grouped by statistical parsimony analysis into nine independent networks, considered here as equivalent to nine MOTUs (Fig. 3).

The MOTUs defined by the three species delimitation methods in the eight-MOTUs scenario, based on the COI alignment, are named I–IV (four-MOTUs scenario: ABGD) and A–H (eight-MOTUs scenario: ABGD, GMYC, TCS) in Figure 3. Concordance was found among the three analyses, with only a single difference: group D was recovered as a single MOTU in the ABGD and GMYC analyses, but split into two by TCS (D1 and D2). In the two-genes (COI + ITS2) GMYC test, the threshold time for the coalescent/Yule boundary was lower (–0.044) than in the single-gene test (–0.01), resulting in the groups A + B + C and D + E + F + G, respectively, clustered into two MOTUs (Fig. S1).

Conserved sites in ITS2 were calculated for the MOTUs suggested in the species delimitation analysis (data not shown). Fixed differences were found only between more distantly related groups (e.g. B–D1, B–D2 and B–F), whereas most sister groups

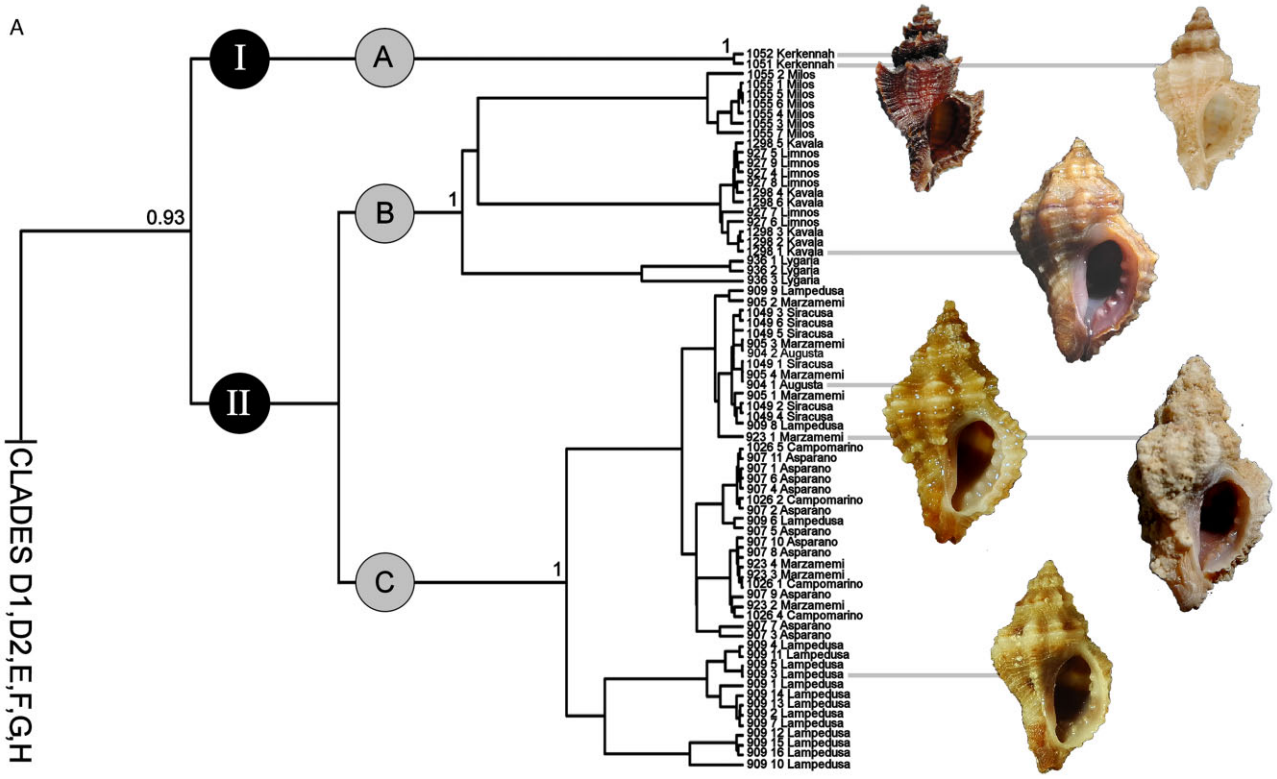

Figure 3. Consensus tree of a final sample of 10^4 trees obtained by BEAST on the cytochrome oxidase subunit I (COI) alignment. The black bars on the right delimit the Molecular Operational Taxonolllmic Units (MOTUs) as defined by the three species delimitation methods employed [Automatic Barcode Gap Discovery (ABGD), Generalized Mixed Yule-Coalescent (GMYC) and TCS]. Posterior supports > 0.95 are reported, and only for the nodes subtending the MOTUs. Voucher shells for each MOTU are figured (shells not to scale). 3A, MOTUs A–C; 3B, MOTUs D1–H.

showed no fixed differences, usable as diagnostic for each MOTU.

Phylogenetic analysis

In the MrBayes analysis, convergence was reached by the sampling chains in almost all analyses, as shown by the average standard deviation of split frequencies lower than 0.01 and by PSRF values of 1.00 for each parameter estimated by the MC^3 algorithm. However, for the single-gene ITS2 analysis, the convergence was difficult to reach even after setting a starting tree, increasing the number of generations, the number of MCMC chains or the λ value. In all the analyses, we employed a 'burnin' of about 25% of the sampled trees, whereas, in the ITS2 analysis, we used a 50% 'burnin'.

The single-gene Bayesian analyses produced three topologies (Supporting Information Fig. S2). In the COI and ITS2 analyses, using *Nucela lapillus* as an outgroup, the *O. aciculate* complex (*O. aciculata* and *O. reinai*) and the *O. edwardsii* complex were reciprocally monophyletic. In particular, in the COI analysis, *O. miscowichae* was defined as the sister taxon to the remaining lineages of the *O. edwardsii* complex. We obtained a high congruence between the COI and 16S trees (Fig. S2A,C), whereas the topology of ITS2 (Fig. S2B) was divergent and less resolved. Groups A–H of the BEAST-GMYC test were also obtained in the COI and 16S trees with high posterior probability (> 0.96), except for group G, which was unresolved in the 16S tree. In the ITS2 tree, none of the clades obtained with the other alignments was recovered. The limited amount of sequence variability observed in ITS2 probably prevented clear resolution of the phylogenetic signal in this alignment. We found no incongruence among highly supported clades in the single-gene trees, and thus also computed the combined-gene trees. In the combined-gene analyses (COI + ITS2, Supporting Information Fig. S3A; COI + ITS2 + 16S, Fig. S3B), we obtained two topologies similar to those of the COI and 16S single-gene analyses. In both cases, groups E and G of the BEAST-GMYC test had a moderate support (between 0.90 and 0.95), whereas all the others had high support values (> 0.96).

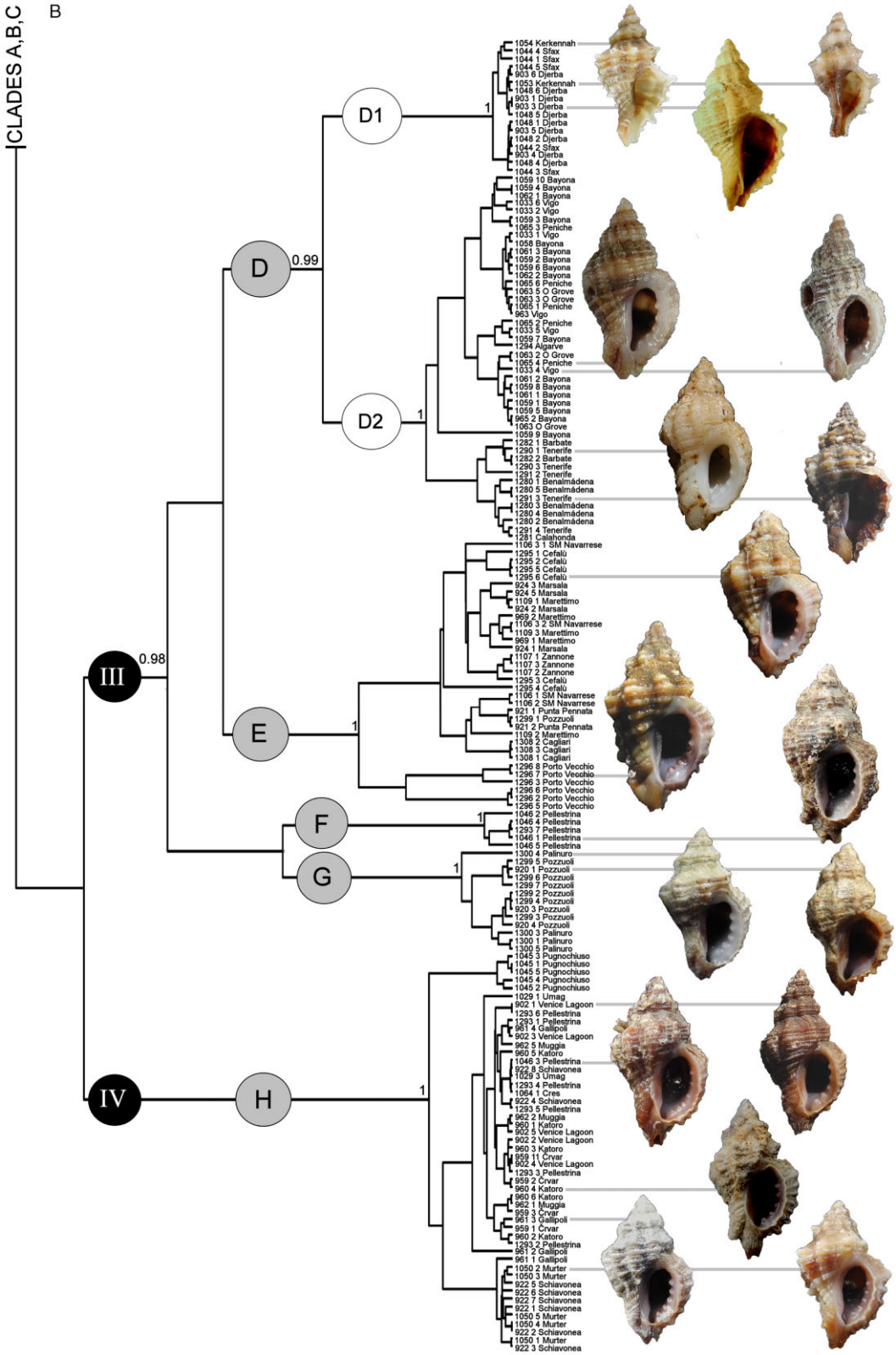

Figure 3. *Continued.*

DISCUSSION

As a starting point to define the threshold between intraspecific and interspecific pairwise comparisons, we searched those values (or range of values) that separated sympatric samples falling in distinct clades. The minima in the multimodal distribution for the frequencies of the pairwise distances (Fig. 2) corresponding to values higher than 0.031, defined in ABGD, partition with a single species for all of our samples. This is an unrealistic scenario which would classify the Tunisian specimens identified as *O. piantoni* and *O. carmelae* as conspecific with the sympatric specimens of *O. hispidula*, despite a K2P distance of > 0.09 in sympatry. A single-species hypothesis would also produce a complete lack of geographical pattern in the phylogenetic trees, with northern Sicily and Tyrrhenian samples more closely related to Adriatic and (some) Tunisian ones than to southern Sicily and Aegean samples. Conversely, the values of the K2P distance between 0.020 and 0.026 corresponded approximately to the partitions 15–17 (K2P distance = 0.014–0.021, eight MOTUs) and 18 (K2P distance = 0.026, four MOTUs). The eight-MOTUs scenario was the same as that depicted by the GMYC method, and the four-MOTUs scenario was within the confidence limits (2–18 MOTUs) of the GMYC results.

For COI, the K2P distance range representing the intra-/interspecific boundary in the eight-MOTUs/four-MOTUs scenarios (0.014–0.026) is similar to that obtained from the analysis of the COI sequences in other studies on marine gastropods (Castelin *et al*., 2010; Reid, Dyal & Williams, 2010; Williams *et al*., 2011; Nuñez *et al*., 2012; Puillandre *et al*., 2012b). This suggests that our COI distance values represent a reliable boundary between congeneric *Ocinebrina* species. However, deep mitochondrial divergences within other mollusc species have been repeatedly observed (e.g. Quattro *et al*., 2001; Wilson, Schrödl & Halanych, 2009), raising the need for support with nuclear data before taking taxonomic decisions. Several previous studies have provided evidence of a general concordance between results from COI and ITS2 sequence analyses and their usefulness in the species delimitation of molluscs (Calvo *et al*., 2009; Puillandre *et al*., 2010; 2011). However, it is also known that too large or too small an intraspecific variation of ITS2 may confound the detection of cryptic species (Vilas, Criscione & Blouin, 2005).

According to the species delimitation methods based on the COI dataset, a set of lineages that possibly represent cryptic species emerged within the *O. edwardsii* complex. A final taxonomic revision of the complex, also including the description of new species, will depend on the detection of a genetic distinction also at the nuclear level, and, eventually, on the identification of diagnostic morphological characters. Unfortunately, the lack of objectively distinguishable shell characters (at least at this point of our study), as well as the absence of a defined phylogenetic pattern in the selected nuclear sequences, prevented us from providing such a taxonomic revision. Therefore, we discuss the pattern emerging from the mitochondrial data, and its congruence with the preliminary shell-based identifications, under the assumption that, in our case, the mitochondrial phylogenetic signal is more reliable than the ITS2 one.

The small mussel drills of the *O. edwardsii* complex are common northeastern Atlantic and Mediterranean gastropods displaying striking morphological variability and, consequently, a complicated taxonomic history. As a probable consequence of their variability, numerous nominal taxa have been erected based exclusively on shell morphology for these gastropods (Houart, 2001; Cecalupo *et al*., 2008). Thirteen species based on a purely morphological approach are accepted currently in the northeastern Atlantic–Mediterranean area (Table 2). The specimens used in our analysis were classified morphologically on collection into seven of these nominal species (*O. carmelae*, *O. edwardsii*, *O. hispidula*, *O. ingloria*, *O. leukos, O. miscowichae*, *O. piantonii*). Of these, *O. miscowichae* proved to be the sister species to the *O. edwardsii* complex (when *Nucella lapillus* was used as an outgroup, and *O. aciculata* was sister to the *O. miscowichae*–*O. edwardsii* pair). In our dataset, specimens morphologically ascribed to *O. helleri*, *O. nicolai* and *O. paddeui* (Table 2) were not available for DNA extraction. Their inclusion in a future dataset will probably affect the taxonomic and nomenclatural patterns. Within the *O. edwardsii* complex, our analyses proposed a number of MOTUs ranging from four to eight, and a preliminary hypothesis of species delimitation only partially concordant with our *a priori* identifications.

THE *OCINEBRINA EDWARDSII* COMPLEX

In the four-MOTUs scenario, supported by the ABGD method only (yet within the confidence limit of the GMYC analysis), the four major lineages (I–IV) comprised the following samples:

MOTU-I: specimens morphologically identified as *O. piantonii* and *O. carmelae* from the Gulf of Gabès.
MOTU-II: specimens morphologically identified as *O. edwardsii* from the Aegean Sea, Ionian Sea, southwestern Sicily and Lampedusa Island.
MOTU-III: specimens morphologically identified as *O. edwardsii* from the Tyrrhenian Sea (including

Table 2. List of available nominal taxa from the northeastern Atlantic and the Mediterranean currently included in the genus *Ocinebrina*. Names currently considered as valid are given in bold. The type locality or, in substitution (in square brackets), the list of localities reported in the original description and the location of the type material are given when known. We have excluded unavailable names, such as infrasubspecific names (ICZN, 1999: Art. 45.5, 45.6) or *nomina nuda* (ICZN, 1999: Art. 12 and Glossary), but also all names introduced by Settepassi (1977), which has sometimes been considered as a consistently non-binomial work, under ICZN, 1999: Art. 11.4 [see, for example, WoRMS (2012) for *Ocinebrina edwardsi var. fasciatus* Settepassi, 1977. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=403955 on 13 March 2013]

Nominal taxon	Type locality [listed localities]	Type material	Currently accepted as	Notes
Murex aciculatus Lamarck, 1822: 176	Vannes area (France)	Not known	***O. aciculata***	Type species of *Ocinebrina* Jousseaume, 1880 and *Corallinia* Bucquoy & Dautzenberg, 1882 (in Bucquoy, Dautzenberg & Dollfus, 1882: 24)
Murex acrisius Nardo, 1847: 59–60	Gulf of Venice (Italy)	14 syntypes MSNVE 21980 (Crocetta *et al.*, 2012)	*O. aciculata*	Originally referring to: Chiereghin (unpublished*): figs. 713–714. Subjective junior synonym (Crocetta *et al.*, 2012)
Ocinebrina edwardsi [sic!] var. *apiculata* Pallary, 1902a: 314	Tanger (Morocco)	Not known	*O. edwardsii*	
Murex baeticus Reeve, 1845: pl. 32 (fig. 162)	Not known	3 syntypes NHMUK 1972024 – one in Houart (2001) as BMNH 1621845	*O. edwardsii*	Probable subjective senior synonym of *Murex hybridus*
Ocinebrina buzzurroi Cecalupo & Mariani, 2008: in Cecalupo *et al.*, 2008: 90, pl. 43 (figs. 1–7)	Borj el Hissar (Tunisia)	Holotype MNHM 33489 (Cecalupo *et al.*, 2008)	*O. corallinoides*	Subjective junior synonym (Crocetta *et al.*, 2012)
Ocinebrina carmelae Cecalupo, Buzzurro & Mariani, 2008: 98, pl. 48 (figs. 1–8)	Borj el Hissar (Tunisia)	Holotype MNHM 33491 (Cecalupo *et al.*, 2008)	***O. carmelae***	New subjective junior synonym of *O. piantonii* (? = *Murex hybridus*)
Amyclina compacta Nordsieck, 1968: 140, pl. 23 (fig. 80.45)	Portovenere (Italy)	Holotype nr./1, Senckenberg Mus. (Houart, 2001)	*O. edwardsii*	
Ocinebrina corallinoides Pallary, 1912: 221, plate (fig. 48)	Sfax, Gulf of Gabès (Tunisia)	Not known	***O. corallinoides***	
Murex corallinus Scacchi, 1836: 12 (fig. 15)	Baia (Italy)	Neotype MZN Z7010 (Crocetta *et al.*, 2012)	*O. aciculata*	Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Murex costulatus Nardo, 1847: 55–56 (sp. 8)	Cres Island area (Croatia)	Not known		Originally referring to: Chiereghin (unpublished*): figs. 693–694. Hereby first considered subjective junior synonym of *Fusus helleri*. Primary junior homonym of *Murex costulatus* Schröter 1805 and *Murex costulatus* Risso, 1826
Ocinebrina edwardsi [sic!] var. *crassata* Pallary, 1902b: 12, pl. 1 (figs. 10–11)	Tanger (Morocco)	Not known		Hereby considered as belonging to the *Ocinebrina edwardsii* complex
Ocinebrina cyclopus Monterosato, 1884: 112	Palermo (Italy)	Syntypes in MCZR (Settepassi, 1977)	*O. edwardsii*	
Purpura edwardsii Payraudeau, 1826: 155, pl. 7 (figs. 19–20)	Corsica (France)	Not known	***O. edwardsii***	Type species of *Dentocenebra* Monterosato, 1917 (= *Ocinebrina* Jousseaume, 1880)
Ocinebrina erronea Cecalupo, Buzzurro & Mariani, 2008: 92, pls. 42 (figs. 5–6), 44 (figs. 7–16) and 45 (figs. 1–10)	Sfax and Kerkennah Islands (Tunisia)	Not designated	*O. hispidula*	Subjective junior synonym (Cossignani & Ardovini, 2011), hereby assigned to Cecalupo *et al.* (2008) since *Muricopsis erroneus* Settepassi, 1977 is unavailable (ICZN, 1999: Art. 11.4)
Ocinebrina aciculata exilis Houart, 2001: 19 (figs. 8–9), 51 (fig. 62), 62, 143 (figs. 167–168), 176 (figs. 324–325)	NW of Bou Grara Sea (Tunisia)	Holotype MNHN 0362 (Crocetta *et al.*, 2012)	*O. corallinoides*	Subjective junior synonym (Bonomolo & Buzzurro, 2006; Cecalupo *et al.*, 2008; Crocetta *et al.*, 2012)
Fusus helleri Brusina, 1865: 8	[Zadar, Šibenik, Hvar, Dubrovnik, Budva] (Croatia and Montenegro)	Not known	***O. helleri***	= *Murex hellerianus* Brusina, 1866: 63 (unnecessary spelling emendation: Houart, 2001)
Ocinebrina edwardsi [sic!] var. *hispidula* Pallary, 1904: 231, pl. 7 (fig. 18)	Gulf of Gabès (Tunisia)	3 syntypes MNHN 1001 (Houart, 2001; Giannuzzi-Savelli *et al.*, 2003; present paper)	***O. hispidula***	
Murex hybridus Aradas & Benoît, 1876: 272, pl. 5 (fig. 9)	Palermo (Italy)	Not known	***O. hybrida***	Probable subjective junior synonym of *Murex baeticus*
Murex inconspicuus Sowerby G.B. II, 1841: 5 (67), plates (figs. 81)	Jersey Island (Bailiwick of Jersey)	Not known	*O. aciculata*	Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Murex inglorius Crosse, 1865: 213, pl.6 (fig.4)	Not known	Holotype MNHN 0993 (Fair, 1976; Houart, 2001; Giannuzzi-Savelli *et al.*, 2003; present paper)	***O. ingloria***	
Ocenebra inordinata Houart & Abreu, 1994: 123, 129 (figs. 11–13)	Madeira Island	Holotype MMF 25429 (Houart & Abreu, 1994)	***O. inordinata***	

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Ocinebrina labiosa Monterosato, 1884: 112	[Chioggia and Venice] (Italy)	Presumably not existing		Originally referring to: Chiereghin (unpublished*): figs. 695–696. New subjective junior synonym of *Murex inglorius*
Merex [sic!] *labiosus* Nardo, 1847: 55–56 (sp. 9)	Kvarner Gulf (Croatia)	Presumably not existing	*Ocenebra erinaceus*	Originally referring to: Chiereghin (unpublished*): figs. 695–696. Currently accepted as *Ocenebra erinaceus*, and here considered as a member of the *O. edwardsii* complex. Primary junior homonym of *Murex labiosus* Gray, 1828 and *Murex labiosus* Wood, 1828
Ocinebrina leukos Houart, 2000: 464 (figs. 7–10)	La Isleta, Lanzarote (Spain)	Holotype MNHN 0966 (Houart, 2000; present paper)	***O. leukos***	
Ocinebrina miscowichi Pallary, 1920: 40, plate (figs. 5–6)	Essaouira (Morocco)	32 syntypes MNHN 0176 and 0177 (Houart, 2001; present paper)	***O. miscowichae***	Spelling emended by Houart (1997)
Ocinebrina nicolai Monterosato, 1884: 112	[Corsica, Sardegna, Lipari] (Italy and France)	Syntypes in MCZR (Settepassi, 1977)	***O. nicolai***	
Murex orcomenus Nardo, 1847: 57–58 (sp. 11)	Northern Adriatic Sea	Not known		Originally referring to: Chiereghin (unpublished*): figs. 699–700.It is probably a senior synonym of *Murex inglorius*, but its status as *nomen oblitum* (ICZN, 1999: Art. 23.9.1.2) should be verified
Ocinebrina paddeui Bonomolo & Buzzurro, 2006: 1 (figs. 1–6)	Off Capo Caccia, Alghero (Italy)	Holotype MNHN 29909 (Bonomolo & Buzzurro, 2006)	***O. paddeui***	
Murex pereger Brugnone, 1873: 10, plate (fig. 17)	African shores	Not known	*O. hybrida*	Subjective junior synonym (Houart, 2001). Primary junior homonym of *Murex pereger* Beyrich, 1854
Murex edwardsi [sic!] f. *perigmus* De Gregorio, 1885: 253	Mediterranean	Not known	*O. edwardsii*	Originally referring to: Bucquoy *et al.* (1882): pl. 2 (fig. 3). Subjective junior synonym (Houart, 2001)
Murex edwardsi [sic!] f. *perilus* De Gregorio, 1885: 253	Mondello (Italy)	Not known	*O. edwardsii*	
Ocinebrina piantonii Cecalupo, Buzzurro & Mariani, 2008: 96, pl. 49 (figs. 1–10), pl. 50 (figs. 1–6)	Borj el Hissar (Tunisia)	Holotype MNHM 33490 (Cecalupo *et al.*, 2008)	***O. piantonii***	Probable subjective junior synonym of *Murex baeticus*
Murex pistacia Reeve, 1845: pl. 34, fig. 174	Not known	3 syntypes NHMUK 1972021 (Crocetta *et al.*, 2012)	*O. aciculata*	Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Murex pumilus Küster, 1869 (in Küster & Kobelt, 1839–1878: 118, pl. 35: figs. 8–10)	Not known	Not known	*O. edwardsii*	Primary junior homonym of *Murex pumilus* Broderip, 1833 and *Murex pumilus* A. Adams, 1853
Ocinebrina purpuroidea Pallary, 1920: 39, plate (figs. 16–17, 24)	Rabat (Morocco)	8 syntypes MNHN 0931 (Houart, 2001)	***O. purpuroidea***	
Ocinebrina reinai Bonomolo & Crocetta, 2012: (in Crocetta *et al.*, 2012: 180, figs. 1 K-1 L, 2A-2F, 2I, 2 L, 3)	Procida Island (Italy)	Holotype MNHN 24566 (Crocetta *et al.*, 2012)	***O. reinai***	
Murex semiclausus Küster, 1869: 111, pl. 34 (figs. 6–7)	Not known	Not known	*O. edwardsii*	
Murex subaciculatus Locard, 1886: 164	Toulon (France)	Not known	*O. aciculata*	Originally referring to: *Murex aciculatus* in Hidalgo (1870): pl. 13 (figs. 7–8); *Murex aciculatus* var. *curta* Bucquoy *et al.* (1882): page 25. Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Fusus titii Stossich, 1865: 31	Northern Adriatic Sea	Not known	*O. aciculata*	Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Ocinebra [sic!] *wardiana* Baker, 1891: 134, pl. 11 (fig. 5)	'Australia' (see discussion in Vokes, 1994)	Holotype CAS 20698 (Vokes, 1994)	*O. aciculata*	Subjective junior synonym (Houart, 2001; Crocetta *et al.*, 2012)
Murex weinkauffianus Crosse, 1866: 274, pl. 8 (fig. 4)	Zadar (Croatia)	Not known	*O. helleri*	Subjective junior synonym (Houart, 2001)

*Chiereghin S. (unpublished). *Descrizione de´crostacei, de´testacei e de´pesci che abitano le lagune e golfo veneto*. [An unpublished manuscript referred to by Nardo, (1847) who then made available several otherwise unavailable names.]

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Corsica, type locality of *O. edwardsii*); specimens morphologically identified as *O. hispidula* (from the Gulf of Gabès); specimens from the Mediterranean and Atlantic coasts of Spain and Portugal, including the Canary Islands; part of the specimens from the Venice Lagoon.
MOTU-IV: specimens from the Adriatic Sea and a few localities in the Ionian Sea.

In the eight-MOTUs scenario, supported by all three methods (ABGD, GMYC, TCS), the eight major lineages maintained a geographical coherence, and only MOTU-A corresponded exactly to MOTU-I.

We cannot decide with the present data between the two scenarios (four-MOTUs or eight-MOTUs). Pending the availability of more reliable and congruent data from the nuclear genes to support such a decision, we discuss the geographical patterns in the *O. edwardsii* complex focusing on the more detailed (albeit admittedly less conservative) eight-MOTUs scenario.

In the eight-MOTUs scenario, all the specimens attributed preliminarily to *O. edwardsii* were distributed among seven MOTUs: clade B, including specimens from localities in the Aegean Sea; clade C from the Ionian Sea, southwestern Sicily and Lampedusa Island; clade D2 from the Atlantic coast of Spain and Portugal, the Canary Islands and Málaga Province; clade E from several localities across the Tyrrhenian Sea; clade F from Pellestrina, a locality within the Venice Lagoon; clade G from Campania (Pozzuoli and Palinuro); and clade H from the Adriatic Sea and a few localities in the Ionian Sea.

Of these groups, we provisionally identify MOTU-E as representing the 'true' *O. edwardsii*, because it included the specimens sampled in Corsica, its type locality (Payraudeau, 1826). Among our samples, this group is apparently restricted to the Tyrrhenian Sea (eastern Corsica, eastern Sardinia, northern Sicily and western Italian coast: Fig. 4).

The sister lineage to clade E is composed of the D1 and D2 lineages (clade D). The D2 lineage included specimens morphologically ascribed to *O. edwardsii* sampled along the Atlantic coasts of Spain and Portugal, from the Canary Islands and from Málaga Province; the specimens ascribed to *O. leukos* from the Canary Islands; and specimens from populations referred in the recent literature to *O.* [cf.] *nicolai* from Galicia (Rolán, 1983) (samples 963, 965, 1033, 1058, 1059, 1061–1063), from Barbate (sample 1282) (Gofas, 2011) and from off Ponta da Piedade–Algarve (sample 1294) (Afonso *et al*., 2011) (Fig. 4). The specimens of *O. edwardsii* sampled along the Atlantic coast of Spain and Portugal displayed a rather distinctive shell morphology with respect to the most common forms found in the Mediterranean. *Ocinebrina leukos* was described from Lanzarote (Canary Islands) as living sympatrically with, yet morphologically easily distinguished from, local *O. edwardsii* (Houart, 2000). The latter, however, has been recorded as highly variable morphologically, even in the same locality (Houart, 2000), and, according to our molecular data, *O. leukos* may fall within the variability of this lineage (D2). *Ocinebrina nicolai* was originally described from Corsica, Sardinia and Lipari Islands (Monterosato, 1884). Specimens from the Monterosato collection (see Settepassi, 1977: 38–44), however, are morphologically different from those figured recently under this name (e.g. Rolán, 1983; Houart, 2001; Afonso *et al*., 2011; Gofas, 2011). Therefore, the association of the name *O. nicolai* with one of our MOTUs is postponed pending the genetic analysis of topotypical specimens.

According to the ABGD and GMYC analyses, clade D also included the specimens from Gabès ascribed to *O. hispidula*, although a distinct well-supported clade (D1) for this lineage has been recovered in all the topologies. The large genetic distance between specimens identified as *O. hispidula* from Gabès and specimens classified preliminarily as *O. edwardsii* from Sicily (clade C) has been reported recently by Barco *et al*. (2013). *Ocinebrina hispidula* has also been reported from other Atlantic and Mediterranean localities, such as Mogador (Ardovini & Cossignani, 2004), Sicily (Settepassi, 1977; Giannuzzi-Savelli *et al*., 2003), Cyprus (Houart, 2001; Öztürk, Buzzurro & Benli, 2004) and the Baleares (Pons-Moyà & Pons, 2002). Although these records may have been based on extremely spiny morphotypes of other taxa of the *O. edwardsii* complex, or on mislabelled specimens, according to our analyses (ABGD and BEAST-GMYC), *O. hispidula* is included in a more widely distributed MOTU (clade D). Even in the less conservative eight-MOTUs scenario, MOTU-D probably represents a single species ranging from the northeastern Atlantic to the western and central Mediterranean Sea, which has recently colonized the Gulf of Gabès with a population displaying a strikingly deviating morphology (Fig. 4), a phenomenon well known in the Gabès molluscan fauna (Cecalupo *et al*., 2008), and with a distribution compatible with that of other intertidal molluscs (Calvo *et al*., 2009). Furthermore, the young age of the Gulf of Gabès (Burollet, Clairefond & Winnock, 1979; Stocchi, Colleoni & Spada, 2009) would not be compatible with the recent evolution of an endemic species. *Ocinebrina erronea* Cecalupo, Buzzurro & Mariani, 2008 (see Table 2 for nomenclatural details) has been synonymized recently with *O. hispidula* by Cossignani & Ardovini (2011). A single specimen of the *O. erronea* morphotype (sample 1053) was included in our dataset and, in all the analyses, it fell

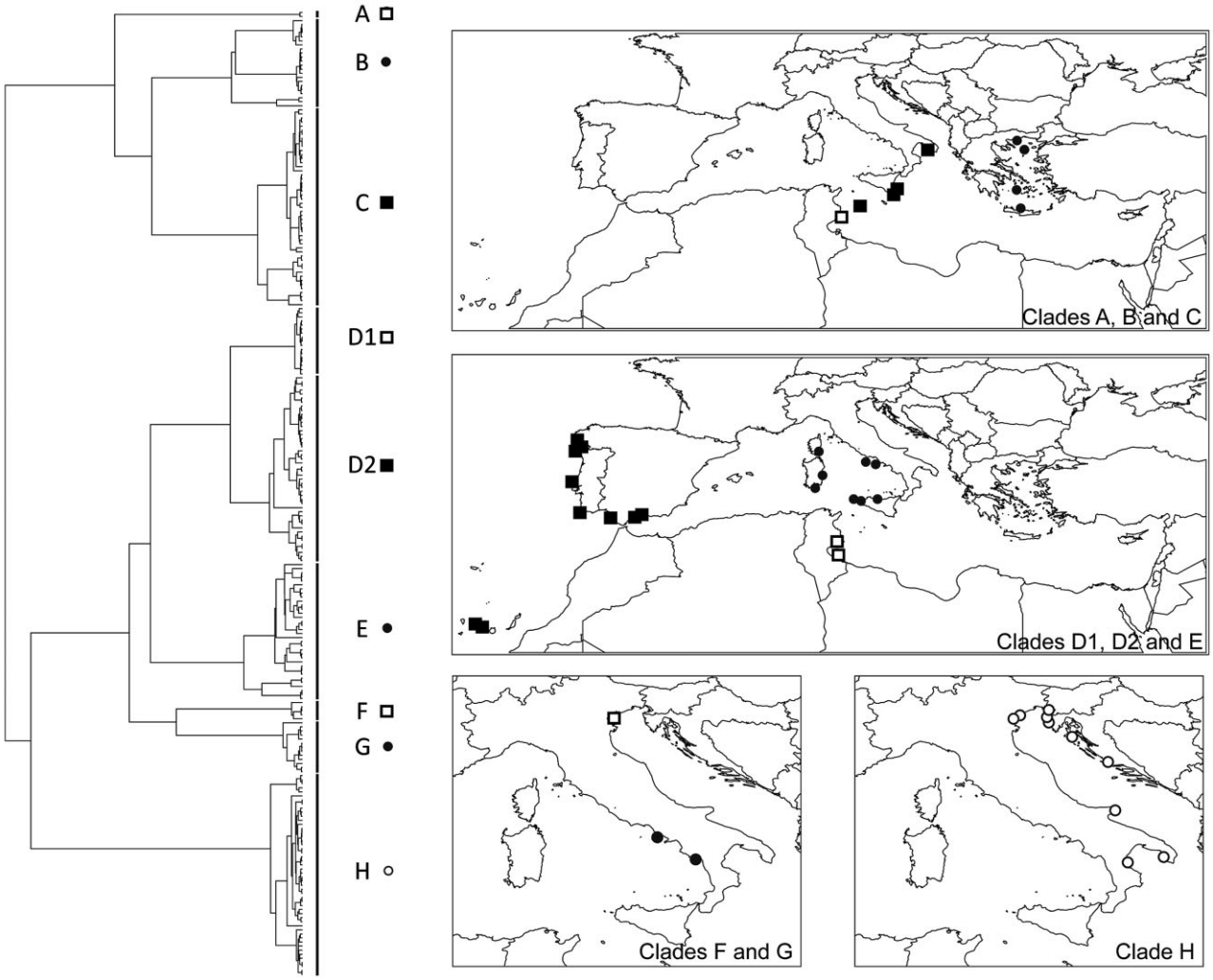

Figure 4. Same tree as in Figure 3 obtained by BEAST on the cytochrome oxidase subunit I (COI) alignment, and distribution maps of the vouchers, grouped in the clades resulting from the COI analysis.

within the *O. hispidula* clade (group D1), indicating that it is not a distinct species.

Ocinebrina piantonii and *O. carmelae* were described as new species from the Kerkennah Islands by Cecalupo *et al*. (2008), living sympatrically (and syntopically) amidst the rhizomes of *Posidonia oceanica* (L.) Delile, 1813. The morphological differences between the two nominal taxa are indeed very subtle, possibly ending up as just two colour forms: a white phenotype (*O. piantonii*) and a dark phenotype (*O. carmelae*). Furthermore, the latter is indistinguishable from *O. hybrida* (Aradas & Benoît, 1876) (which is possibly a synonym of *O. baetica* (Reeve, 1845): see Table 2), a rare species for which we had no specimens for DNA extraction. We assayed two specimens from the Gulf of Gabès, representing the two morphotypes, which proved clearly to belong to a single species (clade A), not related phylogenetically to *O. hispidula*. They fall into an eastern Mediterranean group including clades B (Aegean Sea) and C (Ionian Sea).

Specimens collected at four localities across the Aegean Sea (clade B) were indistinguishable morphologically from those classified preliminarily as *O. edwardsii* from other Mediterranean localities. The COI and 16S sequences of these specimens were particularly divergent from all the others, allowing the identification of a well-distinct mitochondrial lineage. Furthermore, the mitochondrial data also suggest a well-defined population structure within the group, with three deeply separated clusters corresponding to distinct sampling localities (northern, central and southern Aegean Sea) (Fig. 4).

Another clade (C) is represented by the specimens collected in the Ionian Sea, southwestern Sicily and Lampedusa Island. Similar to group B, clade C

is well defined geographically (Fig. 4), but the shells are rather indistinguishable from specimens ascribed morphologically to *O. edwardsii* from other Mediterranean localities. The sister group relationship with the Aegean clade suggests an allopatric origin of both lineages from an ancestral eastern Mediterranean clade (see below).

Ocinebrina ingloria was described originally by Crosse (1865) without specifying any locality. Other names may apply to this entity (e.g. *Murex orcomenus* Nardo, 1847, see Table 2), but the assessment of the status was beyond the scope of this study. Neglected for over a century, it was recorded as *O. ingloria* recently from the Adriatic Sea, from Grado and the Venice Lagoon (which was interpreted as the most likely locality of the type material) by Houart (2001) and from Palermo by Giannuzzi-Savelli *et al*. (2003). The population at Grado seems to be extinct, together with other intertidal molluscan species, as a result of the intense human activity in the area (see Crocetta, 2011), whereas the record from Palermo seems likely to be incorrect because such a morphotype has never been found there (G. Bonomolo, unpublished). We have assayed specimens from the Venice Lagoon (morphologically and geographically representing the nominal taxon *O. ingloria*) which, despite evident morphological differences, were grouped in clade H with other specimens classified morphologically as *O. edwardsii* from the Adriatic Sea and a few localities in the Ionian Sea (Fig. 4). Apparently all specimens of the *O. edwardsii* complex from the Adriatic Sea belong in the same lineage as *O. ingloria*, again suggesting that shell variation, as currently approached within this group, is rather misleading, and that a single species is involved in this lineage.

The sole geographical exception to clade H is represented by some specimens sampled at Pellestrina (clade F), within the area of the Venice Lagoon. All specimens from this site were indistinguishable from each other morphologically (see, for example, vouchers 1046_1 and 1046_3: Fig 3B, Supporting Information Fig. S4). After the first analyses split the first lot of specimens from this locality (sample 1046) into groups F and H, we collected other specimens from the same locality (sample 1293) in order to rule out the possibility of contamination, and obtained the same results. Thus, according to this evidence, two morphologically indistinguishable MOTUs live sympatrically at this site in the Venice Lagoon. The sister clade to MOTU-F from Pellestrina is clade G from Pozzuoli and Palinuro (central Tyrrhenian Sea). Clades F and G were clearly distinct MOTUs in all of our species delimitation analyses, and both lineages possibly represent relict populations isolated on the two sides of the Italian peninsula.

Biogeographical patterns

Comparing our molecular data with the fossil record of *Ocinebrina* would allow the drawing of a time-calibrated tree and a comparison between the estimated origin of clades and the history of the Mediterranean. Unfortunately, although fossil *Ocinebrina* shells are relatively abundant, their species classification based on shell morphology would be rather misleading. What has emerged from the present study is that shell characters used in the taxonomy of *O. edwardsii* and related species do not always define monophyletic lineages, and we have therefore no basis for using fossils to calibrate node ages in the phylogeny. Such an analysis may become possible only after a thorough revision of shell characters used in this group. A comparison with previous studies, however, might be helpful in understanding the observed geographical distribution of molecular lineages.

The present-day Mediterranean marine fauna is the result of a long and troubled geological history (Taviani, 2002) peaking in the Messinian Salinity Crisis (MSC) (Krijgsman *et al*., 1999) and the following Pliocene and Quaternary glacial/interglacial cycles. These events contributed to the shaping of the current fauna into defined biogeographical categories (Bianchi & Morri, 2000), and the geographical structure among populations of Mediterranean organisms can generally be traced back to these major events (Patarnello, Volckaert & Castilho, 2007). Species with short or no planktonic larval stage (such as the north-eastern Atlantic *Ocinebrina* species) are supposed to show a more structured distribution across a reduced geographical range (Cunha *et al*., 2005, 2008; Meyer *et al*., 2005; Paulay & Meyer, 2006) than those with planktonic larvae. Thus, in the Mediterranean Sea, the phylogeography of marine organisms, and especially of those with reduced planktonic stages, is expected to reflect these events. This is the case for reef-building gastropods of the *Dendropoma petraeum* (Monterosato, 1884) complex, which has been shown to include four cryptic species with a clear east–west Mediterranean subdivision (Calvo *et al*., 2009). This geographical structure has been attributed to vicariant cladogenesis favoured by the reduced planktonic larval stage of the species. We observed a high similarity between the geographical distributions of the lineages of *Ocinebrina* and that found by Calvo *et al*. (2009) in the *D. petraeum* complex.

Our group D has a distribution similar to that of the western Mediterranean *Dendropoma* clade. The continuous range across the Atlanto-Mediterranean border found in many marine species is generally explained as a re-colonization process completed by propagules from extant Atlantic populations

establishing new populations in the Mediterranean. However, restricted gene flow through the Straits of Gibraltar has also been detected in species with high dispersal potential (Sá-Pinto *et al*., 2012). In species with a short-lived planktonic stage, the dispersal across long distances possibly has been accomplished by rafting or with floating debris (Martel & Chia, 1991; Cunningham & Collins, 1998). The combination of these factors and the effect of the Algerian current flowing along the northern coast of Africa could explain the presence of this lineage in the Gulf of Gabès and its genetic distinctiveness.

The repeated instances of separation of the western and eastern Mediterranean basins by the Sicilian sill during the Quaternary glacial cycles are often correlated with the present-day distinction between corresponding lineages (e.g. Oliverio, 1994, 1996; Calvo *et al*., 2009; Sá-Pinto *et al*., 2010; Mejri *et al*., 2011). We found that three of our groups (MOTU-A, MOTU-B and MOTU-C) formed a well-supported clade (MOTU-I + MOTU-II) in both the BEAST and MrBayes analyses, supporting the existence of an eastern Mediterranean lineage. In the three-gene Bayesian analysis (Fig. S3B), the Adriatic clade (all the Adriatic specimens with the exception of group F) is closely related to groups A, B and C, suggesting an early separation of the ancestor of these four groups from a western Mediterranean clade ancestor.

The phylogenetic relationships of groups F and G with the others are still unclear, making it difficult to draw their position in this scenario. According to BEAST analysis (Fig. 2), both lineages are closely related to the western Mediterranean group (clades D and E), but, in other analyses, support for such a position is lower. The long branches of these groups and their sympatry with other clades (F with H in the Adriatic Sea and G with E in the Tyrrhenian Sea) would suggest early allopatric isolation followed by secondary contact. The actual distribution of these two groups, however, is still unknown, and further studies with denser sampling are required to understand their status.

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REFERENCES

Afonso CML, Bonomolo G, Monteiro P, Bentes L, Oliveira F, Veiga P, Rangel MO, Sousa I, Leite L, Gonçalves JMS. 2011. First record of *Ocinebrina nicolai* (Mollusca: Gastropoda: Muricidae: Ocenebrinae) in north-eastern Atlantic waters. *Marine Biodiversity Records* **3:** 1–4.

Appeltans W, Bouchet P, Boxshall GA, De Broyer C, de Voogd, Gordon, NJ, Hoeksema DP, Horton BW, Kennedy T, Mees M, Poore J, Read GCB, Stöhr G, Walter S, Costello TC, MJ, eds. 2012. World register of marine species. Available at: http://www.marinespecies.org [accessed 13 March 2013].

Aradas A, Benoît L. 1876. Conchigliologia vivente marina della Sicilia e delle isole che la circondano. Parte terza. *Atti dell'Accademia Gioenia di Scienze Naturali in Catania* **6:** 1–329.

Ardovini R, Cossignani T. 2004. *West African seashells*. Ancona: L'Informatore Piceno.

Baker FC. 1891. Description of new species of Muricidæ, with remarks on the apices of certain forms. *Proceedings of the Rochester Academy of Science* **1:** 129–137.

Barco A, Corso A, Oliverio M. 2013. Endemicity in the Gulf of Gabès: the small mussel drill *Ocinebrina hispidula* is a distinct species in the *Ocinebrina edwardsii* complex (Muricidae: Ocenebrinae). *Journal of Molluscan Studies* **79:** 273–276.

Bianchi CN, Morri C. 2000. Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. *Marine Pollution Bulletin* **40:** 367–376.

Blaxter ML. 2004. The promise of a DNA taxonomy. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359:** 669–679.

Boisselier-Dubayle MC, Gofas S. 1999. Genetic relationships between marine and marginal-marine populations of *Cerithium* species from the Mediterranean Sea. *Marine Biology* **135:** 671–682.

Bonomolo G, Buzzurro G. 2006. Description of a new Muricid for the Mediterranean sea: *Ocinebrina paddeui* (Mollusca, Gastropoda, Muricidae, Ocenebrinae). *Triton* **13:** 1–4.

Brugnone GA. 1873. *Miscellanea Malachologica. Pars prima*. Palermo: Michele Amenta.

Brusina S. 1865. Conchiglie dalmate inedite. *Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien* **15:** 3–42.

Brusina S. 1866. Contribuzione pella fauna dei molluschi dalmati. *Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien* **16:** 1–134.

Bucquoy EJ, Dautzenberg P, Dollfus GF. 1882. *Les Mollusques marins du Roussillon. Tome I (Gastropodes)*. Paris: Baillière J.B. and Fils.

Burollet PF, Clairefond P, Winnock E. 1979. La mer pélagienne. Etude sédimentologique et écologique du plateau tunisien et du golfe de Gabès. *Annales de l'Université de Provence* **5:** 1–345.

Calvo M, Templado J, Oliverio M, MacHordom A. 2009. Hidden Mediterranean biodiversity: molecular evidence for a cryptic species complex within the reef building vermetid gastropod *Dendropoma petraeum* (Mollusca: Caenogastropoda). *Biological Journal of the Linnean Society* **96:** 898–912.

Caron DA, Countway PD, Jones AC, Kim DY, Schnetzer A. 2012. Marine protistan diversity. *Annual Review of Marine Science* **4:** 467–493.

Carreras-Carbonell J, Macpherson E, Pascual M. 2005. Rapid radiation and cryptic speciation in Mediterranean triplefin blennies (Pisces: Tripterygiidae) combining multiple genes. *Molecular Phylogenetics and Evolution* **37:** 751–761.

Castelin M, Lambourdiere J, Boisselier MC, Lozouet P, Couloux A, Cruaud C, Samadi S. 2010. Hidden diversity and endemism on seamounts: focus on poorly dispersive neogastropods. *Biological Journal of the Linnean Society* **100:** 420–438.

Cecalupo A, Buzzurro G, Mariani M. 2008. Contributo alla conoscenza della malacofauna del Golfo di Gabès (Tunisia). *Quaderni della Civica Stazione Idrobiologica di Milano* **31:** 1–267.

Claremont M, Williams ST, Barraclough TG, Reid DG. 2011. The geographic scale of speciation in a marine snail with high dispersal potential. *Journal of Biogeography* **38:** 1016–1032.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1659.

Cossignani T, Ardovini R. 2011. *Malacologia Mediterranea. Atlante delle conchiglie del Mediterraneo – 7.500 foto a colori*. Ancona: L'Informatore Piceno.

Costello MJ, Wilson S, Houlding B. 2012. Predicting total global species richness using rates of species description and estimates of taxonomic effort. *Systematic Biology* **61:** 871–883.

Crocetta F. 2011. Marine alien Mollusca in the Gulf of Trieste and neighbouring areas: a critical review and state of knowledge (updated in 2011). *Acta Adriatica* **52:** 247–260.

Crocetta F, Bonomolo G, Albano PG, Barco A, Houart R, Oliverio M. 2012. The status of the northeastern Atlantic and Mediterranean small mussel drills of the *Ocinebrina aciculata* complex (Mollusca: Gastropoda: Muricidae), with the description of a new species. *Scientia Marina* **76:** 177–189.

Crosse H. 1865. Description d'espèces nouvelles. *Journal de Conchyliologie* **13:** 213–215.

Crosse H. 1866. Description d'un Murex nouveau de l'Adriatique. *Journal de Conchyliologie* **14:** 274–276.

Cunha RL, Castilho R, Rüber L, Zardoya R. 2005. Patterns of cladogenesis in the venomous marine gastropod genus *Conus* from the Cape Verde Islands. *Systematic Biology* **54:** 634–650.

Cunha RL, Tenorio MJ, Afonso C, Castilho R, Zardoya R. 2008. Replaying the tape: recurring biogeographical patterns in Cape Verde *Conus* after 12 million years. *Molecular Ecology* **17:** 885–901.

Cunningham CW, Collins TM. 1998. Beyond area relationships: extinction and recolonization in molecular marine biogeography. In: De Salle R, Schierwater B, eds. *Molecular approaches to ecology and evolution*. Basel: Birkhaeuser Verlag, 297–321.

De Gregorio A. 1885. *Studi su talune conchiglie Mediterranee viventi e fossili*. Siena: Tipografia all'insegna dell'ancora.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Ezard T, Fujisawa T, Barraclough TG. 2009. Splits: species' limits by threshold statistics. Available at: http://R-Forge.R-project.org/projects/splits/

Fair RH. 1976. *The murex book: an illustrated catalogue of recent Muricidae (Muricinae, Muricopsinae, Ocenebrinae)*. Honolulu, HI: Sturgis Printing Co.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Franc A. 1940. Recherches sur le développement d'*Ocinebra aciculata*, Lamarck (Mollusque Gastéropode). *Bulletin biologique de la France et de la Belgique* **74:** 327–345.

Gascuel O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* **14:** 685–695.

Gelman A, Rubin DB. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* **7:** 457–472.

Giannuzzi-Savelli R, Pusateri F, Palmeri A, Ebreo C. 2003. *Atlante delle conchiglie marine del Mediterraneo. Vol. 4 (Neogastropoda: Muricoidea)*. Rome: Evolver.

Goetze E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proceedings of the Royal Society B: Biological Sciences* **270:** 2321–2331.

Gofas S. 2011. Familia muricidae. In: Gofas S, Moreno D, Salas C, eds. *Moluscos marinos de Andalucía – I*. Málaga: Servicio de Publicaciones e Intercambio Científico, Universidad de Málaga, 278–286.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Hart MW, Sunday J. 2007. Things fall apart: biological species form unconnected parsimony networks. *Biology Letters* **3:** 509–512.

Hasegawa M, Kishino H, Yano T. 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22:** 160–174.

Hayashi S. 2005. The molecular phylogeny of the Buccinidae (Caenogastropoda: Neogastropoda) as inferred from the complete mitochondrial 16S rRNA gene sequences of selected representatives. *Molluscan Research* **25:** 85–98.

Hidalgo JG. 1870. *Moluscos marinos de España, Portugal y las Baleares*. Madrid: Impr. de M. Ginesta.

Hillis DM, Moritz C, Mable BK. 1996. *Molecular systematics*. Sunderland, MA: Sinauer Associates.

Houart R. 1997. The west African Muricidae. II. Ocenebrinae, Ergalataxinae, Tripterotyphinae, Typhinae, Trophoninae & Rapaninae. *Apex* **12:** 49–91.

Houart R. 2000. New species of Muricidae (Gastropoda) from the northeastern Atlantic and the Mediterranean Sea. *Zoosystema* **22:** 459–469.

Houart R. 2001. *A review of the recent Mediterranean and Northeastern Atlantic species of Muricidae*. Rome: Evolver.

Houart R, Abreu AD. 1994. The Muricidae (Gastropoda) from Madeira with the description of a new species of Ocenebrinae. *Apex* **9:** 119–130.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

ICZN [International Commission on Zoological Nomenclature]. 1999. *International code of zoological nomenclature, Fourth edn*. London: International Trust for Zoological Nomenclature.

Jousseaume FP. 1880. Division méthodique de la famille des Purpuridae. *Le Naturaliste: Journal des échanges et des nouvelles* **2:** 335–336.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules. In: Munro HM, ed. *Mammalian protein metabolism*. New York: Academic Press, 21–132.

Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9:** 286–298.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

Kimura M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences* **78:** 454–458.

Knowlton N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* **24:** 189–216.

Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilson DS. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature* **400:** 652–655.

Kumar S, Skjæveland T, Orr RJS, Enger P, Ruden T, Mevik B, Burki F, Botnen A, Shalchian-Tabrizi K. 2009. AIR: a batch-oriented web program package for construction of supermatrices ready for phylogenomic analyses. *BMC Bioinformatics* **10:** 357.

Küster HC, Kobelt W. 1839–1878. Die geschwänzten und bewehrten Purpurschnecken (*Murex, Ranella, Tritonium, Trophon, Hindsia*) in abbildungen nach der Natur mit Beschreibungen. *Systematisches Conchylien-Cabinet von Martini und Chemnitz* **3:** 1–99, 110–336, pls A, B, 1–77, 4b, 37a, 38a, 39a.

Lamarck JBM. 1822. *Histoire naturelle des animaux sans vertèbres, présentant les caractères généraux et particuliers de ces animaux, leur distribution, leurs classes, leurs familles, leurs genres, et la citation des principales espèces qui s'y rapportent; précédée d'une introduction offrant la détermination des caractères essentiels de l'animal, sa distinction du végétal et des autres corps naturels; enfin, l'exposition des principes fondamentaux de la zoologie. Vol. 7*. Paris.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25:** 1451–1452.

Locard A. 1886. *Prodrome de malacologie française. Vol. 2. Catalogue général des Mollusques vivants de France. Mollusques marins*. Lyon: Librairie Henri Georg.

Martel A, Chia FS. 1991. Drifting and dispersal of small bivalves and gastropods with direct development. *Journal of Experimental Marine Biology and Ecology* **150:** 131–147.

Mejri R, Arculeo M, Ben Hassine OK, Lo Brutto S. 2011. Genetic architecture of the marbled goby *Pomatoschistus marmoratus* (Perciformes, Gobiidae) in the Mediterranean Sea. *Molecular Phylogenetics and Evolution* **58:** 395–403.

Meyer CP, Geller JB, Paulay G. 2005. Fine scale endemism on coral reefs: archipelagic differentiation in Turbinid gastropods. *Evolution* **59:** 113–125.

Monaghan MT, Wild R, Elliot M, Fujisawa T, Balke M, Inward DJG, Lees DC, Ranaivosolo R, Eggleton P, Barraclough TG, Vogler AP. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology* **58:** 298–311.

di Monterosato TA. 1884. *Nomenclatura generica e specifica di alcune conchiglie mediterranee*. Palermo: Virzi.

di Monterosato TA. 1917. Molluschi viventi e quaternari raccolti lungo le coste della Tripolitania dall'ing Camillo Crema. *Bollettino della Società Zoologica Italiana* **3:** 1–28.

Nardo GD. 1847. *Sinonimia moderna delle specie registrate nell' opera intitolata: Descrizione de' crostacei, de' testacei e de' pesci che abitano le lagune e golfo veneto, rappresentati in figure, a chiaro-scuro ed a colori dall' Abate Stefano Chiereghin Ven. Clodiense*. Venezia.

Nordsieck F. 1968. *Die europäischen Meeres-Gehäuseschnecken (Prosobranchia)*. Stuttgart: G. Fischer.

Nuñez JJ, Vejar-Pardo A, Guzmán BE, Barriga EH, Gallardo CS. 2012. Phylogenetic and mixed Yule-coalescent analyses reveal cryptic lineages within two South American marine snails of the genus *Crepipatella* (Gastropoda: Calyptraeidae). *Invertebrate Biology* **131:** 301–311.

Oliverio M. 1994. Developmental vs genetic variation in two Mediterranean rissoid gastropod complexes. *Journal of Molluscan Studies* **60:** 461–465.

Oliverio M. 1996. Life-histories, speciation and biodiversity in Mediterranean prosobranch gastropods. *Vie et Mileu* **46:** 163–169.

Oliverio M, Mariottini P. 2001a. Contrasting morphological and molecular variation in *Coralliophila meyendorffii* (Muricidae, Coralliophilinae). *Journal of Molluscan Studies* **67:** 243–246.

Oliverio M, Mariottini P. 2001b. A molecular framework for the phylogeny of *Coralliophila* and related muricoids. *Journal of Molluscan Studies* **67:** 215–224.

Öztürk B, Buzzurro G, Benli HA. 2004. Marine molluscs from Cyprus: new data and checklist. *Bollettino Malacologico* **39:** 49–78.

Pallary P. 1902a. Diagnoses de quelques coquilles nouvelles provenant du Maroc. *Journal de Conchyliologie* **49:** 226–228.

Pallary P. 1902b. Liste des mollusques testacés de la Baie de Tanger. *Journal de Conchyliologie* **50:** 1–39.

Pallary P. 1904. Addition à la faune malacologique du Golfe de Gabès. *Journal de Conchyliologie* **52:** 212–248.

Pallary P. 1912. Sur la faune de l'ancienne lagune de Tunis. *Bulletin de la Société d'histoire naturelle d'Afrique du Nord* **3:** 215–228.

Pallary P. 1920. *Mission zoologique. Malacologie. In: Exploration scientifique du Maroc organisée par la Société de Géographie de Paris et continuée par la Société des Sciences Naturelles du Maroc. Deuxième fascicule*. Rabat/Paris: Institut Scientifique Chérifien.

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 2002. *The simple fool's guide to PCR Version 2.0*. Honolulu, HI: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Patarnello T, Volckaert FAMJ, Castilho R. 2007. Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology* **16:** 4426–4444.

Paulay G, Meyer C. 2006. Dispersal and divergence across the greatest ocean region: do larvae matter? *Integrative Comparative Biology* **46:** 269–281.

Payraudeau BC. 1826. *Catalogue descriptif et méthodique des Annélides et des Mollusques de l'île de Corse*. Paris.

Pons J, Barraclough TG, Gomez-Zurita J, Cardoso A, Duran DP, Hazell S, Kamoun S, Sumlin WD, Vogler AP. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* **55:** 595–609.

Pons-Moyà J, Pons GX. 2002. Primera cita d'*Ocinebrina hispidula* (Pallary, 1904) (Mollusca: Gastropoda: Muricidae) per a les aigües Ibero-Balears. *Bolletí de la Societat d'Història Natural de les Balears* **45:** 81–85.

Posada D. 2003. Using MODELTEST and PAUP* to select a model of nucleotide substitution. In: *Current Protocols in Bioinformatics*. New York: John Wiley & Sons, Inc.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Posada D, Crandall KA. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution* **16:** 37–45.

Puillandre N, Baylac M, Boisselier MC, Cruaud C, Samadi S. 2009. An integrative approach to species delimitation in *Benthomangelia* (Mollusca: Conoidea). *Biological Journal of the Linnean Society* **96:** 696–708.

Puillandre N, Lambert A, Brouillet S, Achaz G. 2012a. ABGD, automatic barcode gap discovery for primary species delimitation. *Molecular Ecology* **21:** 1864–1877.

Puillandre N, Meyer CP, Bouchet P, Olivera BM. 2011. Genetic divergence and geographical variation in the deep-water *Conus orbignyi* complex (Mollusca: Conoidea). *Zoologica Scripta* **40:** 350–363.

Puillandre N, Modica MV, Zhang Y, Sirovich L, Boisselier MC, Cruaud C, Holford M, Samadi S. 2012b. Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology* **21:** 2671–2691.

Puillandre N, Sysoev AV, Olivera BM, Couloux A, Bouchet P. 2010. Loss of planktotrophy and speciation: geographical fragmentation in the deep-water gastropod genus *Bathytoma* (Gastropoda, Conoidea) in the western Pacific. *Systematics and Biodiversity* **8:** 371–394.

Quattro J, Chase M, Rex M, Greig T, Etter R. 2001. Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod *Frigidoalvania brychia*. *Marine Biology* **139:** 1107–1113.

Radwin GE, D'Attilio A. 1976. *Murex shells of the world*. Stanford, CA: Stanford University Press.

Rambaut A, Drummond AJ. 2003. Tracer v. 1.5.0. Available at: http://evolve.zoo.ox.ac.uk/software/

Reeve LA. 1845. *Conchologia Iconica, or, illustrations of the shells of Molluscous animals. Vol. 3*. London.

Reid DG, Dyal P, Williams ST. 2010. Global diversification of mangrove fauna: a molecular phylogeny of *Littoraria* (Gastropoda: Littorinidae). *Molecular Phylogenetics and Evolution* **55:** 185–201.

Richards TA, Jones MDM, Leonard G, Bass D. 2012. Marine fungi: their ecology and molecular diversity. *Annual Review of Marine Science* **4:** 495–522.

Rolán E. 1983. Moluscos de la Ria de Vigo – I. Gasteropodos. *Thalassas* **1:** 1–383.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Sá-Pinto A, Baird SJE, Pinho C, Alexandrino P, Branco M. 2010. A three-way contact zone between forms of *Patella rustica* (Mollusca: Patellidae) in the central Mediterranean Sea. *Biological Journal of the Linnean Society* **100:** 154–169.

Sá-Pinto A, Branco MS, Alexandrino PB, Fontaine MC, Baird SJE. 2012. Barriers to gene flow in the marine environment: insights from two common intertidal limpet species of the Atlantic and Mediterranean. *PLoS ONE* **7:** e50330.

Scacchi A. 1836. *Catalogus Conchyliorum Regni Neapolitani quae usque adhuc reperit A. Scacchi*. Napoli: Typis Filiatre-Sebetii.

Schwarz G. 1978. Estimating the dimension of a model. *Annals of Statistics* **6:** 461–464.

Settepassi F. 1977. *Atlante Malacologico. I molluschi marini viventi nel Mediterraneo volume II*. Roma: Museo di Zoologia.

Sowerby GB II. 1832–1841. *The conchological illustrations, or coloured figures of all the hitherto unfigured Recent shells*. London.

Stocchi P, Colleoni F, Spada G. 2009. Bounds on the time–history and Holocene mass budget of Antarctica from sea-level records in SE Tunisia. *Pure and Applied Geophysics* **166:** 1319–1341.

Stossich A. 1865. Enumerazione dei Molluschi del Golfo di Trieste. *Programma della Civica Scuola Reale Superiore di Trieste*. **1865:** 21–58.

Taviani M. 2002. The Mediterranean benthos from late Miocene up to present: ten million years of dramatic climatic and geologic vicissitudes. *Biologia Marina Mediterranea* **9:** 445–463.

Templeton AR. 2001. Using phylogeographic analyses of gene trees to test species status and processes. *Molecular Ecology* **10:** 779–791.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132:** 619–633.

Vermeij GJ. 1978. *Biogeography and adaptation: patterns of marine life*. Cambridge: Harvard University Press.

Vermeij GJ, Vokes EH. 1997. Cenozoic Muricidae of the Western Atlantic region. Part XII–the subfamily Ocenebrinae (in part). *Tulane Studies in Geology and Paleontology* **29:** 68–118.

Vilas R, Criscione CD, Blouin MS. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology* **131:** 839–846.

Vogler AP, Monaghan MT. 2007. Recent advances in DNA taxonomy. *Journal of Zoological Systematics and Evolutionary Research* **45:** 1–10.

Vokes EH. 1994. The muricid types of Frank Collins Baker. *The Nautilus* **107:** 118–123.

Williams S, Apte D, Ozawa T, Kaligis F, Nakano T. 2011. Speciation and dispersal along continental coastlines and Island arcs in the Indo-West Pacific turbinid gastropod genus *Lunella*. *Evolution* **65:** 1752–1771.

Wilson NG, Schrödl M, Halanych KM. 2009. Ocean barriers and glaciation: evidence for explosive radiation of mitochondrial lineages in the Antarctic sea slug *Doris kerguelenensis* (Mollusca, Nudibranchia). *Molecular Ecology* **18:** 965–984.

Xavier R, Zenboudji S, Lima FP, Harris DJ, Santos AM, Branco M. 2011. Phylogeography of the marine isopod *Stenosoma nadejda* (Rezig, 1989) in North African Atlantic and western Mediterranean coasts reveals complex differentiation patterns and a new species. *Biological Journal of the Linnean Society* **104:** 419–431.

Zou S, Li Q, Kong L. 2012. Monophyly, distance and character-based multigene barcoding reveal extraordinary cryptic diversity in *Nassarius*: a complex and dangerous community. *PLoS ONE* **7:** e47276. doi:10.1371/journal.pone.0047276.

SUPPORTING INFORMATION

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

Figure S1. Result of the Generalized Mixed Yule-Coalescent (GMYC) analysis for the combined COI + ITS2 dataset.

Figure S2. Results of the single-gene Bayesian analyses (by MrBayes). From left to right: COI, ITS2, 16S.

Figure S3. Results of the multiple-gene Bayesian analyses (by MrBayes). From left to right: COI + ITS2 and COI + ITS2 + 16S.

Figure S4. Shells of representative voucher specimens from each sampling site. ID numbers as in Table 1. Scale bar, 10 mm.

Figure S5. Shells of representative voucher specimens from each sampling site. ID numbers as in Table 1. Scale bar, 10 mm.

Figure S6. Shells of representative voucher specimens from each sampling site. ID numbers as in Table 1. Scale bar, 10 mm.

Table S1. Sequence Accession Numbers of the sequences of *Ocinebrina* spp. used in this work, within the framework of the BOLD Project 'BOCI' (Barcoding of *Ocinebrina*).