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The Naticidae (Mollusca: Gastropoda) of Giglio Island (Tuscany, Italy): Shell characters, live animals, and a molecular analysis of egg masses

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Abstract

We investigated the occurence of members of the predatory caenogastropod family Naticidae in the littoral of the island of Giglio, Tuscany, Italy. We recorded a total of 8 species, all but one represented by both empty shells and living specimens. As most studies of Mediterranean naticids are based solely on empty shells, we here provide images of living animals for 7 out of the 8 species encountered; for several of these species this is the first photographic documentation of the animal. Our survey included a systematic collection of egg masses (”sand collars”) which were hatched in the laboratory. The larvae obtained as well as the sand collars themselves were used for molecular analysis of the species based on gene fragments of mitochondrial cytochrome oxidase subunit I (COI), histone 3 (H3), the mitochondrial 16S rRNA (16S), and 18S rRNA (18S). We show that such molecular analysis allows the confirmation of the identity of naticid species without having access to adult specimens or shells. This approach identified one additional naticid species for which no adult specimens or shells were found. Additionally, our molecular analysis allows consideration of naticid phylogeny.

Key words: Naticidae, Naticinae, Naticarius, Notocochlis, Tectonatica, Polinicinae, Euspira, Neverita, gastropod biodiversity, phylogenetic relationship, taxonomy, molecular identification, sand collars, egg masses, Mediterranean Sea

Introduction

The Naticidae are a cosmopolitan gastropod family that lives from the intertidal zone to several thousand meters depth. The animals are equipped with a large muscular foot and use its front part, the propodium, to bury into sand and mud bottoms. The naticids are predators that have developed a characteristic mode of feeding on their prey, commonly bivalves but also other gastropods including other naticids, in enveloping their prey with their foot and drilling a hole into the shells to reach the soft parts with their proboscis. It has been estimated that worldwide there are about 260–270 Recent species in this family (Kabat 1996), which is assumed to have originated in the late Triassic (Wenz 1941, Bouchet & Warén 1993) or in the early Jurassic (Carriker & Yochelson 1968, Marincovich 1977). The greatest species and generic diversity is found in tropical regions (Kabat 1996), but Naticidae are also abundant in moderately temperate as well as Arctic and Antarctic waters. Members of the Naticidae can easily be recognized by their shell shape, distinctive animals and their peculiar predatory behavior. The biodiversity of the Naticidae in the Mediterranean Sea has been described by several authors (e.g. Kobelt 1901, Hidalgo 1917, Settepassi 1972, Schiró 1977–1978, Sabelli & Spada 1977–1980, Nordsieck 1982, Riedel 1983, Villa 1985–1986, Doneddu & Manunza 1989, Sabelli *et al.* 1990, Poppe & Goto 1991, Barash & Danin 1992, De Smit & Bába 2001, Demir 2002) during the last two centuries. In 1993, Alf *et al.* published a species list of Mollusca for Giglio Island, Italy, that included three naticid species: *Naticarius hebraeus* (Martyn, 1786) [as *Natica cruentata* (Gmelin, 1791)], *Notocochlis dillwynii* (Payraudeau, 1826) [as *Natica dillwynii* (Payraudeau, 1826)], and either *Euspira nitida* (Donovan, 1804) or *Euspira macilenta* (Philippi, 1844) [as *Lunatia* cf. *guillemini* (Payraudeau, 1826)]. Just one specimen of *Naticarius hebraeus* was reportedly found alive (Alf *et al.* 1993), while only broken shells were found of each of the other two species. Since only one shell picture was published (*N. hebraeus*) and, unfortunately, no voucher specimens have been deposited (Alf, in litt.), it is impossible to confirm the identification of these specimens. Terreni (1980) mentioned 10 naticid species in his compilation of the molluscs of the *Archipelago Toscano* of which 2 are reported from Giglio Island: *Payraudeautia intricata* (Donovan, 1804) and *N. hebraeus*. While the biodiversity of the Mediterranean Sea is well known, only few live pictures of species of the Naticidae have been published. Mostly, shells and, if available, their operculi have been figured. Sabelli *et al.* (1990) listed 21 naticid species for the entire Mediterranean Sea of which 20 are currently believed to belong to the Naticidae [all but *Bulbus globosus* (Jeffreys, 1885); see Bouchet & Wáren 1993]. The 20 species were assigned to three subfamilies and 8 genera. Some pictures of living animals of Naticidae have also been published by Settepassi (1972) who showed images of *Naticarius stercusmuscarum* (Gmelin, 1791) [as *Naticarius punctatus* (Karsten, 1798)] and *Neverita josephinia* (Risso, 1826), by Schiró (1977–1978) who showed photos of *Naticarius stercusmuscarum* and *Neverita josephinia*, and by Ziegelmeier (1955) who showed drawings of *Euspira nitida* from the North Sea.

During our field work at the *Institut für Marine Biologie* (*IfMB*) on Giglio Island, a total of 8 species of Naticidae were collected alive and/or as empty shells. Pictures of shells and living specimens are provided together with a detailed description. As the morphological shell characters alone often present difficulties for unequivocal species determination, characters of the living specimens can be helpful in species identification and surveys of naticid biodiversity. On Giglio Island, the snails were mostly found on pure sand bottoms and sand flats between sea weeds of *Posidonia oceanica* (Linnaeus, 1758) and *Zostera marina* (Linnaeus, 1758), and in small sand patches between rocks. All naticid species bury in the sand, but to different depths. This makes it very difficult to estimate specimen density solely on randomly collected individuals. Previous studies have shown that densities of naticids in intertidal habitats rarely exceed 1–2 m^{-2} (Richardson *et al.* 2005). As *Tectonatica sagraiana* (Orbigny, 1842) and both *Naticarius hebraeus* and *Naticarius stercusmuscarum* crawl on the sand or directly below the sand surface, these species were found predominantly during collecting compaigns. By contrast, *Notocochlis dillwynii*, *Euspira nitida*, *Euspira macilenta*, *Neverita josephinia*, and *Payraudeautia intricata* (Donovan, 1804) bury deeper in the sand. Thus, they rarely were seen alive. Unlike the adult animals, the egg masses of the Naticidae can easily be found on sand bottoms at all depths. This indicates that even species regarded as uncommon may be more abundant than is realized. The egg masses are collar-shaped [hence the name 'sand collars'] and consist of sand grains cemented together by a gelatinous matrix with embedded eggs. Generally, the egg masses of the Naticidae were very abundant, which demonstrates the wide distribution of Naticidae from pure sand areas to small sand patches found in shallow waters between rocks and sea weeds. The morphology of egg masses and the development of the larvae have been analyzed by several scientists (e.g. Giglioli 1955, Ziegelmeier 1961–1963, Bandel 1976, Kingsley-Smith *et al.* 2003). Unfortunately, collected egg masses cannot unequivocally be assigned to a particular species. Nevertheless, the egg masses of the Naticidae were separated into two distinctive subgroups based on their general appearance (Giglioli 1955, Bandel 1976). Because the egg masses have only few characters to differentiate them, definite correlations between egg masses and naticid species require aquaria or *in situ* observations (Giglioli 1955, Ziegelmeier 1961–1963, Bandel 1976, Kingsley-Smith *et al.* 2003).

The molecular identification method [DNA barcoding, (Hebert *et al.* 2003)] aims to compare short, specific DNA fragments from unidentified specimens with sequences of previously identified voucher specimens. Together with the classical, morphology-based taxonomy this technique can help in completing biodiversity inventories (Padial & De La Riva 2007). In this study, we used molecular analysis of four DNA fragments (histon 3 [H3], cytochrome oxidase subunit I [COI], 16S rRNA [16S], and 18S rRNA [18S]) from randomly collected egg masses and live-collected specimens to obtain an inventory of naticid biodiversity of Giglio Island as complete as possible. We found a clear correlation of gene fragments obtained from egg masses with live-collected species by molecular comparison, demonstrating an alternative, versatile technique for estimating naticid biodiversity that could easily be extended to studies of biogeographical distribution and phylogenetic patterns.

In the last two centuries, assumed phylogenetic relationships within the Naticidae were based mainly on shell morphology (Philippi 1849–1853, Cossmann 1925, Finlay & Marwick 1937, Wenz 1941, Marincovich 1977, Kabat 1991, Riedel 2000). Although shell shape, sculpture, coloration, and opercular sculpture are the traditional characters used in naticid systematics, a few analyses deal with other, additional characters (Schileyko 1977: reproductive tract; Cernohorsky 1971: radula; Strong 2003: gut anatomy; Aronowsky 2003: egg mass morphology).

Characters considered to be the main distinguishing features of this family included the composition and the surface structure of the operculum, the umbilical area, the protoconch, the aperture, and the general shell morphology (height, width, color pattern). In addition, morphological characteristics of the radular teeth (e.g., Powell 1933, Dell 1990, Cernohorsky 1971, Villa 1986 Fernandes & Rolán 1993) were included by some researchers. For a critical assessment of the usefulness of these characters see Powell (1937), Dell (1990), Bouchet & Warén (1993), and Bandel (1999). Generally, most authors suggested a distinct separation into four groups at the subfamiliar level: Ampullospirinae, Naticinae, Polinicinae and Sininae (Marincovich 1977, Kabat 1991, 1996, Riedel 2001). This arrangement is mainly based on the material composition (calcareous: Naticinae, corneous: Polinicinae, Sininae) and size of the operculum. Nevertheless, some authors expressed skepticism about the operculum-based arrangement within the Naticidae (Powell 1933, Marincovich 1977, Kabat 1996, Bandel 2000) since *Eunaticina insculpta* (Carpenter, 1865) has a partially calcified operculum (Cernohorsky 1977) while in general members of the genus *Eunaticina* have a corneous operculum. Other taxonomic characters (e.g. protoconch, funicle), which often show similarities across all subfamilies, differ radically within each of these groups. With on the present molecular analysis we provide evidence suggesting that a phylogenetic separation based on the material composition of the operculum may not be possible (Figure 1).

Materials and Methods

a) Material examined

Naticid specimens and sand collars used in molecular analysis (Figures 2, 3) were collected by diving from up to 40 m depths at several dive spots around Giglio Island (Figure 4, Table 4). Several sand collars deposited by *T. sagraiana* and *N. josephinia* under observation in aquaria were included to verify the reliability of our egg mass identification. Additional live-collected Mediterranean naticid species from the Michael Hollmann Collection (MHC), in Witten, Germany, were analyzed for comparison with unknown egg masses. Altogether, our sequence data are based on 11 species representing 7 traditional genera, including 7 live-collected species from Giglio Island and 4 additional Mediterranean species (*Euspira catena* (da Costa, 1778), *Euspira fusca* (Blainville, 1825), *Natica vittata* (Gmelin, 1791), *Natica prietoi* Hidalgo, 1873) not found on Giglio Island (see Figure 2). Furthermore, 24 sand collars from Giglio Island were included in the analysis (see Figure 3). A detailed overview of all analyzed tissues are listed in Table 1. The material examined has been deposited in the collections of the Department of Biochemistry I, Ruhr University Bochum, Bochum, Germany, and is used in the ongoing PhD project of the first author. Images of all specimens used in molecular analyses are deposited on Morphobank (O'Leary & Kaufmann 2007) where the study is saved as *Project 189* (http://morphobank.geongrid.org). Morphobank-based *Image Voucher Numbers* are listed in table 1 (M14565-M14636).

b) Processing of material

First, pictures of the living specimens were taken in a glass aquarium with a black bottom; afterwards, the snails were anasthetized with 0.25 M $MgCl_2$ and transferred to 100% EtOH. The egg masses were kept in small jars and the sea water was changed daily until the larvae had hatched. All larvae analyzed in this study showed planctonic stages. As the larvae were processed for DNA extraction immediately after hatching, we cannot report observations on their further development. Except for one egg mass found in the aquaria of *T. sagraiana* (L76), the time to hatching of the randomly collected egg masses could not be determined due to the unknown deposition date.

The larvae were collected, centrifuged at 5000 rpm, and stored in 100% EtOH or in RNAlater (Qiagen, Hilden, Germany) for subsequent DNA extraction. Aditionally, small pieces of each egg mass were also stored in 100% EtOH or in RNAlater (Qiagen, Hilden, Germany). The morphology of the protoconch is a widely used character within naticid classification (e.g., Cernohorsky 1970, Marincovich 1977, Bouchet & Warén 1993, Bandel 1999) and was also analyzed in this study. Protoconchs were measured by counting the number of whorls starting with the first embryonal whorl.

TABLE 1: Specimens, egg masses, Morphobank voucher numbers, and the GenBank accession numbers for the appendant sequences analyzed in this study. Morphobank voucher numbers refer to images deposited in Morphobank (http://morphobank.geongrid.org/, project_id: 189). Accession numbers with the prefix EU refer to specimens collected for this study. Gene segments lacking from the data and unknown origins of specimens are indicated by dashed cells. As outgroup taxa the H3, COI, and 18S sequences of *Tonna cerevisina* and *Cypraea annulus* as listed in the NCBI database (see Colgan *et al*. 2007) were used. Missing data was coded as "?". On average 1367 bp ± 224 [SD] were analyzed. The shortest data set was obtained for *N. prietoi* with 556 bp. DNA samples from egg masses were marked with C followed by a reference number when DNA was extracted directly from an egg mass. they were marked with L followed by a reference number if DNA extraction was performed from hatched larvae.

Analyzed specimens (Morphobank voucher No.)	**Origin / Collection site**	**COI gene**	**H3 gene**	**16S gene**	**18S gene**	**Length (bp)**
Tonna cerevisina	----------	DQ916506	DQ916453	-----------	DQ916534	955
Cypraea annulus	----------	AY296832	AF033681	-----------	DQ916532	882
Natica prietoi (M14575, M14618, M14619, M14620)	Strait of Gibralter, Southern Spain (15-20 m depth)	----	EU332675	----	EU332608	556
Natica vittata (M14573, M14621, M14622, M14623)	Nossa Senhora da Rocha, Algarve, Portugal (7-10 m depth)	EU332642	EU332676	EU332575	EU332609	1354
Naticarius hebraeus (M14567, M14583, M14584, M14585)	Campese Bay, Giglio Island, Italy (6 m depth)	EU332643	EU332677	EU332576	EU332610	1491
Naticarius stercus-muscarum (M14574, M14586, M14587, M14588)	Campese Bay, Giglio Island, Italy (6 m depth)	EU332644	EU332678	EU332577	EU332611	1527
Notocochlis dillwynii (M14566, M14627, M14628, M14629)	Pt. del Morto, Giglio Island, Italy (3 m depth)	EU332646	EU332680	EU332579	EU332613	1483
Tectonatica sagraiana (M14565, M14624, M14625, M14626)	Campese Bay, Giglio Island, Italy (13 m depth)	EU332648	EU332682	EU332581	EU332615	1480
Euspira catena (M14572, M14615, M14616, M14617)	Island of Terschelling; Netherlands	EU332639	EU332672	----	EU332606	1055
Euspira nitida (M14570, M14577, M14578, M14579)	Cala dell´Allume, Giglio, Italy (9 m depth)	EU332641	EU332674	EU332574	EU332607	1529
Euspira fusca (M14571, M14630, M14631, M14632)	off Olhão, Algarve, Portugal (380 – 400 m depth)	EU332640	EU332673	EU332573	----	1216
Neverita josephinia (M14569, M14589, M14590, M14591)	Campese Bay, Giglio Island, Italy (7 m depth)	EU332645	EU332679	EU332578	EU332612	1394

...... *continued*

TABLE 1 (continued)

Analyzed specimens (Morphobank voucher No.)	**Origin / Collection site**	**COI gene**	**H3 gene**	**16S gene**	**18S gene**	**Length (bp)**
Payraudeautia intricata (M14568, M14592, M14593, M14594)	Cala dell´Allume, Giglio Island, Italy (6 m depth)	EU332647	EU332681	EU332580	EU332614	1521
L61 (*Tectonatica sagraiana*, M14597)	Campese Bay, Giglio Island, Italy (12 m depth)	EU332616	EU332649	EU332550	EU332582	1450
L62 (*Tectonatica sagraiana*, M14598)	Campese Bay, Giglio Island, Italy (14 m depth)	EU332617	EU332650	EU332551	EU332583	1446
L64 (*Notocochlis dillwynii*, M14610)	Cala dell´Allume, Giglio Island, Italy (6 m depth)	EU332618	EU332651	EU332552	EU332584	1441
L65 (*Notocochlis dillwynii*, M14611)	Cala dell´Allume, Giglio Island, Italy (8 m depth)	EU332619	EU332652	EU332553	EU332585	1289
L69 (*Tectonatica sagraiana*, M14599)	Campese Bay, Giglio Island, Italy (8 m depth)	EU332620	EU332653	EU332554	EU332586	1493
L70 (*Tectonatica sagraiana*, M14600)	Campese Bay, Giglio Island, Italy (11 m depth)	EU332621	EU332654	EU332555	EU332587	1529
L71 (*Notocochlis dillwynii*, M14612)	Cala dell´Allume, Giglio Island, Italy (7 m depth)	EU332622	----	EU332556	EU332588	1184
L73 (*Notocochlis dillwynii*, M14613)	Cala dell´Allume, Giglio Island, Italy (8 m depth)	EU332623	EU332655	EU332557	EU332589	1495
L74 (*Tectonatica sagraiana*, M14601)	Campese Bay, Giglio Island, Italy (10 m depth)	----	EU332656	EU332558	EU332590	1241
L75 (*Notocochlis dillwynii*, M14614)	Cala dell´Allume, Giglio Island, Italy (9 m depth)	EU332624	EU332657	----	EU332591	934
L76 (*Tectonatica sagraiana*, M14602)	Aquarium, ex. Campese Bay, Giglio Island, Italy (10 m depth)	EU332625	EU332658	EU332559	EU332592	1463
L79 (*Tectonatica sagraiana*, M14603)	Campese Bay, Giglio Island, Italy (10 m depth)	EU332626	EU332659	EU332560	EU332593	1529
L81 (*Tectonatica sagraiana*, M14604)	Campese Bay, Giglio Island, Italy (11 m depth)	EU332627	EU332660	EU332561	EU332594	1485
L82 *(Tectonatica spec.* probably *T. rizzae,* M14636)	Pt. delle Secche, Giglio Island, Italy (18 m depth)	EU332628	EU332661	EU332562	EU332595	1530

...... *continued*

TABLE 1 (continued)

Analyzed specimens (Morphobank voucher No.)	Origin / Collection site	COI gene	H3 gene	16S gene	18S gene	Length (bp)
L83 (*Tectonatica sagraiana*, M14605)	Campese Bay, Giglio Island, Italy (7 m depth)	EU332629	EU332662	EU332563	EU332596	1529
C111 (*Tectonatica sagraiana*, M14596)	Aquarium, ex. Campese Bay, Giglio Island, Italy (10 m depth)	EU332630	EU332663	EU332564	EU332597	1465
C126 *(Tectonatica spec.* probably *T. rizzae,* M14635)	Pt. delle Secche, Giglio Island, Italy (24 m depth)	EU332631	EU332664	EU332565	EU332598	1489
C127 *(Tectonatica spec.* probably *T. rizzae,* M14633)	Pt. delle Secche, Giglio Island, Italy (18 m depth)	EU332632	EU332665	EU332566	EU332599	1441
C128 (*Notocochlis dillwynii*, M14608)	Fenaio, Giglio Island, Italy (5 m depth)	EU332633	EU332666	EU332567	EU332600	1439
C129 (*Neverita josephinia*, M14606)	Campese Bay, Giglio Island, Italy (5 m depth)	EU332634	EU332667	EU332568	EU332601	1443
C130 (*Neverita josephinia*, M14607)	Aquaria, ex. Campese Bay, Giglio Island, Italy (4 m depth)	EU332635	EU332668	EU332569	EU332602	1391
C131 *(Tectonatica spec.* probably *T. rizzae,* M14634)	Pt. delle Secche, Giglio Island, Italy (20 m depth)	EU332636	EU332669	EU332570	EU332603	1520
C132 (*Notocochlis dillwynii*, M14609)	Pt. delle Secche, Giglio Island, Italy (16 m depth)	EU332637	EU332670	EU332571	EU332604	1458
C133 (*Naticarius hebraeus*, M14595)	Pt del Morto, Giglio Island, Italy (24 m depth)	EU332638	EU332671	EU332572	EU332605	1435
					MEAN	1366.5 ± 223.67

As outgroup taxa we used *Tonna cerevisina* (Hedley, 1919) and *Cypraea annulus* (Linnaeus, 1758) of which the H3, COI, and 18S gene fragments were included. We selected Tonnidae and Cypraeidae as outgroup taxa based on the molecular study by Colgan *et al.* (2007) who showed that these two gastropod families are closely related to the Naticidae.We additionally calculated the ratios (r) of height (h) and width (w) of shells as a measure of differences in shell morphology. The standard error of the mean (SEM) was calculated for all values. The significance of differences between the ratios of the various species was verified by a two-paired non-parametric t-test (Mann-Whitney) using the Program PRISM v3.0.

c) Nucleic acid isolation, subcloning, and sequence analysis

Total DNA was extracted from ethanol/RNAlater-preserved tissue using a modified protocol of the DNeasy Extraction Kit (Qiagen, Hilden, Germany) and stored in 0.1 mM Tris-EDTA pH 7.4. DNA samples were marked with C when DNA was extracted directly from the egg masses. They were marked with L if DNA extraction was performed on hatched larvae (Table 1). A 423 bp fragment of the COI gene, 244 bp of the H3 gene, 470 bp of the 16S rRNA gene, and 405 bp of the 18S rRNA gene were sequenced from each species and each egg mass, resulting in a total alignment of 1542 bp. On average, we were able to analyze 1367 bp ± 224 [SD]. The shortest data set was obtained from *N. prietoi* with 556 bp (see Table 1). Amplification reactions using iPoof-Polymerase (Bio-Rad Laboratories, Munich, Germany) were performed in MJ Research thermocyclers (Watertown, MA, USA). Amplification primers used were P388 (5´-gcttttgttataattttytt-3´) and P390 (5´-cgatcagttaaaartatwgtaat-3´) for COI, P263 (5´-cctcatcgttacaggcccgg-3´) and P266 (5´-actggatgtccttgggcatg-3´) for H3, P213 (5´-cgcctgttaccaaaaacat-3´) and P214 (5´- ccggtctgaactcagatcacgt-3´) for 16S, and P398 (5´-cgtgttgatyctgccagt-3´) and P399 (5´- tctcaggctccytctccgg-3´) for the partial 18S rRNA gene. The PCR products were purified using the JETSORB Gel Extraction Kit (Genomed, Löhne, Germany), and both strands were sequenced on an ABI 3130xl automated sequencer using the PCR primers and a BigDye® Terminator v3.1 sequencing kit (both Applied Biosystems, Foster City, CA, USA).

d) Phylogenetic calculations

All sequences were checked via BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) and subsequently aligned using the MegAlign program (DNAStar). The alignments were checked by eye, corrected and assembled in a combined data set using MacClade 4.08 (Maddison & Maddison 2006). Missing sequence data was coded as ”?”. Sequence divergencies were calculated separately for each data set (H3, COI, 16S, 18S), and in addition for the combined data set (ALL) using PAUP*4.0b10 (Swofford 2003). In a second step the heterogeneity of base composition was determined, using the *chi*-square test. Additionally, the permutation tail probability test (PTP) was performed, which assesses the randomness of the data structure. Both test are implemented in PAUP*4.0b10. Each single sequence analysis showed homogeneous base compositions (*chi*-square test: P = 0.999 –1.000) while the combined data set displays a heterogeneous base composition (P = 0.217) that prompted us to define all parameters as unlinked. 100 permutation test replicates resulted in P < 0.01 for all data sets, demonstrating absence of randomness. A detailed description of the results of the tests are shown in Table 2.

TABLE 2: Alignment characteristics for the four gene fragments amplified (H3, COI, 16S, 18S). Each set of fragments was analyzed separately, and in a combined data set (ALL) yielded 1542 bp. The heterogeneity of base composition (chi-square test) and permutation test (PTP) were performed with Paup*4.0b10 (df = degree of freedom; P = probability).

Alignment		**Positions**			**Tests**	
Gene	**Length**	**Informative**	**Uninformative**	**Constant**	**PTP**	**Chi-square**
COI [tree length 486]	423 bp	138	31	254	P=0.01	Chi-square = 47.358 (df=108), P = 0.999
H3 [tree length 85]	244 bp	31	15	198	P=0.01	Chi-square = 9.794 (df=108), P = 1.000
16S [tree length 254]	470 bp	115	23	332	P=0.01	Chi-square = 46.792 (df=108), P = 0.999
18S [tree length 78]	405 bp	41	7	357	P=0.01	Chi-square = 18.113 (df=108), P = 1.000
TOTAL [tree length 893]	1542 bp	325	76	1141	P=0.01	Chi-square = 119.220 (df=108), P = 0.217

An unrooted phylogenetic analysis was performed using MrBayes 3.1 (Huelsenbeck & Ronquist 2001), calculating 780000 generations. The phylogenetic model used in this analysis was estimated by MrModeltest (Nylander 2004) using PAUP*4.0b10 (Swofford 2003) executing the MrModelblock. The model selected was GTR+I+G. The protein-coded data sets of H3 and COI were coded as ”CODON” due to their protein-coding sequences. Owing to the heterogeneous base compositions (chi-square test) in the combined data set (see above), all parameters were defined as unlinked. Thus, each partition has its own set of parameters. The whole nexus file comprised of the four gene fragments is available from the author upon request.

For egg mass species identification, the absolute differences were transformed into distances for each data set. Absolute and relative distances were calculated with PAUP*4.0b10 (Swofford 2003), whereas average distances were calculated by a pairwise sequence comparison of each terminal taxon including egg masses. Standard deviation (SD) was calculated for all distance values. Table 3 shows the resulting values and their standard deviation. A total of 325 positions in the whole data set were parsimony-informative, 76 were parsimony-uninformative, while 1141 were constant. For reasons of simplicity, „gene sequence“ in the following refers to the sequence of the respective fragment of that gene as specified above under c). Additionally, „BayInf“ in the following refers to the Baysian Inference of bifurcating branches calculated by the program MrBayes.

Results

a) Egg mass species identification by molecular comparison

The egg masses of the Naticidae are well known, widely distributed in their habitat and thus easy to collect. Because the shells and living animals of some of the species are rarely found, we examined molecular data from egg masses and compared the gene sequences of H3, COI, 16S, and 18S with those of the live-collected specimens for a molecular species identification of each egg mass. For this we collected larvae from egg masses, which were hatched under controlled conditions in small aquaria, and extracted DNA from them as well as directly from the sand collars. For the molecular markers used in this study, no sequence differences were found between the DNA from hatched larvae and DNA directly extracted from the respective sand collars (Table 3). The molecular data were compared to data from specimens collected in the waters around Giglio Island as well as data from additional specimens of Mediterranean species not found on Giglio Island. Those sequences of additional Mediterranean species were added in an attempt to identify those egg masses with no obvious sequence correlation to species live-collected on Giglio Island.

TABLE 3: Absolute and relative distances (calculated with PAUP*4.0b10) between the investigated sequences (H3, COI, 16S rRNA, 18S rRNA, and the combined data set: ALL) of the analyzed egg masses and their respective species according to the phylogenetic tree shown in Figure 1. Abs, absolute distances [total distance, in bp]; Rel, relative distances [in %]. A, Average distances calculated for sequence comparisons of egg masses and their respective adult specimens; B, Average sequence distances between *T. sagraiana* and *T. spec.* (probably *rizzae*) based on their egg masses and an adult specimen of *T. sagraiana*. Values were calculated by a pairwise sequence comparison of all sequences from each terminal taxon which includes egg masses (* = average).

A

Pairwise comparison of sequences from egg masses and adult specimens of five naticid species

	H3 [244 bp]		**COI [423 bp]**		**16S [470 bp]**		**18S [405 bp]**		**ALL [1542 bp]**	
	abs.[bp]	rel. [%]	abs.[bp]	rel. [%]	abs.[bp]	rel. [%]	abs.[bp]	rel. [%]	abs.[bp]	rel. [%]
Naticarius hebraeus	1.00 ± ---	0.4 ± ---	1.00 ± ---	0.23 ± ---	0.00 ± ---	0.00 ± ---	0.00 ± ---	0.00 ± ---	2.00 ± ---	0.10 ± ---
Neverita josephinia	0.33* ± 0.6	0.17* ± 0.03	0.67* ± 0.58	0.16* ± 0.13	0.00* ± 0.00	0.00* ± 0.00	2.00* ± 1.00	0.63* ± 0.35	3.00* ± 1.73	0.23* ± 0.01
Notocochlis dillwynii	0.21* ±0.41	0.11* ±0.02	4.43* ± 3.65	1.27* ± 0.99	1.29* ± 1.15	0.31* ± 0.27	0.00* ± 0.00	0.00* ± 0.00	5.92* ± 4.24	0.53* ± 0.34
Tectonatica sagraiana	0.00* ± 0.00	0.00* ± 0.00	1.33* ± 0.86	0.36* ± 0.23	0.53* ± 0.61	0.15* ± 0.15	0.19* ± 0.40	0.05* ± 0.1	2.27* ± 1.26	0.20* ± 0.09
Tectonatica cf. *rizzae*	0.00* ± 0.00	0.00* ± 0.00	1.67* ± 0.82	0.39* ± 0.02	1.00* ± 0.63	0.22* ± 0.13	0.00* ± 0.00	0.00* ± 0.00	2.67* ± 1.37	0.18* ± 0.09

B

Sequence comparison *T. sagraiana* and *T.* spec. (probably *T. rizzae*)

Tectonatica cf. *rizzae* vs. *Tectonatica sagraiana*	6.53* ± 0.61	2.79* ± 0.27	36.39* ± 7.55	8.90* ±0.66	36.67* ±5.95	8.64* ±0.93	6.11* ± 0.32	1.66* ± 0.16	85.69* ± 8.49	5.98* ± 0.34

The phylogenetic tree (Figure 1) of the combined data set shows distinct terminal taxa supported by high statistical significances. The two outgroup taxa are separated by 100% from the ingroup. The sequences from *N. dillwynii* were similar to sequences from L71, L73, L75, 80, L85, L86, C128, and C132, showing average pairwise distances of 0.11 ± 0.02% for the H3 gene fragment (0.21 ± 0.41 total substitutions), 1.27 ± 0.99% for the COI gene fragment (4.43 ± 3.65 total substitutions), 0.31 ± 0.27% for the 16S gene fragment (1.29 ± 1.15 total substitutions), and 0.00% for the 18S gene fragment (0.00 total substitutions). The combined data set yielded in a relative distance of 0.53 ± 0.34%. This corresponds to 5.92 ± 4.24 total substitutions among 1542 bp analyzed (Table 3).

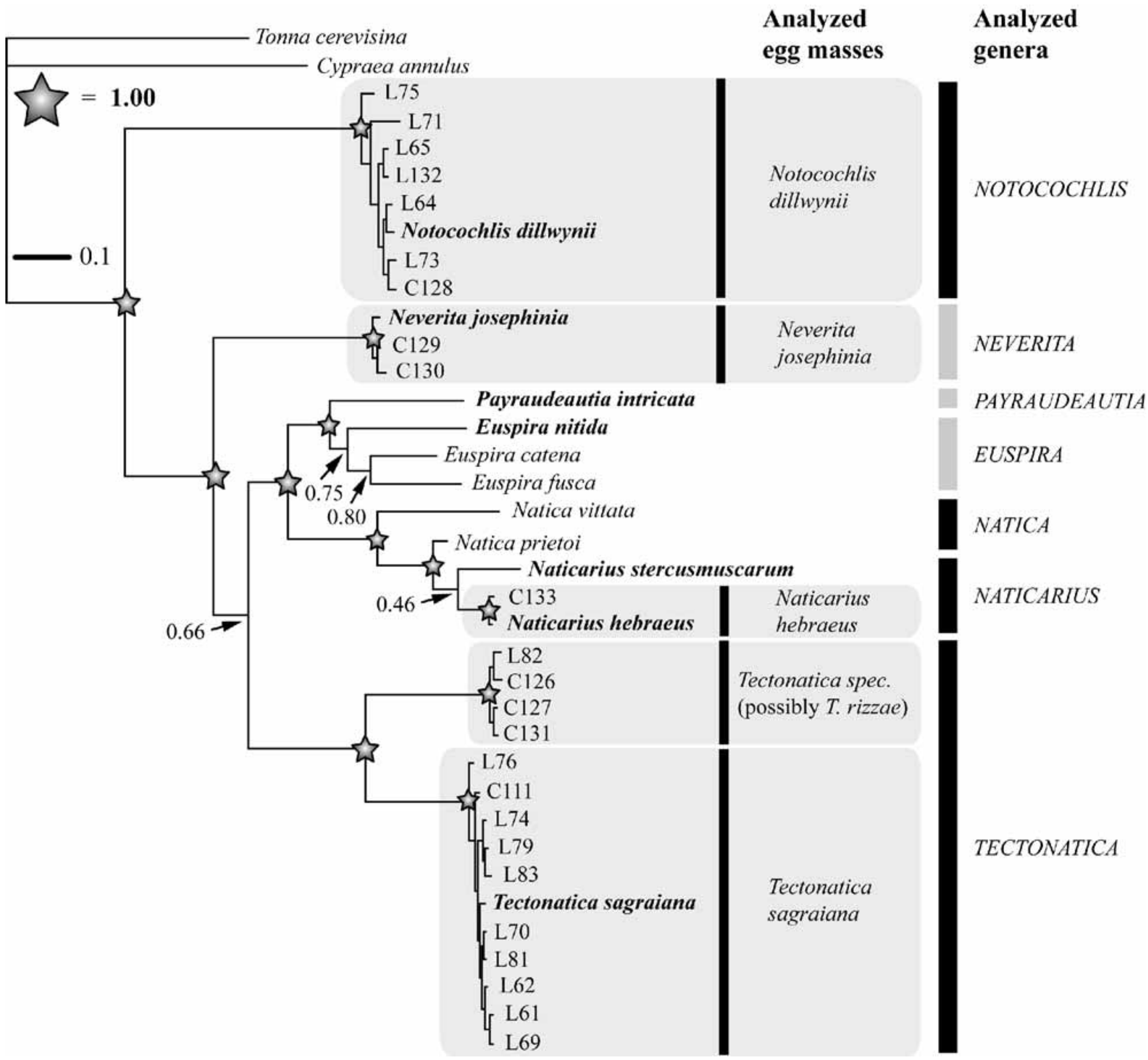

FIGURE 1: Phylogenetic tree based on an analysis of the entire data set (H3, COI, 16S, and 18S sequences) of all specimens listed in Table 2. The phylogenetic model (GTR+I+G) was estimated by MrModeltest (Nylander 2004) performed with Paup*4.0b10 (Swofford 2003). Protein coding data sets were coded as ”CODON”. Based on different base compositions (chi-square test) in each of the single data sets, all parameters were defined as unlinked. Paup*4.0b10 tree characteristics: RI=0.851, CI=0.579. 325 positions were parsimony-informative, 76 were parsimony-uninformative, and 1141 were constant (1542 bp). *Tonna cerevisina* (Hedley, 1919) and *Cypraea annulus* (Linnaeus, 1758) were used as outgroup.

FIGURE 2: Apertual views of all adult naticid specimens used for molecular analysis (see Figure 1) in this study. For more details concerning collection sites and the sequences amplified see Table 2. Scale bars represent 0.5 cm.

FIGURE 3: Photos of all egg masses used for molecular analysis (see Figure 1) in this study. For details concerning collection sites and the sequences amplified see Table 2. The pictures were taken immediately after the collars has been collected. DNA samples from egg masses were marked with C followed by a reference number when DNA was extracted directly from an egg mass; they were marked with L followed by a reference number if DNA extraction was performed from hatched larvae. Scale bars represent 0.5 cm.

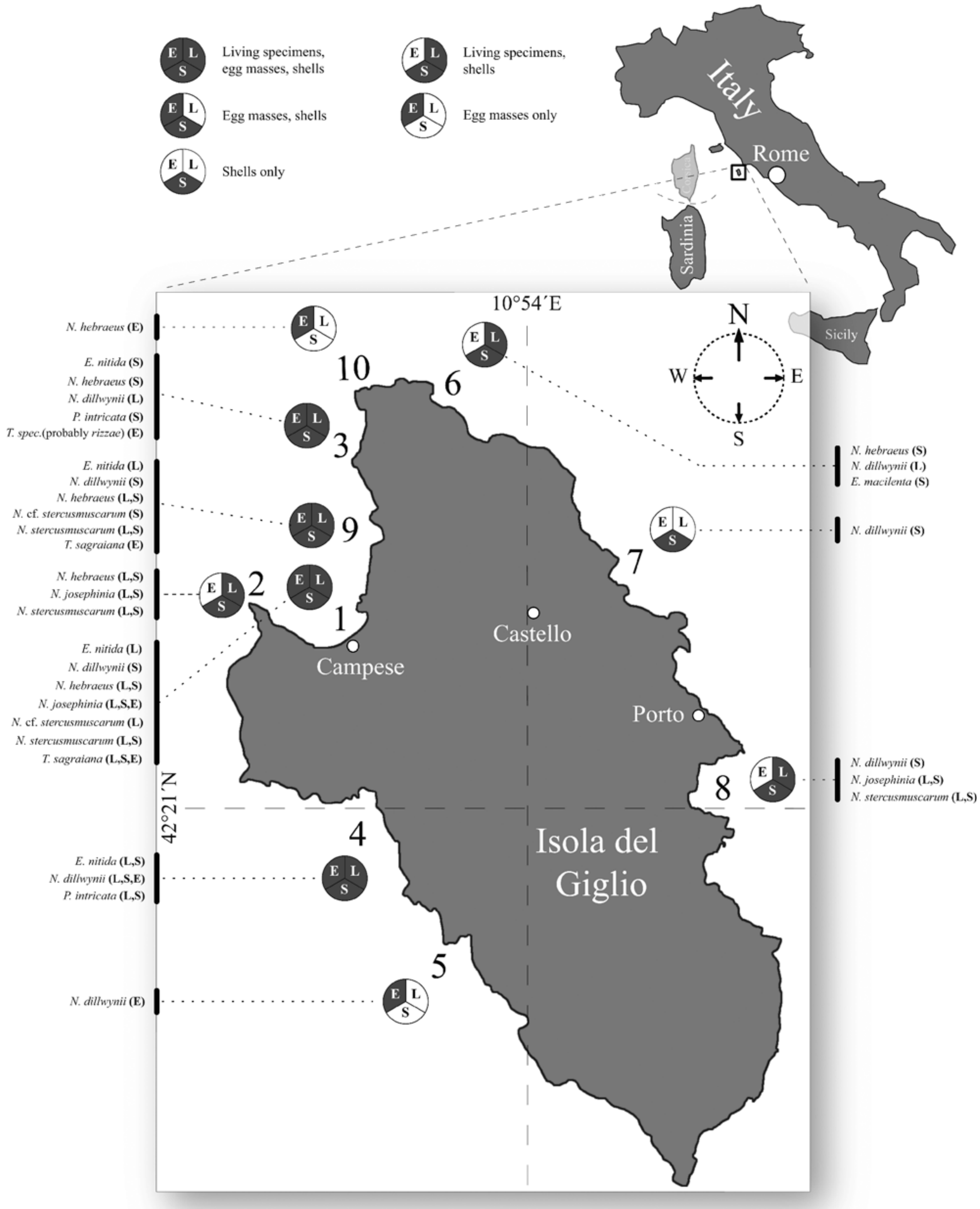

FIGURE 4: Schematic map of Giglo Island, Grosseto County, Tuscany, Italy (42°21.000´´N 10°54.000´´E), including all collecting sites with naticid occurence: 1, Campese Bay; 2, Pt. del Faraglione; 3, Pt. delle Secche; 4, Cala dell´Allume; 5, Pt. del Corvo; 6, Pt. del Morto; 7, Pt. della Campana; 8, Cannelle Bay; 9, *´Swiss House´*; 10, Pt. del Fenaio. Pure shallow sandy sites are Campese Bay, Pt. del Faraglione, and Canelle Bay, while the remaining sites are bluffs with rocks, coarse sand flats, and sea weeds. The circular charts show the material (A, living specimens; E, egg masses; S, empty shells) collected at each site in a qualitative manner. *N. dillwynii* is distributed widest. Collected egg masses listed here were included in the molecular analysis.

The sequences from L61, L62, L69, L70, L74, L76, L79, L81, L83, L87, L88, L89, and C111 are virtually identical to those from *T. sagraiana*, showing average pairwise distances of 0.00 ± 0.00% for the H3 gene fragment (0.00 total substitutions), 0.36 ± 0.23% for the COI gene fragment (1.33 ± 0.86 total substitutions), 0.15 ± 0.15% for the 16S gene fragment (0.53 ± 0.61 total substitutions), and 0.05 ± 0.1% for the 18S gene fragment (0.19 ± 0.40 total substitutions). The combined data set yielded a relative distance of 0.20 ± 0.09%. This corresponds to 2.27 ± 1.26 total substitutions out of 1542 bases. The egg masses C111 and L76 (Figure 3) had been collected from aquaria holding specimens exclusively of *T. sagraiana*. For C111, DNA was extracted directly from the egg mass while the DNA from L76 was extracted from the hatched larvae. In the resulting phylogenetic tree these taxa are arranged together with *T. sagraiana* and a number of additional egg masses in a terminal taxon. This illustrates that the egg mass sequences are virtually identical to those of the animals. This result indicates that sequence comparison can serve to reliably determine the species producing a given egg mass (Table 3).

Two collected egg masses of *N. josephinia*, and one egg mass of *N. hebraeus* could similarly be identified by molecular comparisons (see Figure 3, Table 3). The sequences of C130 and C129 are clearly correlate with to those of *N. josephinia*, having an average pairwise distance of only 0.17 ± 0.03% for the H3 gene fragment (0.33 ± 0.6 total substitutions), 0.16 ± 0.13% for the COI gene fragment (0.67 ± 0.58 total substitutions), 0.00% for the 16S gene fragment (0.00 total substitutions), and 0.63 ± 0.35% for the 18S gene fragment (2.00 ± 1.00 total substitutions). The combined data set yielded a relative distance of 0.23 ± 0.01%, corresponding to 3.00 ± 1.73 total substitutions out of 1542 bases. C130 was retrieved from an aquarium containing exclusively *N. josephinia* and adding further proof that the egg mass sequences are virtually identical to those of the animals (Table 3).

The sequences obtained from C133 (Figure 3, Table 3) are almost identical to those from *N. hebraeus* showing distances of only 0.4% for the H3 gene fragment (1.00 total substitution), 0.23% for the COI gene fragment (1.00 total substitution), 0.00% for the 16S gene fragment (0.00 total substitution), and 0.00% for the 18S gene fragment (0.00 total substitution). The combined data set yielded a relative distance of merely 0.10%, corresponding to 2.00 total substitutions within 1542 bp.

Four of the egg masses (see Figure 3) investigated here were arranged in a separate terminal taxon (L82, C126, C127, C131). The sequences show average distances of 0.00% for the H3 gene fragment (0.00 total substitution), 0.39 ± 0.02% for the COI gene fragment (1.67 ± 0.82 total substitutions), 0.22 ± 0.13% for the 16S gene fragment (1.00 ± 0.63 total substitutions), and 0.00% for the 18S gene fragment (0.00 total substitution). The combined data set yielded a relative distance of only 0.18 ± 0.09% corresponding to 2.67 ± 1.37 total substitutions out of 1542 bases among those four egg masses. The sequences from those four egg masses were closest to the sequences of *T. sagraiana* and its confirmed sand collars. They show average pairwise distances of 2.79 ± 0.27% for the H3 gene fragment (6.53 ± 0.61 total substitutions), 8.90 ± 0.66% for the COI gene fragment (36.39 ± 7.55 total substitutions), 8.64 ± 0.93% for the 16S gene fragment (36.67 ± 5.95 total substitutions), and 1.66 ± 0.16% for the 18S gene fragment (6.11 ± 0.32 total substitutions). The combined data set thus yielded a relative distance of 5.98 ± 0.34% which corresponds to 85.69 ± 8.49 total substitutions (Table 3).

Accordingly, the terminal taxon defined by these four egg masses is supported by 100% of the trees as a sister taxon of *T. sagraiana*. None of the remaining species is more closely related with these egg masses. Due to the close relationship to *T. sagraiana*, we assume that these egg masses belong to another Mediterranean *Tectonatica* species, *T. rizzae* (Philippi, 1844). *T. rizzae* is a rarely found deeper water species living below 30 m depths.

b) Phylogenetic tree

A phylogenetic tree was constructed from all data available (Figure 1). Species which are marked in bold in the tree were collected alive in waters around Giglio Island. The outgroup taxa (*Tonna cerevisina*, *Cypraea annulus*) are separated from the ingroup in 100% of all calculated trees (BayInf: 1.00). The phylogenetic relationships among the ingroup taxa show that the data from egg masses were distinctly grouped, and each group was unequivocally arranged with a certain species in a well-separated terminal taxon; each of these taxa was supported by 100% of all calculated trees (BayInf: 1.00).

These arrangements show that the egg masses can be assigned to distinct species. Notably, egg masses for which the depositing species was positively known due to aquarium-observed sand collar deposition (L76, C111: *T. sagraiana*, C130: *N. josephinia*) were arranged unambiguously with their expected species (Figure 1). *N. hebraeus* is arranged together with *N. stercusmuscarum* (BayInf: 0.46), *N. prietoi* (BayInf: 1.00), and *N. vittata* in a terminal taxon (BayInf: 1.00). *T. sagraiana* is placed as a sister group to a 100%-supported (BayInf: 1.00) terminal taxon comprising the egg masses L82, C126, C127, C131. These egg masses cannot be arranged together with any of the identified naticid species. The bifurcating monophyletic branch that includes these two sister taxa is supported by 100% of all calculated trees (BayInf: 1.00), indicating a very close relationship (Figure 1), and prompting us to predict that those egg masses may represent a second *Tectonatica* known to occur in the Mediterranean, *T. rizzae* (see above).

The phylogenetic tree shows solid sister taxa arranged together in terminal taxa (Figure 1). The first taxon diverting from the outgroup taxa includes *N. dillwynii* in 100% of all calculated trees. The next taxon includes *N. josephinia* [BayInf: 1.00]. The *Natica*/*Naticarius* taxon is supported in 100% of all trees as a sister taxon of a monophyletic taxon including the species *E. fusca*, *E. catena*, *E. nitida*, and *P. intricata*. This *Euspira* taxon is also supported by 100% in the overall tree [BayInf: 1.00]. All species of the genus *Euspira* analyzed here are arranged together with the species hitherto determined as belonging to a distinct genus *Payraudeautia*, in the same terminal taxon. This shows that *Payraudeautia intricata* is closely related to the genus *Euspira* and diverts early in this terminal taxon. It appears questionable if *Payraudeautia* species are distinct enough to merit separation at the generic level.

The bifurcating branch including the terminal taxa *Euspira*/*Payraudeautia*-*Natica*/*Naticarius* and the *Tectonatica* taxon is supported by 66% of all calculated trees [BayInf: 0.66].

Conclusions from molecular studies

In this study we were able to show that naticid biodiversity can be reliably determined by molecular analysis of egg masses in addition to live-collected specimens. Egg masses for 4 distinct species could be identified (Figs. 1, 3, Table 3): *N. hebraeus*, *N. josephinia*, *N. dillwynii*, and *T. sagraiana*. Additionally, we were able to identify egg masses of another species which has not been found alive, yet, on Giglio Island. Due to their low genetic distance and their close phylogenetic relationship to *T. sagraiana*, we assume, that the egg masses L82, C126, C127, and C131 belong to a very closely related species. Only one additional Mediterranean *Tectonatica* species is known, *Tectonatica rizzae*, (Kobelt 1901, Settepassi 1972, Schiró 1977, Fernandes & Rolán 1993). Morphologically, it is quite similar to the same-sized *T. sagraiana* (Figs. 8A/B). *T. rizzae* is a rarely collected species which is known predominantly from deeper water. The egg masses believed to belong to *T. rizzae* (Figure 3; L82, C126, C127, and C131) were found in 16 – 28 m depth on coarse sand flats between rocks and sea weeds. Thus, the collecting site fits the general description of the habitat of this species as reported in the literature (Kobelt 1901, Settepassi 1972, Villa 1977). Furthermore, the sequence data from the four egg masses did not cluster with any of the remaining species analyzed here. Altogether, 21 naticid species have been reported for the Mediterranean Sea (Schiró 1977, Sabelli *et al.* 1990). The analysis presented here lack the following positively confirmed species: *Euspira guillemini* (Payraudeau, 1826), *Euspira grossularia* (Marche-Marchad, 1957), *Cryptonatica affinis* (Gmelin, 1791), *Notocochlis gualtieriana* (Recluz, 1844), and *Sinum bifasciatum* (Recluz, 1851). None of these taxa show morphological similarities to *T. sagraiana*. *C. affinis* has recently been listed as a *Tectonatica* (Settepassi 1972, Schiró 1978, Terreni 1980, Sabelli & Spada 1980, Cecalupo & Giusti 1988), but generally is regarded as a *Cryptonatica* (Bouchet & Warén 1993). More importantly, this species has always been collected at more than 100 m depth. We therefore conclude that the unknown egg masses likely belong to *T. rizzae*. Genetic distances of two closely related Northern Atlantic *Neverita* species, *N. delessertiana* and *N. duplicata* yielded 9.9% in the COI gene fragment (Huelsken *et al.* 2006). Those sequence distances for COI lie in the range of the average COI sequence divergence reported for congeneric species of Mollusca (Hebert *et al.* 2003). A similar number of substitutions (8.9%) in the sequences between *T. sagraiana* and the unknown egg masses L82, C126, C127 and C131 suggest a similarly close relationship (Figure 1, Table 3). Furthermore, *T. rizzae* shows many morphological homologies to *T. sagraiana,* such as the umbilicus, the operculum, the size of the shell, and a very similar color pattern (see descriptions of *T. rizzae* and *T. sagraiana,* and Figs. 8A,B). Thus, we identified a total of 9 naticid species (see Figs. 1, 2, 3, 5B, 6, 8, 9A, 10) in 6 traditional genera for Giglio Island based on live-collected specimens, collected shells, and molecular analysis of egg masses (Table 4). A detailed description of these taxa is presented below.

TABLE 4: Naticid species collected on Giglio Island. Collection sites with global positioning data: 1, Campese Bay (42°21'59.43"N 10°52'35.92"E); 2, Pt. del Faraglione (42°22'10.37"N 10°52'1.41"E); 3, Pt. delle Secche (42°23'1.15"N 10°52'42.64"E); 4, Cala dell´Allume (42°21'0.88"N 10°52'56.14"E); 5, Pt. del Corvo (42°20'18.04"N 10°53'25.45"E); 6, Pt. del Morto (42°23'16.60"N 10°53'22.98"E); 7, Pt. della Campana (42°22'11.98"N 10°54'51.17"E); 8, Cannelle Bay (42°21'4.99"N 10°55'15.40"E); 9, *´Swiss House´* (42°22'39.64"N 10°52'54.80"E); 10, Pt. del Fenaio (42°23'19.29"N 10°52'49.33"E).

Genus	Species	Author, Year	Collection site	Figures
Euspira	*macilenta*	(Philippi, 1844)	6	9B
Euspira	*nitida*	(Donovan, 1804)	1, 3, 4, 9	9A
Naticarius	*hebraeus*	(Martyn, 1786)	1, 2, 3, 6, 9, 10	6A, 11G/g
Naticarius	*stercusmuscarum*	(Gmelin, 1791)	1, 2, 4, 8, 9	5A,B
Neverita	*josephinia*	(Risso, 1826)	1, 2, 8	10B, 11A/a, J/K
Tectonatica	*sagraiana*	(Orbigny, 1842)	1, 9	8A, 11F/f, H/I
Tectonatica	spec. (probably *rizzae)*	(Philippi, 1844)	3 (egg mass)	8B, 11B/b
Notocochlis	*dillwynii*	(Payraudeau, 1826)	1, 3, 4, 5, 6, 7, 8, 9	6B, 11C/c, D/d, E/e
Payraudeautia	*intricata*	(Donovan, 1804)	3, 4	10A

Description of species

Family Naticidae Guilding, 1834
Subfamily Naticinae Guilding, 1834

The species of the Naticinae are characterized by a calcareous operculum.

The genus *Naticarius* Duméril, 1806 on Giglio Island

Two distinct species encountered in the waters of Giglio Island are usually described to the genus *Naticarius*: *N. hebraeus* and *N. stercusmuscarum* (Figs. 5A,B; 6A). Additionally, one living specimen and three empty shells of an interesting color form of *N. stercusmuscarum* (Fig. 5B) were collected at Campese Bay. To our knowledge this color form has not been described in the literature, yet. However, photos of this form have appeared on a website (Gelatolo 2007; www.naturamediterraneo.com). The specimen figured there was also collected on Giglio Island. This color form is so distinct in appearance that the possibility of it being a separate species could not be ruled out entirely. However, initial sequence analysis uncovered no significant differences to typical *N. stercusmuscarum*.

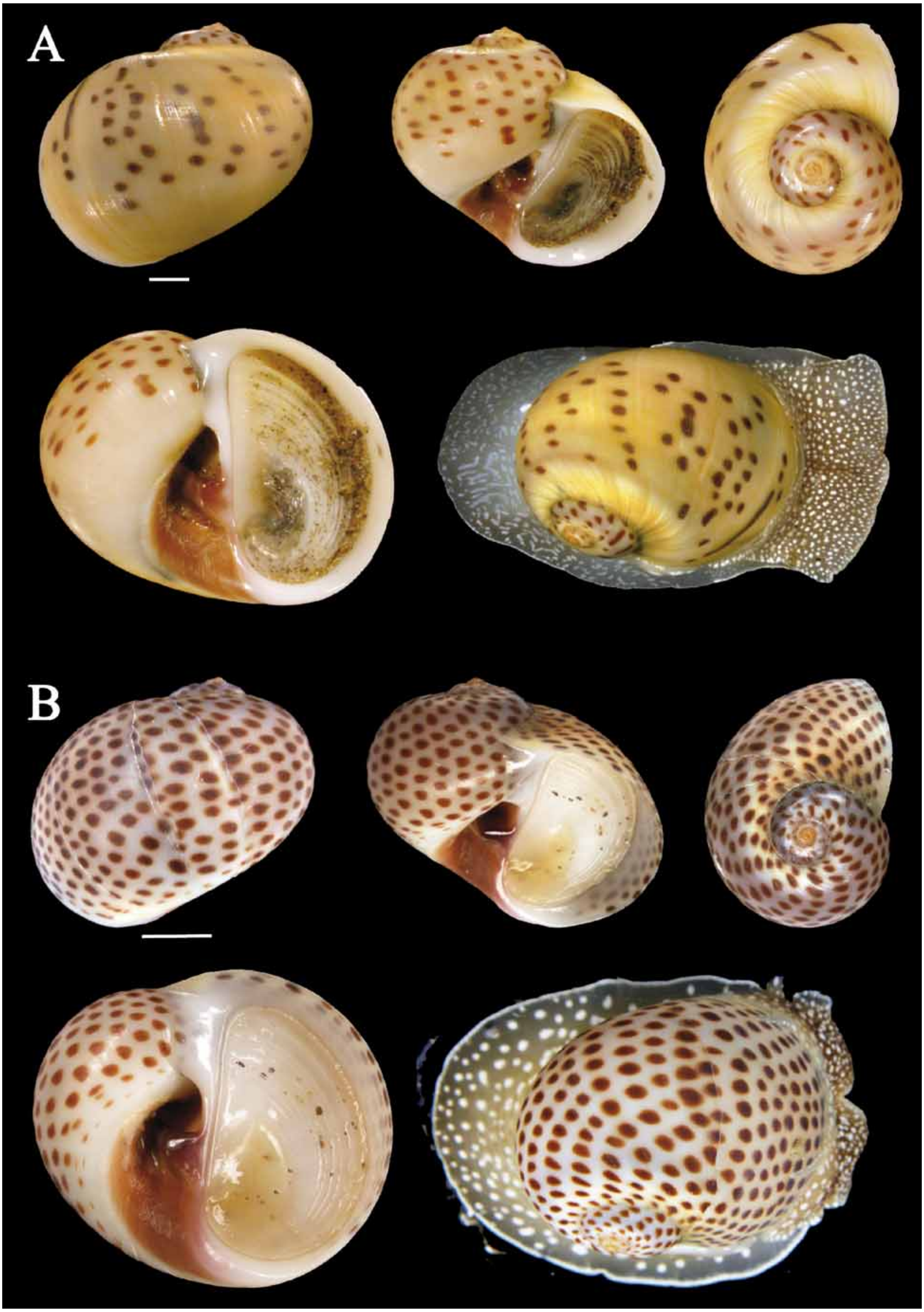

FIGURE 5: A, Sparsely dotted form of *Naticarius stercusmuscarum* (Gmelin, 1791); B, *Naticarius stercusmuscarum* (Gmelin, 1791). All specimens are shown in four standardized views (dorsal, apertural, apical, umbilical) as well as alive. The pictures of living specimens were taken in an aquarium with a black bottom. Scale bars represent 0.5 cm.

The two species of *Naticarius* were found living sympatrically on sand bottom in the Bay of Campese and were encountered during day and night dives. Some living *N. stercusmuscarum* were also collected off the Pt. del Faraglione, and some empty shells were found at Cala dell´Allume. Almost all of the living *Naticarius* specimens collected were found crawling on the sand surface. The remaining specimens were found burrowed in the sand at depths of 3–5 cm. Both species are very common in the Mediterranean Sea. Unfortunately, the two *Naticarius* species are often confused with each other, in museum collections as well as in field guides (e.g. Melone & Picchetti 1980). While both species are very similar in shell shape and color pattern, opercular surface and umbilical area (Figs. 5A–B, 6A), living specimens can easily be differentiated by their foot color pattern as well as the coloration of their tentacles (see below). *N. stercusmuscarum* and *N. hebraeus* are most frequent on circumlittoral and infralittoral sea beds from 10 to 100 meters in the Mediterranean Sea. Reported occurances in Eastern Atlantic regions (Spain and North Africa: Kobelt 1901, Settepassi 1972, Sabelli 1977, Schiró 1977) remain to be confirmed. Both *N. hebraeus* and *N. stercusmuscarum* show many homologies to certain forms of the Eastern Atlantic species *Natica multipunctata* Blainville, 1825 (syn.: *Natica variolaria* Recluz, 1844). Thus, published distribution patterns may have included the latter species. On Giglio Island, *N. hebraeus* and *N. stercusmuscarum* were found from 2 to 18 meters depths with an average depth of 6 m for *N. hebraeus* and 8 m of *N. stercusmuscarum*. One report records *N. stercusmuscarum* also from the Black Sea (Demir 2002).

***Naticarius hebraeus* (Martyn, 1786)—Fig. 6A [egg mass: Figs. 3, 11G, g]**

Nerita hebraea Martyn, 1786. The universal conchologist, exhibiting the figure of every known shell, accurately drawn, and painted afternature with a new systematic arrangement by the author Thomas Martyn. London, T. Martyn. 4, Vol. 3, pl. 109
+*Nerita stercusmuscarum sensu* auct. [non Gmelin 1791]. Sabelli *et al.* (1990), p. 170
+*Natica maculatus* von Salis, 1793, p. 379. Sabelli *et al.* (1990), p. 170
+*Natica millepunctata sensu* auct. [non Lamarck, 1822]. Sabelli *et al.* (1990), p. 170
+*Nacca maxima* Risso, 1826. Sabelli *et al.* (1990), p. 170
+*Natica adspersa* Menke, 1830. Sabelli *et al.* (1990), p. 170
Naticarius hebraeus (Martyn, 1786). Kobelt (1901), pp. 76–78, pl. 52, figs. 1–8; Sabelli & Spada (1977), p. 9, fig. 2; Schiró (1978b), p. 5, fig. 2 (second row); Nordsieck (1982), p. 186, pl. 57, fig. 63.11; Riedel (1983), pp. 287–288, pl. 98 figured; Barash & Danin (1992), pp. 107–108, fig. 115, Terreni (1981), p. 31
Natica hebraea (Martyn, 1786). Hidalgo (1917), p. 486; Villa (1985), pp. 106–107
Natica (*Naticarius*) *hebraea* (Martyn, 1786). Settepassi (1972), vol. III, p. 26, pl. 3; Demir, (2002), p. 110
+*Naticarius cruentatus* (Gmelin, 1791). Poppe & Goto (1991), p. 119, pl. 16, figs. 18–20
+*Natica cruentata* (Gmelin, 1791). Alf *et al.* (1993), p. 190, fig. 4

Description

Size: Up to 59.2 mm maximal obtainable diameter (m.o.d.) (Italy; Hutsell *et al.* 2001). Specimens (n = 58) from Giglio Island: 8.1–47.5 mm (mean: 29.6 ± 0.8 mm) height; 7.8–47.1 mm (mean: 29.4 ± 0.7 mm) width. Ratio [h/w] = 1.01 ± 0.005. Aperture approximately 79% of shell height.

General shape: Globose, large bodywhorl, relatively thin-shelled for its size, with 4.5 convex, slightly tabulated whorls and adpressed sutures [teleoconch: 3.5].

Sculpture: Minute barely visible axial growth striae, stronger and easy visible below suture.

Shell color: White, cream or yellowish background with dense irregularly arrranged, blurred, brownish dots that have a tendency to coalesce into larger blotches and bands.

Protoconch: Uncolored, one embryonal whorl.

Aperture & outer lip: Aperture half moon-shaped, oblique, angled anteriorly, rounded at the bottom, fairly thick basal callus; external lip simple, sharp. Fairly thick parietal callus, covering 1/4 to 1/3 of inner lip.

Umbilical area: Wide open, brownish, with well-defined strong, U-shaped, funicle with growth marks, positioned slightly below middle of the umbilical area; funicular callus not enlarged, inner lip without callus.
Operculum: Calcareous with numerous narrow, sharply-raised ribs; ribs sometimes mushroom-shaped, with base more narrow than top. Number of ribs varies considerably, independently of body size, from below 10 to > 25, innermost ribs sometimes fused to form flat areas.
Animal: Mesopodium and propodium surround shell to same width during crawling; irregularly arrranged, blurred, brownish dots and bands distributed over the entire foot; tentacles colored dark; animal unable to cover entire shell with mesopodium; only lower fringe of shell covered.
Egg mass: Flexible, inwardly cambered, paucispiral coiled band of mucus-cemented sand grains (Figs. 3, 11G/g), 1.25 circles; outer diameter 7.5 mm (n = 1).
Differential diagnosis: Irregular brownish dots, strong funicle, operculum with numerous sharp ribs, and large size easily distinguish the species from all other Mediterranean naticids except for *N. stercusmuscarum*. For differences to *N. stercusmuscarum*, see under that species.

Geographical distribution
Giglio Island: Campese Bay (1), ´*Swiss House*´ (9), Pt. del Faraglione (2), Pt. delle Secche (3)
Alf *et al.* (1993): ´Found singularly at the beach after storm at Bay of Campese, Giglio Island.´
General distribution: Contrary to several publications (Hidalgo 1917, Schirò 1977), this species is probably strictly Mediterranean, ubiquitous and common from the Strait of Gibraltar to the Aegean Sea (Demir 2002).

Naticarius stercusmuscarum (Gmelin, 1791)—Figs. 5A, B

+*Natica canrena sensu* auct. non Linnaeus, 1767. Sabelli *et al.* (1990), p. 170
+*Nerita punctata* Karsten, 1789 [non *punctata* Müller, O.F., 1776]. Sabelli *et al.* (1990), p. 170
Nerita stercusmuscarum Gmelin, J.F. 1791. Tomus 1, pars VI, Vermes testacea. Lipsiae. *in:* Editio decima tertia, reformata, cura J.F. Gmelin (Ed.), *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonomis, locis.* G. E. Beer, p. 3673.
+*Natica punctata* (Karsten, 1789). Hidalgo (1917), p. 490
+*Natica millepunctata* Lamarck, 1822. Weinkauff (1867), pp. 242–245; Kobelt (1901), Vol II, p. 74, pl. 51, figs. 1–9
+*Nacca punctata* Risso, 1826. Sabelli *et al.* (1990), p. 170
+*Natica sanguinolenta* Brusina, 1865 [non *sanguinolenta* Deshayes, 1839]. Sabelli *et al.* (1990), p. 170
+*Natica* (*Naticarius*) *punctata* (Chemnitz in Karsten, 1789). Settepassi (1972), p. 24, pls. 3, 4; Villa (1986b), pp. 134–136
+*Naticarius punctatus* (Chemnitz in Karsten, 1789). Sabelli & Spada (1980), pp. 1, 3, pl. 3, fig. 1; Schirò (1978b), p. 4, fig. 2 (first row); p. 5 fig. 1; Poppe & Goto (1991), pp. 119–120, pl. 16, figs. 21–23, Terreni (1981), p. 31
Naticarius stercusmuscarum (Gmelin, 1791). Nordsieck (1982), p. 186, pl. 57, fig. 63.10; Riedel (1983), p. 288, pl. 98
+*Naticarius millepunctatus* (Lamarck, 1822). Barash & Danin (1992), p. 107, fig. 114
Natica (*Naticarius*) *stercusmuscarum* (Gmelin, 1791). Demir (2002), p. 110, Gelatolo (2004), http://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=4259, [shell was found at the beach of Isola del Giglio: listed as form „*pocopunctatus*“]

Description
Shell, apex, operculum and umbilical area similar to *N. hebraeus*; generally smaller in size, with a slightly more elevated spire.
Size: Up to 54.1 mm m.o.d. (off Isole Egadi, Sicily, Italy; MHC#030511.23). Specimens (n = 23) from Giglio Island: 12.2–29.8 mm (mean: 18.6 ± 1.0 mm) height; 13.0–30.5 mm (mean: 19.6 ± 1.0 mm) width. Ratio [h/w] = 0.95 ± 0.006. Aperture approximately 82% of shell height.
General shape: Globose with slightly elevated spire, large body whorl, relatively thin-shelled for its size, 4.25 convex, slightly tabulated whorls, adpressed at sutures [teleoconch: 3.5].
Sculpture: Same as *N. hebraeus*.

FIGURE 6: A, *Naticarius hebraeus* (Martyn, 1786); B, *Notocochlis dillwynii* (Payraudeau, 1826). Further details as in Figure 3. Scale bars represent 0.5 cm.

Shell color: Distinct perfectly rounded dots distributed regularly on whitish, cream or yellowish shell; a distinctive sparsely dotted color form known only from Giglio Island has less dots distributed irregulary over yellowish shell, sparing an uncolored band underneath the suture.

Protoconch: Uncolored, one embryonal whorls.

Aperture & outer lip: Aperture half moon-shaped, oblique, angled anteriorly, base rounded, thickened; external lip simple, sharp. Fairly thick parietal callus, covering 1/4 to 1/3 of inner lip.

Umbilical area: Same as *N. hebraeus*.

Operculum: Same as *N. hebraeus*.

Animal: Mesopodium and propodium surround shell to same width during crawling; only lower fringe of shell covered by mesopodium, tentacles whitish grey. Mesopodium of sparsely dotted color form has thin whitish lines while typical form shows distinct white dots (Figs. 5A/B); fringe of mesopodium of typical form shows distinct white line which is absent in sparsely dotted form; color patterns of propodium and tentacles identical in both forms (whitish-grey).

Differential diagnosis: *N. stercusmusarum* is most easily distinguished from *N. hebraeus* by the distinct color pattern of the animal, but also of the shell. *N. hebraeus* has dark tentacles, and a dark foot with radial, irregularly, whitish to cream-colored, distributed lines, while *N. stercusmuscarum* has lightly colored tentacles and a dark foot showing distinct whitish to cream-colored dots of different size distributed irregularly across its surface. On the shell, *N. hebraeus* has irregularly arranged, blurred, brownish dots that have a tendency to coalesce into bands and/or larger blotches, while *N. stercusmuscarum* has distinct, brownish, well-separated dots distributed regularly over its shell. Average ratios of height to width of 58 shells of *N. hebraeus* is 1.01 ± 0.005 (SEM), and 0.95 ± 0.006 (SEM) for 23 shells of *N. stercusmuscarum*. The difference is significant in a non-parametric two-tailed t-test including 23 pairs (p-value < 0.0001). However, despite the statistical significance of the differences in ratio of width to height, individual specimens from Giglio Island cannot be identified unambiguously based on size alone, due to the overlapping size distribution.

The sparsely dotted form of *N. stercusmuscarum* has significantly fewer dots than the typical form. The dots are not as regularly spaced as in the typical form, and may touch each other to form short streaks. The mesopodium of the sparsely dotted form has thin, short, whitish lines while that of the typical *N. stercusmuscarum* has distinct dots; color patterns of the propodium and tentacles are indistinguishable. A distinct white fringe which surrounds the mesopodium of the typical *N. stercusmuscarum* is absent in the sparsely dotted form. Molecular comparison between these two color forms of *N. stercusmuscarum* was complicated by the fact that only tissue of one partly decomposed specimen of the sparsly dotted form of *N. stercusmuscarum* was available. The data obtained from that tissue did not show differences in a partial 18S rRNA gene (ca. 350 bp). However, the analyzed fragment lies in a well conserved region of the 18S gene and may not be sufficiently informative to differentiate closely related species. A specimen of the sparsely dotted form of *N. stercusmuscarum* observed in an aquarium fed exclusively on other naticids and ignored Venus mussels, even when starved for several days. None of several specimens of the typical form of *N. stercusmuscarum* showed such a behavior. Additional molecular analysis will have to be performed to verify that the sparsely dotted form is indeed a distinct species.

Geographical distribution

Giglio Island: Campese Bay (1), P. del Faraglione (2), Cala dell´Allume (4), Pt. del Morto, (6).

The sparsely dotted form of *N. stercusmuscarum* was found exclusively in the Bay of Campese (Figure 4).

General distribution: Contrary to many publications (Hidalgo 1917, Settepassi 1972, Schirò 1977), this species is probably strictly Mediterranean, widespread from the Strait of Gibraltar to the Aegean Sea with reports from the Sea of Marmara and from the Black Sea (Demir 2002).

The genus *Notocochlis* Powell, 1933 on Giglio Island

One species of the genus *Notocochlis* is found in upper infralittoral sand bottom around Giglio Island. This species was originally described as *Natica dillwynii* Payraudeau, 1826, but due to its striking morphological similarity to the Pacific species *Notocochlis cernica* (Jousseaume, 1874), which Kabat (1996) placed in Notocochlis, we transferred the Mediterranean species to that genus as well. *N. dillwynii* is a typical Mediterranean species and has been found in Spain, Northern Africa, Sicily, and the Thyrrenian Sea (Kobelt 1901, Hidalgo 1917, Schiró 1977), as well as in Turkish and Greek waters (Villa 1986, Demir 2002). It prefers coarse granular sand bottoms and debris (Villa, 1986). These bottom characteristics are often present at bluffs on Giglio Island. Bluffs with coarse-grained sand bottoms were predominantly found in the northern (Pt. del Faraglione, Pt. del Morto, Pt. delle Secche, Fenaio) and western areas (Cala dell´Allume, Scoglio del Corvo) of Giglio Islands where two specimens, several egg masses and many empty shells of this species were found. The living specimens were crawling directly under the sand surface in the sand between weeds of *Posidonia oceanica*, leaving a distinct trail behind. While many empty shells were found in Cala dell´Allume and Pt. del Morto, only one living specimen was found at Pt. del Morto and one was found on a bluff on the shoal Pt. delle Secche. Probably, *N. dillwynii* burrows deep in the sand. According to the number of empty shells found, *N. dillwynii* is one of the most common naticid species on Giglio Island.

***Notocochlis dillwynii* (Payraudeau, 1826) [new comb.]—Fig. 6B [egg mass: Figs. 3, 11C, D, E, c, d, e]**

Natica dillwynii Payraudeau, B.-C. 1826. *Catalogue descriptif et méthodique des annelides et des mollus*- ques de l'ile de Corse; avec huit planches representant quatre-vingt-huit especes, dont soixante-huit *nouvelles*. Béchet, Paris, 218 pp., p. 120, pl. 5, figs. 27-28.

Natica dillwynii Payraudeau, 1826. Weinkauff (1867), p. 248; Kobelt (1901), p. 81, pl. 49, Figs. 11-10; Hidalgo (1917), p. 483; Villa (1986b), pp. 179-180, p. 180; Alf *et al.* (1993), p. 190; Demir (2002), p. 110; Terreni (1981), p. 31

+*Nacca fasciata* Risso 1826. Sabelli et al. (1990), p. 169

Natica (*Naticarius*) *dillwynii* (Payraudeau, 1826). Settepassi (1972), p. 23, pls. 4, 8

+*Tectonatica operculata farolita* Nordsieck, 1973. Bouchet & Warén (1993), p. 759

Naticarius (*Naticarius*) *dillwini* (Payraudeau, 1826). Schiró (1978c), p. 3, fig. 1 (first row)

Naticarius dillwynii (Payraudeau, 1826). Nordsieck (1982), p. 186, pl. 57, fig. 63.13 (Ibiza, Mediterraneo); Sabelli & Spada (1980), pp. 2-3, figs. 4a-b; Poppe & Goto (1991), p. 119, pl. 17, fig. 23, Barash & Danin (1992), p. 108

Description

Size: Up to 19.7 mm height (Djidjelli, France; MNHN, Paris). Specimens (n = 30) from Giglio Island: 5.5 - 17.0 mm (mean: 11.0 mm ± 0.6 mm) height; 5.2 – 16.8 mm (mean: 10.8 ± 0.6 mm) width. Ratio [h/w] = 1.03 ± 0.006. Aperture approximately 80% of shell height. The larval shell has a diameter of approx. 135 µm.

General shape: Globose, slightly elevated spiral whorls, rather thin, 5.5 [teleoconch: 4] whorls.

Sculpture: Axial wrinkles on shoulder; fine growth striae.

Shell color: Whitish-cream, yellowish-grey or light-brown with three distinct horizontal white bands with chevron-shaped brown marks; one band on shoulder, one around periphery, broadest band with somewhat fuzzy marks encircles umbilicus; in addition, unmarked yellowish band below suture.

Protoconch: Dark protoconch, 1.5 embryonal whorls.

Aperture & outer lip: Aperture half-moon shaped, oblique, angled at the top; outer lip simple, sharp; basal lip slightly drawn out.

Umbilical area: Open umbilicus; relatively large, centrally located whitish funicle; funicular callus slightly enlarged, merging anteriorly with columellar callus which is tapering towards basel lip; distinctive sulcus; distinct, whitish parietal callus.

Operculum: Calcareous, glossy, with a deep, broad (as broad as ribs) furrow in front of two distinctive, massive ribs separated by a narrow ridge next to outer lip.

Animal: Propodium and mesopodium translucent whitish-grey, speckeled with red, irregularly shaped dots; dots more dense on propodium, which appears darker colored than mesopodium; when extended, mesopodium twice the length of the shell; propodium about as long as the shell; tentacles nearly as long or longer than propodium, colored with red dots on a whitish grey background, similar to coloration of foot.
Egg mass: Rigid, multispiral, even-walled coil of mucus-cemented sand grains (Figs. 3, 11C,D,E/c,d,e), 3–5 circles; outer diameter 20–40 mm (N = 6; mean: 30.25 mm ± 7.961 [SD]).
Differential diagnosis: This species cannot be confused with any other Mediterranean species; distinct morphological features include the operculum with the furrow in front of two distinctive, thick ribs next to the outer lip; the reddish color pattern of the shell and foot, the long tentacles, and the elongated mesopodium.

Geographical distribution
Giglio Island: Cala dell´Allume (4), Pt. del Corvo (5), Pt. del Morto (6), Cannelle Bay, Pt. della Campana (7), (8), Pt. delle Secche (3)
General distribution: Common in the entire Mediterranean Sea and the Eastern Atlantic, South to Ghana (Villa 1986), and offshore to the Cape Verde Islands (Rolán 2005).

The genus *Tectonatica* Sacco, 1890 on Giglio Island

So far, one species of the genus *Tectonatica* has been found alive on Giglio Island. *T. sagraiana* is common in sublittoral areas at depths of 10 m and below. It is well known for the Mediterranean Sea and lives infaunal directly under the sand surface. Specimens of *T. sagraiana* were found crawling in the sand in the Bay of Campese between 7 and 15 meters depths in large groups of 20 to 30 individuals. The specimens were collected both at day and night. The animals leave an easily observable trail on the sand surface. In contrast to other naticid species, *T. sagraiana* does not bury very deep in the sand.

Molecular analysis of larval stages of Naticidae indicated a related additional species to be present in the waters of Giglio Island. Four independent marker gene sequences obtained from randomly collected egg collars suggest a close relationship to *T. sagraiana* (Figs. 1, 11B/b). One further species of the genus *Tectonatica* closely related to *T. sagraiana* is known from the Meditteranean: *T. rizzae* (Philippi, 1844) which has been described as a rare species in the Mediterranean Sea living at greater depth (> 10–100 m). Unfortunately, no shells were found on Giglio Island, so far, and no live-collected adult *T. rizzae* was available for sequencing. The sequences were obtained from larvae hatched from collected sand collars.

Tectonatica sagraiana **(Orbigny, 1842)—Figs. 7, 8A [egg mass: Figs. 3, 11F, H, I, f]**

Natica sagraiana Orbigny, 1842, *in* Sagra, M. R. de la (1841–1853) *Histoire physique, politique et naturelle de l´Ile de Cuba*, vol. 2 p. 34, pl. 17, figs. 20–22
Natica sagraiana Orbigny, 1842. Weinkauff (1867), pp. 246–247; Tryon (1886), p. 19, pl 3, fig. 45; Hidalgo (1917), p. 491
+*Natica lineolata* Philippi, 1844 [non Deshayes, 1832]. Sabelli *et al.* (1990), p. 170, as *T. filosa* Philippi, 1845
+*Natica filosa* Philippi, 1845. Vol. 2(2):42, pl. 2, fig. 4
+*Natica flammulata* Requien, 1848. Kobelt (1901), p. 78, pl. 52, Figs. 11–12; Jeffreys 1885, p. 36; Dautzenberg 1883, p. 316, *fide* Kobelt 1901
+*Tectonatica abbreviata* (Sowerby, 1883). Nordsieck (1982), p. 106, pl. 17, fig. 63.25
+*Natica* (*Tectonatica*) *filosa* (Philippi, 1845). Settepassi (1972), p. 24, pl. 4, 9–10, Demir (2002), p. 110
+*Natica filosa* Philippi, 1845. Schiró (1978c), p. 4, fig. 1 (third row); Poppe & Goto (1991), pl. 18, fig. 3
+*Tectonatica filosa* (Philippi, 1845). Sabelli & Spada (1977), p. 10, pl. 3, fig. 7; Bouchet & Warén (1993), p. 765, fig. 1824
+*Tectonatica flammulata* (Requien, 1848). Barash & Danin (1992), p. 108, fig. 116

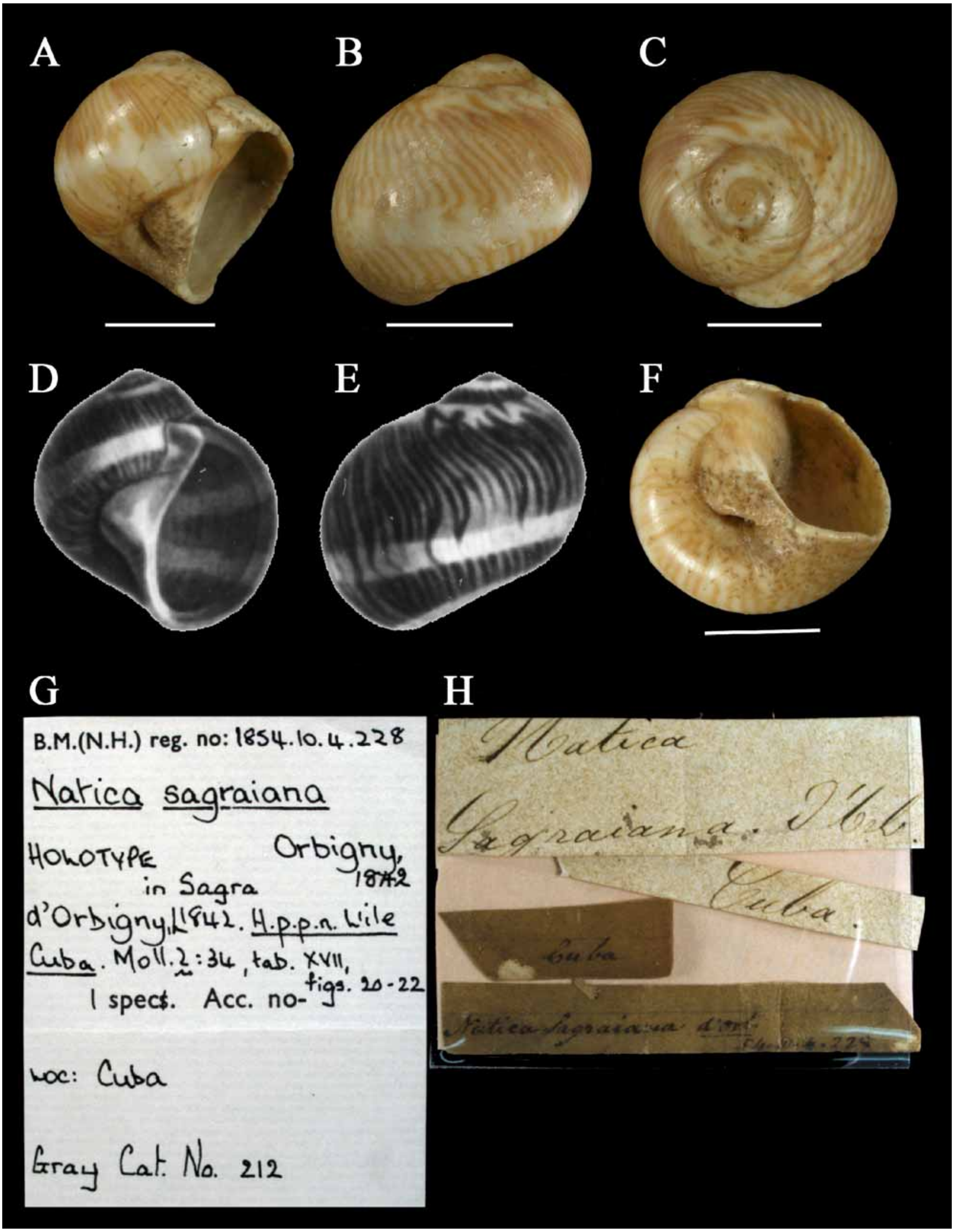

FIGURE 7: Photos of the holotype of *Natica sagraiana* Orbigny, 1842 (A–C, F), held at the Natural History Museum, London, BM(NH)#1854.10.4.228, including its labels (G, H), and figured specimen (D, E) of *Natica sagraiana* Orbigny, 1842 (Orbigny in Sagra 1842, Mollusques, vol. 2, pl. 17, page 34). A, apertual view; B, dorsal view; C, umbilical view; F, apical view; G, BM(NH) label of holotype; H, original labels of Orbigny, indicating the type locality to be Cuba. The figured specimen (D, E) appears to represent the holotype (A-C, F). Scale bars represent 0.5 cm.

The earliest available name for the species which is most commonly known as *Tectonatica filosa* (Philippi, 1845) is actually *Tectonatica sagraiana* (Orbigny, 1842) which makes *T. filosa* (Philippi, 1845) a junior synonym. This was already noted by Weinkauff (1867), Tryon (1886), Kobelt (1901), and Hidalgo (1917), and was also mentioned in recent studies (e.g., Sabelli & Spada 1977, Sabelli *et al.* 1990, Kabat 1990). To this date, the priority of *N. sagraiana* has not been generally accepted despite numerous discussions in the past (e.g., Weinkauff 1867, Settepassi 1972, Schiró 1978, Kabat 1990). The preferred name *T. filosa* (Philippi, 1845) was retained mainly based on the fact that the published type locality of *T. sagraiana* did not fit a Mediterranean species. Additionally, there were uncertainties about the date of release of Orbigny´s work (revised in Keen 1971).

While *T. sagraiana* was described from Cuba and not the Mediterranean Sea, that locality may be an error of Sagra who provided the specimen to Orbigny (see original description). The holotype in the Natural History Museum of London (BM(NH)#1854.10.4.228) is shown in Figure 5 (A–C, F) and was published in 1842 (d´Orbigny in Sagra; our Figs. 5D–E). Comparison of the the original descriptions of Orbigny (*Natica sagraiana*) and Philippi (*Natica filosa*) leave no doubt that both authors described the same species (see below). In addition, the holotype at the BM(NH) is virtually identical to the species described by Philippi (1845) and the specimens commonly found in the Mediterranean as well as those collected by us on Giglio Island (see Figure 7). Thus, the correct name for the species hitherto described as *T. filosa* (Philippi, 1845) is *T. sagraiana* (Orbigny, 1842). Unfortunately, the type specimen of *T. filosa* could not be located. It might be present at the *Museo Nacional de Historia Natural*, Santiago de Chile, Chile, but this could not be verified.

Original description of *Natica sagraiana* Orbigny in Sagra, 1842—Figs. 7A–F

Orbigny, 1842, *in* Sagra, M. R. de la (1841–1853) *Mollusques*, vol. 2, p. 34, pl. 17, figs. 20–22

> "Natica testa globosa, tenui, laevigata, albescente, lineolis fuscis longitudinaliter, undulatis, confertim interruptus ornata; spira brevi, obtusa, anfractibus quatuor convexiusculis, ultimo magno; umbilico calloso, antice fissurato, apertura ovali. "
>
> Dimensions: Lengths..11 mm
> Diameter...10 mm

Translation of the French original description:

"*Shell*. The shell is globular, somewhat compressed, slender, smooth. The spire is very short, very blunt, and consists of 4 slightly convex whorls, the last one being much larger than the others. The mouth is oval, a little bit callous at its posterior angle, with this callosity being separated from the umbilical callus by a small sulcus; the umbilicus is covered by the callus, leaving only a slit-like opening anteriorly which opens up to the inside of the umbilicus.

Color. Whitish, with reddish-brown oblique, wavy longitudinal lines, sometimes bifurcated, covering the whole shell; however, they are interrupted close to the suture where they form blotches, and in the middle of the shell where they leave a horizontal white band.

This charming species, really distinct by its umbilical callus and its lines, lives in Cuba, from where it has been brought to us by Mr. de la Sagra."

Original description of *Natica filosa* Philippi, 1845

Philippi (1845). *Diagnosen einiger nicht oder wenig bekannter Conchylien*, Cassel, Theodor Fischer. x, 231 pp., 48 hand-colored pls. Vol 2(2) p. 42, pl. 2, fig. 4

„*N*. testa ventricosa-globosa, albida, lineis longitudialibus undulatis ferrugineis confertissimis picta; fasciis duabus albis interruptis, una suturali, altera in medio unfractu ultimo; spira prominula; callo magno, rufo, umbilicum fere totum obtegente. Alt. 7 1/3’’’; diam. 8’’’; alt. apert. 6’’’
N. lineolata Ph. in Menke Zeitschrift für Malacozoologie 1844. p. 107 (I overlooked that this name had already been used for a fossil species, thus I had to change it.)
Patria: M. Mediterraneum ad Panorum rara, ad Graeciam ut videtur frequentior. “

Translation of the german original description:
“This species seems to be more frequently found in Greece than in Sicily, because Bergrath Koch got more specimens from Greece. It shows nearly the size and shape of *N. dillwynii*, only the whorls are less convex, the umbilical callus is much wider, covering almost entirely the umbilicus, and the coloration is totally different. Densely packed, angled, reddish-brown lines on a white background run parallel to the growth marks and are interrupted by a white band in the middle of the last whorl. A second somewhat wider band runs along the suture. Both bands are occasionally interrupted by crossing vertical, brownish lines. The umbilicus is white, the umbilical callus and the inner lip are reddish-brown. Unfortunately, the artist drew most of the figures of this plate too dark so that the characteristic color pattern does not emerge.”

Description
Size: Up to 20.9 mm m.o.d. (Malaga, Spain, MHC#080306.3; Hutsell *et al.* 2001). Specimens (n = 47) from Giglio Island: 5.7–11.80 mm (mean: 8.62 ± 0.22 mm) height; 5.80–11.80 mm (mean: 9.02 ± 0.22 mm) width. ratio [h/w] = 0.95 ± 0.007. Aperture approximately 78% of shell height. The larvae hatched after ~13 days in small aquaria. The planctonic larval shells have a diameter of approx. 300 µm.
General shape: Globose, solid shell with 5 slightly convex whorls [teleoconch: 2.5].
Sculpture: Minute axial and concentric striae.
Shell color: Glossy, whitish to cream background color, marked with regularly arranged, slightly wavy, brown axial lines, interrupted by a clear band below midline of body whorl which in some specimens is traversed by some but not all axial lines. Similar band below suture sparsely marked with extensions of some axial lines.
Protoconch: Slightly reddish, 2.5 embryonal whorls.
Aperture & outer lip: Aperture half-moon shaped, oblique, angled at the top, rounded at bottom; outer lip simple, sharp.
Umbilical area: Columellar callus almost completely overhanging umbilicus from columellar side, leaving narrow cleft to the left; funicle not evident; columellar callus button-shaped, brownish, reddish or purple, rarely uncolored (probably fading); rather thin, uncolored parietal callus.
Operculum: Calcareous, smooth, glossy; nucleus thickened.
Animal: Mesopodium and propodium show white background color; mesopodium marked irregularly with few reddish or brown irregular dots; propodium reddish, with irregular whitish dots and lighter colored areas at its fringe.
Egg mass: Flexible, inwardly cambered, coiled, paucispiral, mucus-cemented bands of sand grains (Figs. 3, 8F/f, 8H,I), one circle; outer diameter 24.0 to 40. mm (N = 8; mean: 34.3 ± 5.4 mm [SD])
Differential diagnosis: See under *T. rizzae*.

Geographical distribution

Giglio Island: Campese Bay (1)

General distribution: Common in most of the Mediterranean Sea with reports from the Aegean Sea; absent in the Adriatic Sea (Schiró, 1977). The species has also been reported from West Africa south to Angola (Fernandez 1993), Cape Verde Islands (Morán *et al.* 1989), and Madeira (Fernandez 1993).

***Tectonatica spec.*, probably *T. rizzae* (Philippi, 1844) – Fig. 8B [egg mass: Figs. 3, 11B, b]**

Natica rizzae Philippi, 1844. Nachtrag zum zweiten Bande der Enumeratio Molluscorum Siciliae. *Zeitschrift für Malakozoologie*, 1, 100–112. p. 108.

Natica rizzae Philippi, 1844. Weinkauff (1867), p. 254; Hidalgo (1917), p. 490; Schiró (1978b), p. 4, pl. 1 (second row); Poppe & Goto (1991), p. 121, pl 17, fig. 13; De Smit & Bába (2001), p. 98

+*Natica pyrrhosticta* Dautzenberg & Fischer, 1896. Settepassi (1972), p. 22, Bouchet & Warén (1993), p. 764–767, figs. 1822–1823, Barash & Danin (1992), p. 109

Natica (*Naticina*) *rizzae* (Philippi, 1844). Kobelt (1901), p. 93, pl. 55, figs. 16–18

Natica (*Lunatia*) *rizzae* (Philippi, 1844). Settepassi (1972), p. 14, pl. 9

Lunatia rizzai (Philippi, 1844). Error of Schiró (1977b), p. 17, for *Lunatia rizzae* (Philippi, 1844)

+*Lunatia macilenta rizzae* (Philippi 1844). Nordsieck (1982); p. 184, pl. 58, fig. 62.27

+*Natica settepassii* Gaglini in Settepassi, 1985. Settepassi (1972), pls. 9, 10; Fernandez & Rolán (1993), p. 3; Bouchet & Warén (1993), pp. 764–767, figs. 1822–1823

+*Natica settepassii* Gaglini in Settepassi, 1985 [= *rizzae* of authors, non *rizzae* Philippi, 1844]. *sensu* Sabelli, Sabelli *et al.* (1990), p. 169

Description

Size: Up to 13,7 mm m.o.d. (off Almeria, Spain; MHC#970515.5). Height 11 mm; width 10.5 mm. Ratio [h/w] = 1.04. Aperture approximately 78% of shell height. The planctonic larval shell has a diameter of approx. 300 µm.

General shape: Globose, solid shell, 5 slightly convex whorls [teleoconch: 2.5]; similar to *T. sagraiana*.

Sculpture: Minute axial and concentric striae.

Shell color: Glossy, whitish background, marked by numerous closely spaced, wavy, brown axial lines interrupted by a band below midline of body whorl; band formed by irregularly distributed, arrowhead-shaped marks, branching off from axial lines; band of large, dark-brown blotches below suture; additional narrow band with densely spaced arrowheads formed by kinks in axial lines occasionally found in upper third of body whorl; a band of fuzzy dark blotches encircles umbilical area.

Protoconch: Uncolored or slightly yellowish; 2.5 whorls (Bouchet & Warén 1993).

Aperture & outer lip: Aperture half-moon shaped, oblique, lightly angled at top, rounded at bottom; outer lip simple.

Umbilical area: Columellar callus partly overhanging umbilicus from above, covering 2/3 of it; funicle absent; columellar callus brown, red, or purple, sometimes uncolored; border of umbilical canal tinged with brown; parietal callus thick, colored like columellar callus.

Operculum: Calcareous, smooth except for single broad rib on outer lip side.

Animal: Unknown

Egg mass: Flexible, coiled, paucispiral, mucus-cemented band of sand grains with inwardly cambered walls (Figs. 3, 11B/b); 1.25 circles; outer diameter: 37.0 to 48.4 mm in four egg masses (N = 4; mean: 43.5 ± 5.9 mm [SD]).

Differential diagnosis: The shell morphology of *T. sagraiana* and *T. rizzae* shows many homologies. While *T. rizzae* seems to live in deeper water, shells collected in shallow water (< 20 m depth) are always *T. sagraiana*. Principal distinguishing feature is the operculum that in *T. sagraiana* lacks a marginal rib that is found in *T. rizzae*; in addition, *T. sagraiana* lacks a subsutural band with regularly-spaced brown blotches that is present in *T. rizzae*.

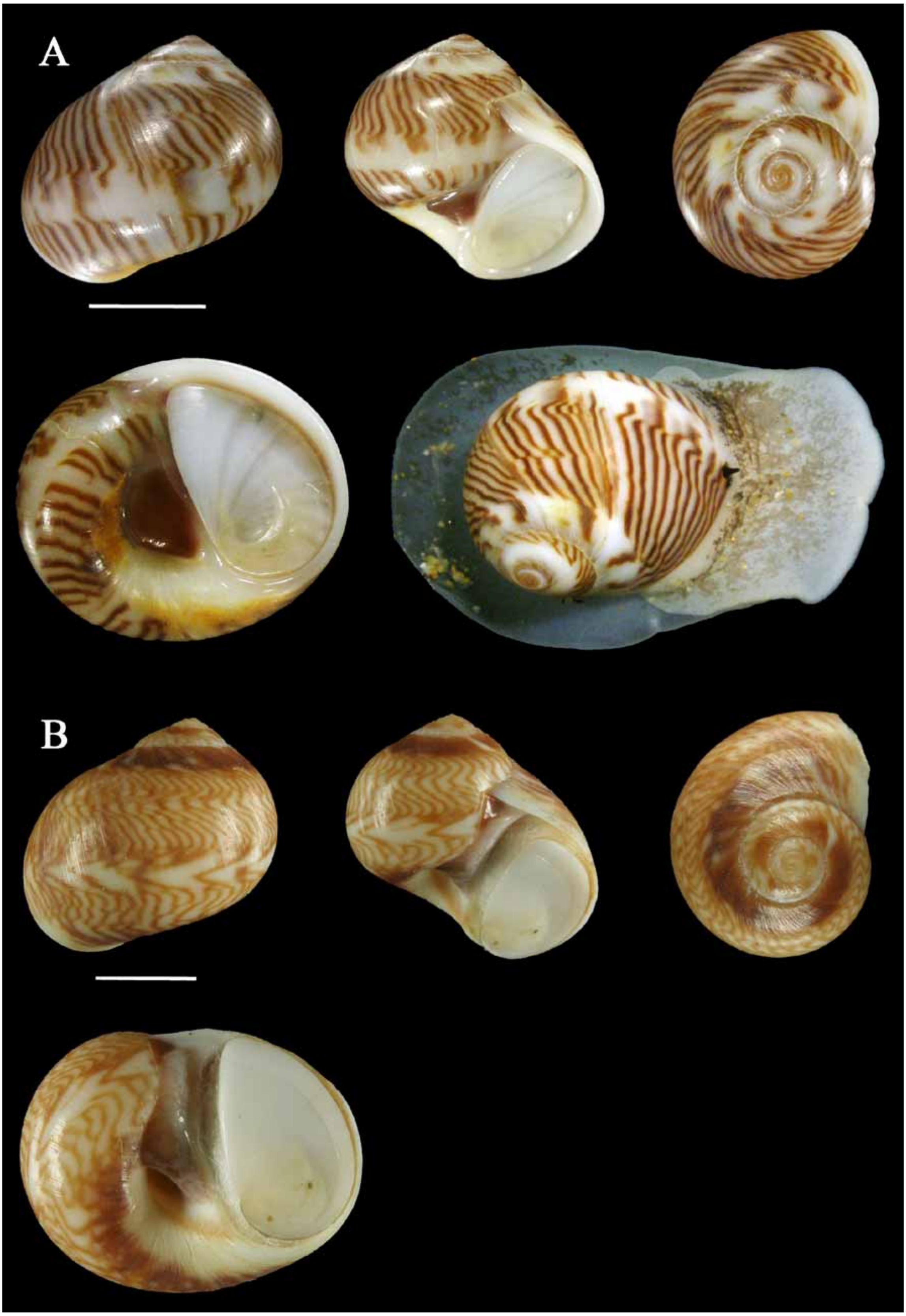

FIGURE 8: A, *Tectonatica sagraiana* (Orbigny, 1842); B, *Tectonatica rizzae* (Philippi, 1844). Further details as in Figure 3. Scale bars represent 0.5 cm.

Geographical distribution
Giglio Island: Pt. delle Secca (3)
General distribution: Distributed in most of the Mediterranean Sea with reports from the Aegean Sea (De Smit & Bába 2001); absent in the Adriatic Sea (Schiró, 1977). The species has also been reported from West Africa south to Angola (Fernandez & Rolán 1993), Cape Verde Islands (Morán *et al.* 1989).

Subfamily Polinicinae Gray, 1847

The species of the Polinicinae are characterized by a corneous operculum.

The genus *Euspira* Agassiz in Sowerby, 1837 on Giglio Island

Two species of the genus *Euspira* were found on Giglio Island. *Euspira nitida* (Donovan, 1804), which is frequently listed under one of its many junior synonyms such as *Lunatia poliana* (Delle Chiaje, 1826) (e.g., Settepassi 1970, Sabelli & Spada 1977, Schiró, 1977, Riedel 1983), and *Euspira macilenta* (Philippi, 1844). Specimens of *E. nitida* were found in the Bay of Campese and in the caves of the Cala delle`Allume in shallow waters up to 8 m depth. Both collecting areas were overgrown with *Posidonia oceanica* or *Zostera spec.* sea weeds. The specimens were found crawling under the sand, leaving well observable trails on its surface. Additionally, many empty shells were found in the Caves at the Cala delle´Allume. The species prefers coarse sediment. The one shell of *E. macilenta* was found at Pt. del Morto in 35 m depth in coarse sand.

***Euspira nitida* (Donovan, 1804)—Fig. 9A**

Nerita nitida Donovan 1804. *The natural history of British Shells*. 5 vols. 1799–1804, Vol. 4, pl. 144 (1804)
+*Natica mammilla* Maton & Rackett, 1807 [non *mammilla* Linnaeus, 1758].
+*Natica mammilla* Dillwyn, 1817 [non *mammilla* Linnaeus, 1758].
+*Natica poliana* Delle Chiaje, 1826, pl. 55, figs. 12, 13. Sabelli *et al.* (1990), p. 171
+*Polinices pulchellus* (Risso, 1826). Kingsley-Smith *et al.* 2005
+*Lunatia pulchella* (Risso, 1826), Terreni (1981), p. 31
+*Natica glaucina* Risso, 1826 [non *glaucina* Linnaeus, 1748]. Hidalgo (1917), pp. 479, 484
+*Natica glaucina* Maravigna, 1836 [non *glaucina* Linnaeus, 1758]. Fernandes & Rolán (1993), p. 3
+*Euspira intermedia* Philippi, 1836. Hidalgo (1917), pp. 479, 484; Fernandes & Rolán (1993), p. 3
+*Euspira intermedia* Philippi, 1836 [non *intermedia* Deshayes, 1832]. Sabelli *et al.* (1990), p. 171
+*Natica marochiensis sensu* Philippi, 1836 [non *marochiensis* Gmelin, 1791]. Tryon (1886), p. 41, pl. 6, fig. 13, 15; Kobelt (1901), pp. 93–96, pl. 54, figs. 12–15, Sabelli *et al.* (1990), p. 171
+*Natica* (*Naticina*) *alderi* (Forbes, 1836). Kobelt (1901), p. 95, pl. 54, figs. 12–15; Sabelli *et al.* (1990), p. 171
+*Natica similis* Koch, 1844
+*Natica lamarckiana* Leach, 1852
+*Natica marochiensis* Petit, 1852 [non *marochiensis* Gmelin, 1791]. Settepassi (1971), p.8, pl. 2
+*Natica parvula* Tapparone-Canefri, 1869. Settepassi (1971), p. 13, pl. 1; Fernandes & Rolán (1993), p. 3
+*Natica lactea* (Marshall, 1875). Tryon (1886), pp. 40–41
+*Natica alexandriae* "Récluz" Sowerby, 1883.
+*Natica americana* "Récluz" Sowerby, 1883. [= nitida? *fide* Sowerby (1883), p. 101]
+*Natica neustriaca* Locard, 1886. Kobelt (1901), pp. 93–96, pl. 54, figs. 12–15; Settepassi (1971), pp. 7–9, pl. 2
+*Natica complanata* Locard, 1886. Kobelt (1901), pp. 93–96, pl. 54, figs. 12–15
+*Lunatia alderi* (Forbes, 1836). Nordsieck (1982), p. 105, pl. 16, fig. 63
+*Natica* (*Lunatia*) *poliana* (Delle Chiaje, 1826). Settepassi (1972), p. 7, pl. 2; Sabelli *et al.* (1990), p. 171
+*Lunatia poliana* (Delle Chiaje, 1826). Sabelli & Spada (1977), p. 2, pl. 1, fig. 4; Schiró (1977b), p. 16, fig. 2 (p. 17, third row); Riedel (1983), p. 287, pl. 98, Barash & Danin (1992), p. 105–106
Euspira nitida (Donovan, 1804). Sabelli et al. (1990), p. 171; Fernandes & Rolán (1993), p. 3; Demir (2002), p. 110

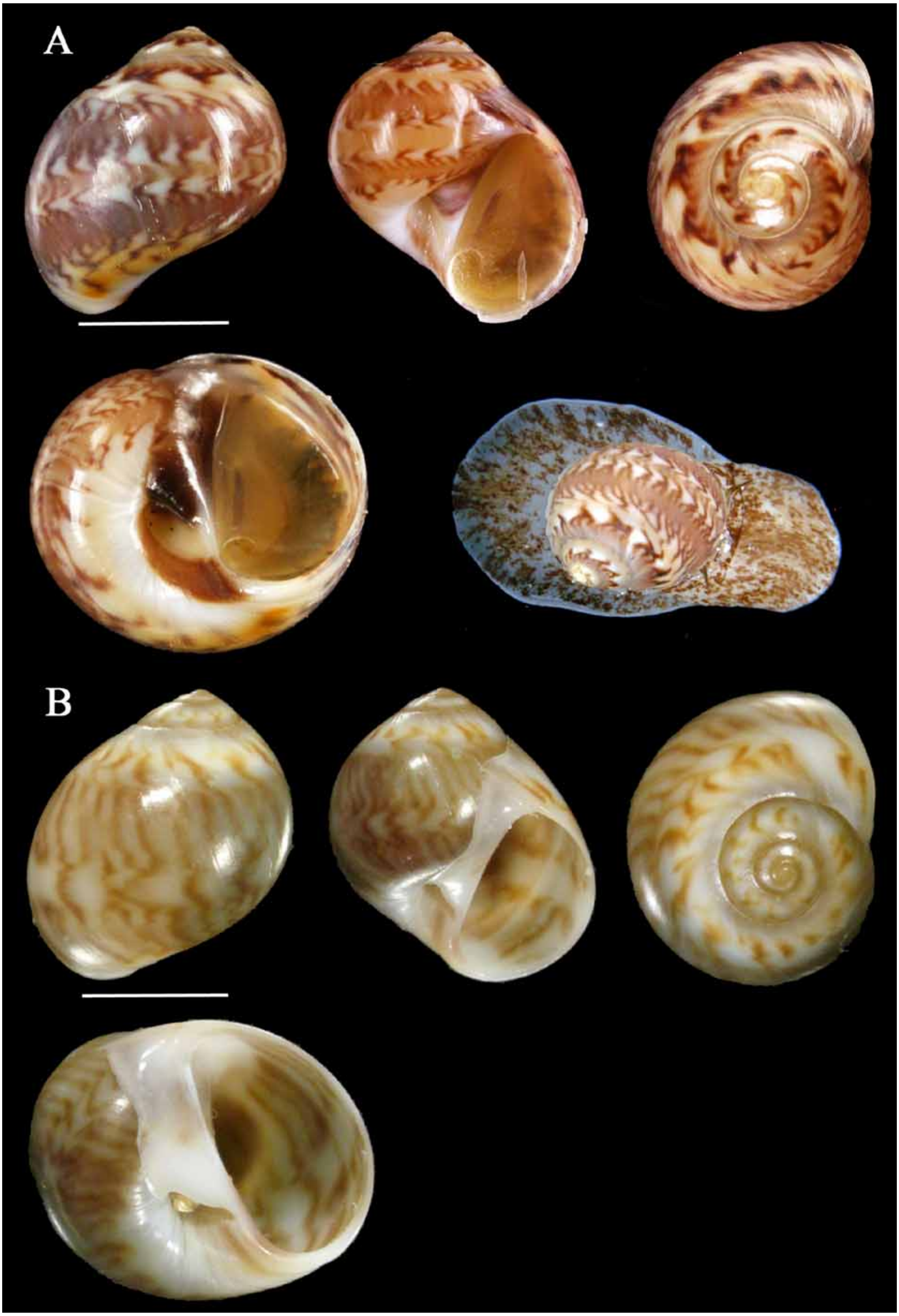

FIGURE 9: A, *Euspira nitida* (Donovan, 1804); B, *Euspira macilenta* (Philippi, 1844). Further details as in Figure 3. Scale bars represent 0.5 cm.

Description

Size: Up to 19.9 mm m.o.d. (Bretagne, France; MHC #030511.21). Specimens (n = 7) from Giglio Island: 6.2– 10.9 (mean: 8.7 ± 0.7 mm) height; 5.4–9.2 mm (mean: 7.8 mm ± 0.6 mm) width. Ratio [h/w] = 1.1 ± 0.01. Aperture approximately 67% of shell height. The planctonic larval shell has a diameter of approx. 200 μm (Thorson 1946, Kingsley-Smith 2005).

General shape: Conical, solid, 5.5 spiral whorls [teleoconch: 3].

Sculpture: Minute axial growth striae.

Shell color: Glossy, yellowish or reddish-brown zones on white background; five bands on lighter colored background with brown marks: a double band separated by a whitish zone below the suture, two smaller bands in the middle of body whorl, one bordering the umbilicus; upper band darkest of the five, 3rd and 4th with true arrowhead marks; parietal callus colored dirty reddish to brownish.

Protoconch: Slightly yellowish; conical, elevated apex, 2.5 embryonal whorls.

Aperture & outer lip: Aperture half-moon shaped, oblique, angled at top, well rounded at bottom; external lip simple, sharp.

Umbilical area: Umbilicus deeply open anteriorly, whitish, with brownish border; no funicle evident; parietal callus connects to columellar callus without sulcus, covering umbilical area triangularly from above, columellar callus tapering towards basal lip.

Operculum: Corneous, honey-colored.

Egg mass: Flexible, coiled, paucispiral band of mucus-cemented sand grains with outwardly cambered walls; 1.5 circles; outer diameter approx. 30 mm (Ziegelmeier 1961–1963, fig. 5, p.101).

Animal: Propodium and mesopodium colored identically; whitish background covered with irregular, fuzzy, reddish or light-brownish lines and blotches; tentacles short, colored reddish, dotted irregularly in white; propodium half the length of the shell, mesopodium 1.5 times shell length; both equal in width; animals cover lower parts of their shells with mesopodium.

Differential diagnosis: This species can most easily be identified by its distinct pattern of five bands of color marks combined with an umbilicus bordered by a brown-colored ridge and lacking a sharply carved-out umbilical canal or furrow.

Geographical distribution

Giglio Island: Campese Bay (1), Secca di Secche (3), Cala dell´Allume (4).

General distribution: Atlantic Ocean and North Sea from Norway, United Kingdom (Kingsley-Smith *et al.* 2005) to Gibraltar (Schiró 1977), common throughout the entire Mediterranean.

Euspira macilenta (Philippi, 1844)—Fig. 9B

Natica macilenta Philippi, R.A. (1844) *Enumeratio molluscorum Siciliae cum viventium tum in tellure tertiaria fossilium, quae in itinere suo observavit.* Vol. 2, p. 140, pl. 24, fig. 14.

Natica macilenta Philippi, 1844. Weinkauff (1867), p. 252; Hidalgo (1917), pp. 480, 488; Schiró (1977b), pp. 16–17, pl. 2 (second row)

Natica (*Naticina*) *macilenta* (Philippi, 1844). Kobelt (1901), p. 92, pl. 52, figs. 13, 14

Lunatia macilenta (Philippi, 1844). Sabelli & Spada (1977), p. 2, pl. 1, figs. 5 a–b; Terreni (1981), p. 31; Nordsieck (1982), p. 184, pl. 57, p. 405, fig. 62.26; De Smit & Bàba (2001), p. 98

+*Lunatia macilenta rizzae* (Philippi, 1844). Nordsieck (1982), p. 184, pl. 57, fig. 62.27

Description

Size: Up to 9.1 mm m.o.d (Torre Vieja, Muricia, Spain; MHC#070908.1). Specimen (n = 1) from Giglio Island: 10 mm height; 8 mm width. Ratio [h/w] = 1.2. Aperture approximately 67% of shell height.

General shape: High spired, conical, 4.25 spiral whorls [teleoconch: 2.75].

Sculpture: None.

Shell color: Glossy, white to cream background color with indistinct irregular, reddish-brown, vertical flames distributed over entire shell; indistinct broad, whitish band directly below suture, marked with brown flecks; another lighter band around middle of body whorl, interrupted by a few of the vertical brown lines, bent into indistinct arrowhead shape; basal area including most of umbilical border white.
Protoconch: Uncolored, whitish; 1.5 embryonal whorls.
Aperture & outer lip: Aperture half-moon shaped, oblique, angled at the top and well-rounded at the bottom; external lip simple, sharp; inner lip tinged with brown.
Umbilical area: Deep, whitish umbilicus, with a carved-out umbilical canal or furrow inside; no funicle evident; parietal callus uncolored at the top, turning brown towards the columellar callus, callus covering the umbilical area triangularly at the top, tapering sharply towards midpoint of columellar, leaving the anterior umbilical area open;
Operculum: Corneous, honey-colored (Schiró 1977, MHC#070908.1).
Animal: Unknown.
Differential diagnosis: This species is the most slender naticid in the Mediterranean. Its color pattern and slender form as well as the carved-out umbilical canal serve to distinguish it from *E. nitida* and *E. guillemini* (Payraudeau, 1826), the latter of which has not been found on Giglio Island.

Geographical distribution
Giglio Island:One empty shell was collected at Pt. del Morto (6) in 38 m depth in coarse sand.
General distribution: *E. macilenta* is spread throughout the western Mediterranean Sea and in the eastern part except for the Aegean Sea and some other limited zones (Settepassi 1972, Schiró 1977). The species is rather uncommon.

The genus *Payraudeautia* Bucquoy, Dautzenberg & Dollfus, 1883 on Giglio Island

One species of the naticid genus *Payraudeautia, P. intricata,* was collected on Giglio Island. The single live collected specimen was found at Cala dell´Allume at sunset between extensive *Posidonia oceanica* weeds, on sand consisting of coarse granular grains and debris. The specimen was found in 5 meter depths crawling directly beneath the sand surface and leaving an observable trail. Additionally, many empty shells were found in the caves on the Cala delle´Allume, demonstrating that there are more specimens of this species living in this area. Additionally, an empty shell was found on a bluff on top of Pt. delle Secche. This species has been reported from the entire Mediterranean Sea and the eastern Atlantic Ocean from northern Spain to the Azores (Kobelt 1901, Hidalgo 1917, Settepassi 1973, Schiró 1978, Demir 2002).

Payraudeautia intricata (Donovan, 1804)—Fig. 10A

Nerita intricata Donovan, 1804. *The natural history of British Shells*. 5 Vols. 1799–1804. Vol. 5, pl. 167.
+*Natica valenciennesi* Payraudeau, 1826. Sabelli *et al.* (1990), p. 171
+*Natica grisea* Requien, 1848. Tryon (1886), p. 42; Sabelli *et al.* (1990), p. 171
Natica intricata (Donovan, 1804). Weinkauff (1867), p. 255; Hidalgo (1917), p. 486–487
+*Payraudeautia similis* Monterosato, 1884. Sabelli *et al.* (1990), p. 171
+*Natica crassatella Locard*, 1886. Settepassi (1971), p. 19
Natica (*Payraudeautia*) *intricata* (Donovan, 1804). Tryon (1886), p. 42; Kobelt (1901), p. 102, pl. 55, fig. 9–15; Settepassi (1972), p. 16, pl. 3
+*Payraudeautia peloritana* Sulliotti, 1889. Sabelli *et al.* (1990), p. 171
+*Payraudeautia alleryana* Sulliotti, 1889. Sabelli *et al*. (1990), p. 171
Payraudeautia intricata (Donovan, 1804). Sabelli & Spada (1977), p. 10, pl. 3, fig. 8a–b; Schiró (1978a), fig. 1 (first row); Terreni (1981), p. 31; Nordsieck (1982), p.188, pl. 57 fig. 63.40; Poppe & Goto (1991), p. 120, pl. 18, fig. 4; Demir, (2002), p. 110, Barash & Danin (1992), pp. 106–107

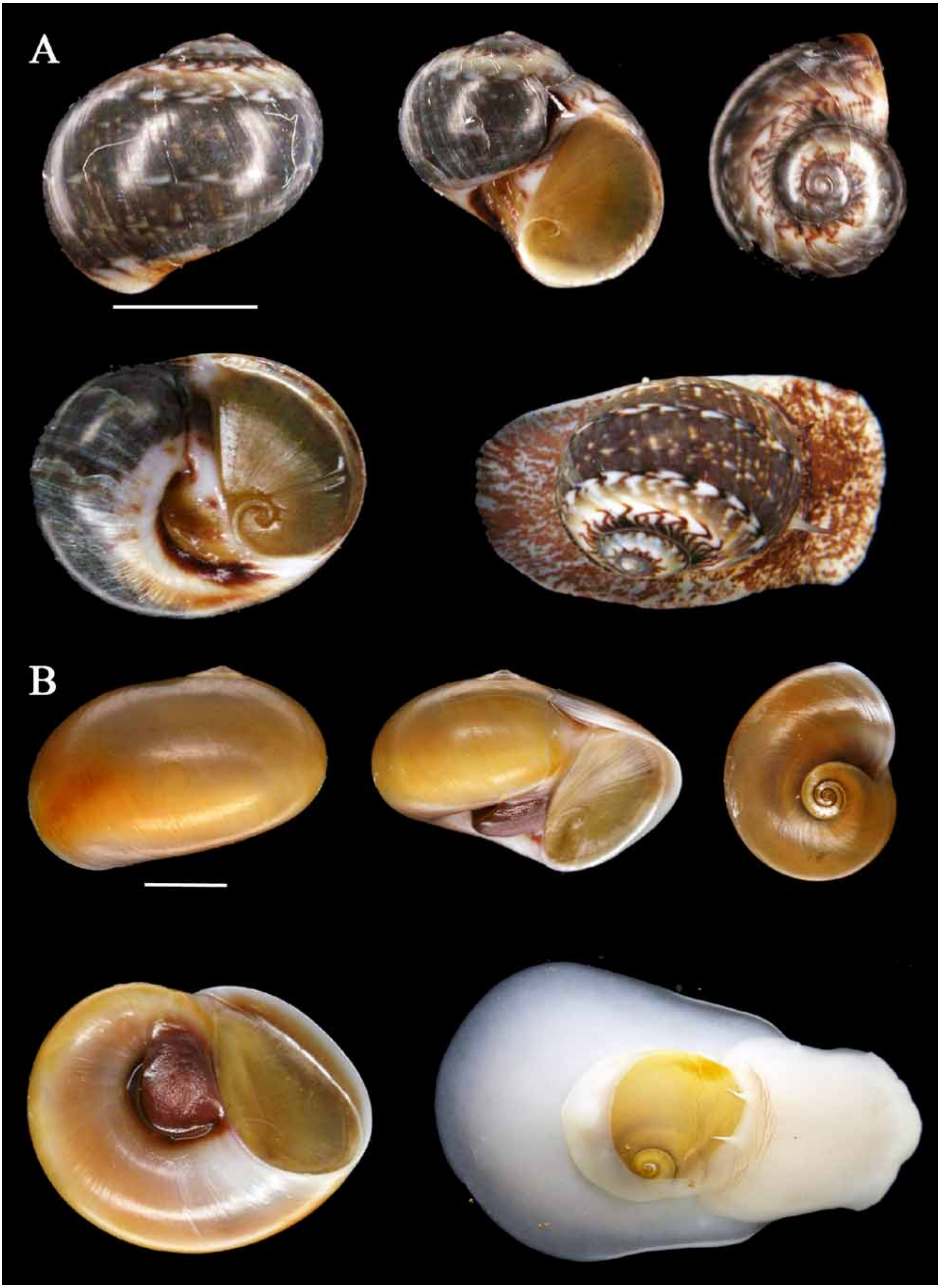

FIGURE 10: A, *Payraudeautia intricata* (Donovan, 1804); B, *Neverita josephinia* (Risso, 1826). Further details as in Figure 3. Scale bars represent 0.5 cm.

Description

Size: Up to 19.7 mm height (“Mediterranean“; MNHN, Paris). Specimens (n = 15) from Giglio Island: 6.9 `–11.2 mm (mean: 8.8 ± 0.4 mm) height; 6.5–11.0 mm (mean: 8.58 ± 0.35 mm) width. Ratio [h/w] = 1.03 ± 0.007. Aperture approximately 80% of shell height.

General shape: Globose, solid, four spiral whorls [teleoconch: 2.25].

Sculpture: Minute axial growth striae.

Shell color: Light brown to light ash-colored, speckled with small, irregular white dots and short steaks; six spiral bands marked by reddish or brown arrowheads, evenly distributed across body whorl; first band below suture, topped by a wider band of wavy brown lines in some specimens; base white, with brown band bordering the umbilicus in some but not all specimens.

Protoconch: Uncolored whitish; 1.75 embryonal whorls.

Aperture & outer lip: Half moon-shaped aperture, slightly oblique, rounded at top and bottom; external lip simple.

Umbilical area: Deep umbilicus, wide open to apex, showing two well-spaced funicles; distinct parietal callus, at least partly brownish in most specimens.

Operculum: Corneous, honey-colored.

Animal: Propodium and mesopodium with irregular, densely packed reddish or brownish lines and spots on a whitish background; tentacles beige, their tips ash colored or brownish; propodium half the length of the shell, mesopodium is 1.5 times shell length, both equal in width; animal covers only small parts of shell with the mesopodium.

Differential diagnosis: The existence of two distinct funicles within the umbilicus is the most obvious and fail-safe distinguishing shell character for species identification.

Geographical distribution

Giglio Island: Pt. della Secche (3), Cala dell´Allume (4) between sea weeds of *Posidonia oceanica*.

General distribution: Locally common in the entire Mediterranean Sea including the Aegean Sea (Settepassi 1972, Schiró 1978) and the Sea of Marmara (Demir 2002). In the Atlantic, this species is found in the Lusitanic Sea and the Azores (Kobelt 1901, Hidalgo 1917, Settepassi 1970).

The genus *Neverita* Risso, 1826 on Giglio Island

Neverita josephinia is the only native *Neverita* species in the Mediterranean and was found on Giglio Island in large numbers. The specimens were collected in the Bay of Campese and on the sand grounds seawards of Pt. del Faraglione in the northern area of the island at a depth of 7 to 15 meters. This species prefers fine sediments—no appearence of debris—at all collecting sites investigated. The specimens were found in groups of 20 or more individuals distributed in close vicinity to each other. Normally, *Neverita josephinia* was burrowed deep in the sand, not leaving an observable trail at the sand surface.

As noted in Doneddu & Manunza (1989) and Schiró (1977), the morphology of *Neverita josephinia* is very similar to that of the North American species *Neverita duplicata*. Schiró (1977) even distinguished the two species only at a subspecific level. In a recent study (Hülsken *et al.* 2006) we showed that the genetic distances between the Mediterranean *N. josephinia* and the North American *N. duplicata* and its sister taxon *N. delessertiana* amount to 9.0—13.3% for COI and thus lie in the range of the average COI sequence divergence reported for congeneric species of Mollusca (Hebert *et al.* 2003). This clearly confirms that *Neverita josephinia* is a distinct *Neverita* species which can be separated from both *Neverita duplicata* and *N. delessertiana*. The species is distributed within the entire Mediterranean Sea (Kobelt 1901, Settepassi 1972, Schiró 1977–1978) and is very common in most regions.

Neverita josephinia **(Risso, 1826)—Fig. 10B [egg mass: Figs. 3, 11A, J, K, a)**

+*Nerita glaucina sensu* auct. - non Linnaeus, 1758. Sabelli *et al.* (1990), p. 171
Natica josephinia Risso, 1826. *Historie naturelle des principales producions de l´Europe méridionale et particulièrement de celles des environs de Nice et des Alpes maritimes*. p. 149, pl. 4, fig. 43
Natica (*Neverita*) *josephinia* (Risso, 1826). Kobelt (1901), p. 103, pl. 56, figs. 1–7; Settepassi (1972), p. 28, pls. 1, 3, 7
+*Neverita olla* de Serres, 1829. Sabelli *et al.* (1990), p. 171
+*Natica albumen sensu* Scacchi, 1836 - non Linnaeus, 1758. Sabelli *et al.* (1990), p. 171
+*Neverita philippiana* Reeve, 1855 - ex Récluz MS. Sabelli *et al.* (1990), p. 171
+*Natica naticoides sensu* Sandri & Danilo, 1856 - non Küster, 1856. Sabelli *et al.* (1990), p. 171
Natica josephinia (Risso, 1826). Weinkauff (1867), pp. 256-257; Hidalgo (1917), pp. 487-488
+*Natica aegyptiaca* Pallary, 1913 [ex Recluz MS]. Sabelli *et al.* (1990), p. 171
Neverita josephinia (Risso, 1826). Sabelli & Spada (1977), p. 2, pl. 1, fig. 1; Schiró (1977a), p. 56, figs. 1, 2 (fifth row); Terreni (1981), p. 30; Nordsieck (1982), p. 102, pl. 16, fig. 62.10; Doneddu & Mananza (1989), pp. 55–60; Poppe & Goto (1991), p. 120, pl. 17, fig. 20; Barash & Danin (1992), p. 105, fig. 113a,b; De Smit & Bába (2001), p. 98; Demir (2002), p. 110; Huelsken *et al.* (2006)

Description

Size: Up to 39.8 mm m.o.d. (Spain, Hutsell *et al.*, 2001). Specimens (n = 38) from Giglio Island 4.5 – 21.7 mm (mean: 11.4 mm ± 0.9 mm) height; 6.0 – 30.5 mm (mean: 15.8 ± 1.2 mm) to width. Ratio [h/w] = 0.72 ± 0.006. Aperture approximately 87% of shell height. The larval shell has a diameter of approx. 780 µm (Giglioli 1955).

General shape: Subglobose, depressed shell with extremely low spire; 4.5 [teleoconch: 2.25] spiral whorls; last whorl extremely wide, constituting most of the shell.

Sculpture: Minute axial growth striae.

Shell color: Glossy; greyish-white to greyish-brown; lighter whitish, indistinct band at periphery; funicular callus greyish (small shells) or reddish (large shells); yellowish brown band beneath suture.

Protoconch: Weakly reddish, flat; 1.75 embryonal whorls.

Aperture & outer lip: Broadly half moon-shaped aperture, oblique, rounded at bottom, slightly angled posteriorly; outer lip simple, thin.

Umbilical area: Wide umbilicus, almost completely (sometimes entirely) occupied by large, thick funicle with button-like, reddish funicular callus.

Operculum: Corneous, honey-colored.

Animal: Propodium and mesopodium completely white, almost entirely covering the shell, including spire; propodium as long as mesopodium, each twice as long as shell; width of mesopodium twice the width of shell, broadens posteriorly; width of propodium equals width of shell.

Egg mass: Rigid, coiled paucispiral band of mucus-cemented sand grains with inwardly cambered walls (Figs. 3, 8A/a, 8J,K), one circle; outer diameter: 51.0 to 59.0 mm (n = 2; mean: 55.0 mm ± 5.7 [SD]).

Differential diagnosis: As a typical *Neverita* species, *N. josephinia* has a very flat outline; its shell is much wider than high (r = 0.72 ± 0.006 [SEM]) which is unique for Mediterranean naticids outside the genus *Sinum*; the animal is very large, and it is able to cover most parts of the shell with the propodium and mesopodium.

Geographical distribution

Giglio Island: Campese Bay (1), Pt. del Faraglione (2), Cannelle Bay (8).

General distribution: Strictly Mediterranean, and very commen in the entire Mediterranean Sea.

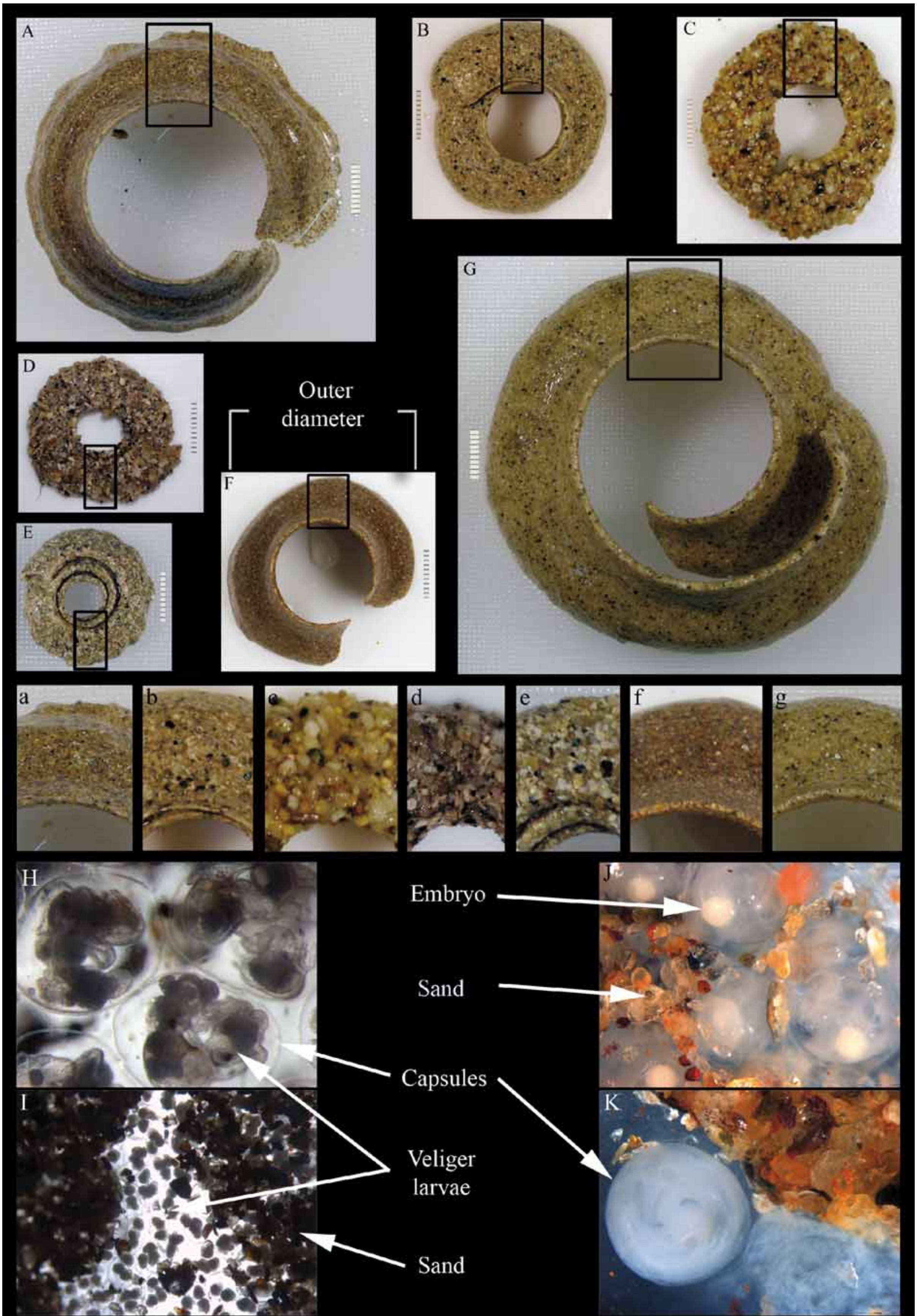

FIGURE 11: Naticid egg masses collected on Giglio Island. A/a, *Neverita josephinia* (Campese Bay); B/b, probably *Tectonatica rizzae* (Pt. delle Secche); C/c, *Notocochlis dillwynii* (Pt. delle Secche); D/d, *Notocochlis dillwynii* (Cala dell´Allume); E/e, *Notocochlis dillwynii* (Fenaio); F/f, *Tectonatica sagraiana* (Campese Bay); G/g, *Naticarius hebraeus* (Pt. del Morto); H-I, egg capsules in egg masses of *T. sagraiana* (10 days old); J-K, egg capsules in egg masses of *N. josephinia* (1 day old).

Phylogenetic considerations

A phylogenetic tree (Figure 1) constructed from sequence data of all the naticid species collected at Giglio Island plus four Mediterranean species not found at Giglio Island (*E. catena*, *E. fusca*, *N. prietoi* and *N. vittata*) does not support a distinct separation between members of the subfamilies Naticinae and Polinicinae. By contrast, species of the subfamilies intermingle in several terminal taxa. *N. dillwynii* (Figs. 2, 6B) is the first taxon which branches off the outgroup taxa including Cypraeidae and Tonnidae (BayInf: 1.00). Next, *N. josephinia* (Figs. 2, 10B) splits off the main branch. In the following, a 100% supported branch (BayInf: 1.00), which includes the *Euspira* (Figs. 2, 9, 10A) taxon and the *Natica*/*Naticarius* (Figs. 2, 5, 6A) taxon, splits off the main branch. Both terminal taxa are arranged as sister taxa. The last terminal taxon includes *T. sagraiana* and *T. spec.* (probably *T. rizzae*) (Figs. 2, 8). This branch is also well supported (BayInf: 1.00). Neither the Polinicinae (genera *Euspira*, *Neverita*, *Payraudeautia*) nor the Naticinae (genera *Natica*, *Naticarius*, *Notocochlis*, *Tectonatica*) were supported as monophyletic groups. The paleogeography of the Mediterranean Sea and the biogeography of its species are very complex. In addition, phylogenetic analysis may be complicated by species immigration, species emigration, and possible endemic occurrence. For example, *N. hebraeus* and *N. stercusmuscarum* may be species endemic to the Mediterranean Sea. By contrast, *N. dillwynii* is postulated to be a later immigrant from the Atlantic ocean (Kobelt 1901). Thus, genetic transfer between these members of the Naticinae might have been disconnected early in naticid evolution. Phylogenetic relationships of such taxa are quite difficult to estimate. For a more rigorous analysis, more naticid taxa have to be included. Currently, 20 naticid species are known for the Mediterranean Sea (Sabelli 1990, see note at page 2). Only 11 could be included here. In addition, some common naticid genera (*Eunaticina*, *Polinices*, *Mammilla*, *Sigatica*, *Tanea*) do not occur in the Mediterranean but would have to be added for a detailed phylogenetic analysis. It has been estimated that the family Naticidae includes 270–300 species (Kabat 1996). Thus, further sequences may include more informative characters and the insertion of additional taxa and characters may change the resulting phylogenetic tree. Nevertheless, in none of the single data sets shown here can the Naticinae be unequivocally separated from the Polinicinae (data not shown). Furthermore, the *Tectonatica*, *Euspira* and *Natica*/*Naticarius* groups are well supported in all data sets, indicating a close relationship of the species united in these genera. Similar to what has been suggested in previous phylogenetic analyses of the Naticidae (Powell 1933, Marincovich 1977, Bouchet & Warén 1993, Kabat 1996, Bandel 1999, Aronowsky 2003), our analysis shows that the subfamiliar groups Polinicinae and Naticinae can not be arranged in monophyletic taxa. The problems are probably the result of their long geological history (Powell 1933, Wenz 1941, Marincovich 1977, Bouchet & Warén 1993). Thus, it is quite likely that features of shell form, operculum calcification, and umbilical characters such as funiculus, funicular callus and callus ridges have appeared in several lineages independently (Bandel 1999). Our data clearly indicate that the material composition of the operculum is a paraphyletic character. The material of the operculum alone thus cannot be used as the sole criterion for genetic affinity in the Naticidae.

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Literature

Alf, A., Brümmer, F. & Koch, I. (1993) Ein Beitrag zur Kenntnis der marinen Molluskenfauna der Insel Giglio (Isola del Giglio, Grosseto, Italien). *Club Conchylia Informationen*, 25, 187–197.

Aronowsky, A. (2003) *Evolutionary biology of naticid gastropods*, PhD thesis, University of California, Berkeley, UMI Dissertation Services, Ann Harbour, Michigan, 1–312.

Bandel, K. (1976) Die Gelege karibischer Vertreter aus den Überfamilien Strombacea, Naticacea und Tonnacea (Mesogastropoda) sowie Beobachtungen im Meer und Aquarium. *Mitteilungen des Instituts Colombo-Alemán, Investigaciones Cientificas*, 8, 105–139.

Bandel, K. (1999) On the origin of the carnivorous gastropod group Naticoidea (Mollusca) in the Cretaceous with description of some convergent but unrelated groups. *Greifswalder Geowissenschaftliche Beiträge*, 6, 134–175.

Barash, A. & Danin, Z. (1992) *Annotated list of Mediterranean molluscs of Israel and Sinai*. The Israel Academy of Science and Humanities, Fauna Palaestina - Mollusca I, Jerusalem, 405.

Bouchet, P. & Warén, A. (1993) Revision of the Northeast Atlantic bathyal and abyssal Mesogastropoda. *Bollettino Malacologico*, Supplement 3, 577–840.

Cecalupo, A. & Giusti, F. (1988) Si conferma la presenza die *Tectonatica affinis* (Gmelin in L., 1791) vivente nel Mediterraneo. *Bollettino Malacologico*, 24, 47–48.

Cernohorsky, W.O. (1971) The family Naticidae (Mollusca: Gastropoda) in the Fiji Islands. *Records of the Auckland Institute and Museum*, 8, 169–208.

Colgan, D.J., Ponder, W.F., Beacham, E. & Macaranas, J. (2007) Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). *Molecular Phylogenetics and Evolution*, 42, 717–37.

Cossmann, M. (1925) *Essais de paléconchologie comparée*. Les Presses Universitaires de France, Paris, 1–345.

De Smit, E. & Bába, K. (2001) Data to the malaocofauna of Katavothres (Kefalinia, Greece). *Malacological Newsletter (Malakológiai Tájékoztató)*, 19, 95–101.

Dell, R.K. (1990) Antarctic Mollusca. With special reference to the fauna of the Ross Sea. *Bulletin of the Royal Society of New Zealand*, 27, 1–311.

Demir, M. (2003) Shells of Mollusca collected from the Seas of Turkey. *Turkish Journal of Zoology*, 27(2), 101–140.

Doneddu, M. & Manunza, B. (1989) La famiglia Naticidae Forbes, 1838 nel Mar Mediterraneo - Part IV. *Argonauta (Roma)*, 5(3–4), 55–60, (December 1989).

Fernandes, F. & Rolán, E. (1993) The family Naticidae in Angola (West Africa). *Argonauta (Roma)*, 7(6–12), 1–21.

Finlay, H.J. & Marwick, J. (1937) The Wangaloan and associated molluscan faunas of Kaitangata-Green Island subdivision. *New Zealand Geological Survey Paleontological Bulletin*, 15, 1–140.

Gelatolo, S. (2007) *Forum: Natica stercusmuscarum (=millepunctatus)*, www.naturamediterraneo.com (pub.). Available from: http://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=4259, (09 Sep 2007).

Giglioli, M.E.C. (1955) The Egg masses of the Naticidae (Gastropoda). *Journal of Fisheries Research Bd. Canada*, 12, 287–327.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society London B*, Supplement, 1–4.

Hidalgo, J.G. (1917) Fauna Malacologica de España: Portugal y las Baleares, Moluscos testáceos marinos. *Trabajos del Muso Nacional de Ciencias Naturales*, 30, pp. 477–491.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–5.

Huelsken, T., Clemmensen, M. & Hollmann, M. (2006) *Neverita delessertiana* (Recluz in Chenu, 1843): a naticid species (Gastropoda : Caenogastropoda) distinct from *Neverita duplicata* (Say, 1822) based on molecular data, morphological characters, and geographical distribution. *Zootaxa*, 1–25.

Hutsell, K.C., Hutsell, L.L. & Pisor, D.L. (2001) *Hutsell, Hutsell and Pisor´s registry of world record size shells*. Snail´s Pace Productions, San Diego, 158.

Kabat, A.R. (1990) *The Western Atlantic Naticidae (Mollusca: Gastropoda) with a catalogue of genera and a review of shell boring predation*. PhD thesis, Harvard University, UMI Dissertation Services, Ann Arbor, MI, 441.

Kabat, A.R. (1991) The classification of the Naticidae (Mollusca: Gastropoda): Review and analysis of the supraspecific taxa. *Bulletin of the Museum of Comparative Zoology*, 152, 417–449.

Kabat, A.R. (1996) Biogeography of the genera of Naticidae (Gastropoda) in the Indo-Pacific. *American Malacological*

Bulletin, 12, 29–35.
Keen, A.M. (1971) *Sea shells of tropical West America*. Stanford University Press, California.
Kingsley-Smith, P.R., Richardson C.A. & Seed, R. (2005) Growth and development of the veliger larvae and juveniles of *Polinices pulchellus* (Gastropoda: Naticidae). *Journal of the Marine Biological Association of the United Kingdom*, 85, 171–174.
Kobelt, W. (1887–1908) *Iconographie der schalentragenden europäischen Meeresconchylien, vols. 1–4*, C. W. Kreidel´s Verlag, Wiesbaden, [Familie Naticidae in vol. 2, 73–111].
Maddison, W.P. & Maddison, D.R. (2006) *MacClade*. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
Marincovich, L.N. (1977) Cenozoic Naticidae (Mollusca: Gastropoda) of the Northeastern Pacific. *Bulletins of American Paleontology*, 70 (No. 294), 169–494.
Melone, N. & Picchetti, G. (1980) *Vita del Mare. Guida illustrata della fauna e della flora del Mediterraneo*. Primaris, Milano, 171.
Morán, R., Gil, J.L., Calvo, M. & Luque, A.A. (1989) Contribucion al conomimiento de los moluscos gasteropodos prosobranquios del Archipielago de Cabo Verde. I. Naticidae, Nassaridae y Terebridae. *Publicações Ocasionais da Sociedade Portuguesa de Malacologia*, 13, 37–50.
Nordsieck, F. (1982) Superfamilia Naticoidea *in:* F. Nordsieck (Eds.), *Die europaeischen Meeres-Gehauseschnecken (Prosobranchia). Vom Eismeer bis Kapverden, Mittelmeer und Schwarzes Meer.* Gustav Fischer Verlag, Stuttgart - New York, 182–188.
Nylander, J.A.A. (2004) *MrModeltest v2. Program distributed by the author.* Evolutionary Biology Centre, Uppsala University.
O'Leary, M.A. & Kaufman, S.G. (2007) *MorphoBank 2.5: Web application for morphological phylogenetics and taxonomy*, http://www.morphobank.org (17. Feb. 2008).
Orbigny, A. d´ (1841–1853) Mollusques, in: Sagra , R. de la (Ed.), *Histoire physique, politique et naturelle de l´Ile de Cuba*. A.Bertrand, Paris, 2 vols. + atlas. Vol.1: pp. 1–16 (1840), 17–240 (1841), 241–264 (1842); vol.2: pp. 1–112 (1842), 113–128 (1844), 129–224 (1847), 225–368 (1853); atlas: 29 pls. (1842) [fide Gary Rosenberg, Malacolog v.4.1.0, http://www.malacolog.org/bibliography.php].
Padial, J.M. & De La Riva, I. (2007) Integrative taxonomists should use and produce DNA barcodes *Zootaxa*, 1586, 67–68.
Philippi, R.A. (1849–1853) Die Gattungen *Natica* und *Amaura in:* Martini, Chemnitz, (Schubert G.H.v., J. A. Wagner and H. C. Küster) (Eds.), *Systematisches Conchylien-Cabinet*. Bauer und Rapse, Nürnberg, 164, 20 pls.
Philippi (1845). *Diagnosen einiger nicht oder wenig bekannter Conchylien*, Cassel, Theodor Fischer. x, 231 pp., 48 hand-colored pls. Vol 2(2), 50.
Poppe, G.T. & Goto, Y. (1991) *European Seashells. 1. (Polyplacophora, Caudofoveota, Solenogastra, Gastropoda)*. Verlag Christa Hemmen, pp. 352.
Powell, A.W. (1933) Notes on the taxonomy of the recent Cymatiidae and Naticidae of New Zealand. *Transactions of the New Zealand Institute*, 63, 154–170.
Richardson, C.A., Kingsley-Smith, P.R., Seed, R. & Chazinikalaou, E. (2005) Age and growth of the naticid gastropod *Polinices pulchellus* (Gastropoda: Naticidae) based on length frequency analysis and statolith rings. *Marine Biology*, 148, 319–326.
Riedel, F. (2000) Ursprung und Evolution der "höheren" Caenogastropoda. *Berliner Geowissenschaftlichen Abhandlungen*, Berlin, 32, 1–240, pls. 1–21.
Riedel, R. (1983) *Fauna und Flora des Mittelmeeres*. Paul Parey Verlag, Hamburg und Berlin, 836.
Risso, A. (1826) *Historie naturelle des principales producions de l´Europe méridionale et particulièrement de celles des environs de Nice et des Alpes maritimes*. F.-G. Levrault, Paris, 438.
Rolán, E. (2005) *The malacological fauna of the Cape Verde archipelago*. ConchBooks, Hackenheim, 88–91.
Sabelli, B., Giannuzi-Savelli R. & Bedulli, D. (1990) *Catalogo annotato dei Molluschi marini del Mediterraneo, vol. 1*. Libreria Naturalistica Bolognese, Bologna, 348.
Sabelli, B. & Spada, G. (1977a) Guida illustrata all’identificazione delle conchiglie del Mediterraneo. No. 3. Fam. Naticidae. – Part I. *Supplemento a Conchiglie*, 13(7–8), [1–4].
Sabelli, B. & Spada, G. (1977b) Guida illustrata all’identificazione delle conchiglie del Mediterraneo. No. 8. Fam. Naticidae. – Part II. *Supplemento a Conchiglie*, 13(11–12), [1–4].
Sabelli, B. & Spada, G. (1980) Guida illustrata all’identificazione delle conchiglie del Mediterraneo. No. 16. Fam. Naticidae. – Part III. *Supplemento a Bollettino Malacologico*, 16(1–2), [1–4].
Schileyko, A.A. (1977) Materials on the morphology of the Naticoidea and problems of the taxonomy of the superfamily (Mollusca: Mesogastropoda). *Trudy Okeanologicheski Instituta, Akademiia Nauk SSSR*, 108, 77–97.
Schiró, G. (1977a) Naticidae (moon shells) of the Mediterranean - Part I. *Riv. "La Conchiglia"*, 9(102–103), 4–6.
Schiró, G. (1977b) Naticidae (moon shells) of the Mediterranean - Part II. *Riv. "La Conchiglia"*, 9(104–105), 16–17.
Schiró, G. (1978a) Naticidae (moon shells) of the Mediterranean - Part III. *Riv. "La Conchiglia"*, 10(106–107), 8–10.
Schiró, G. (1978b) Naticidae (moon shells) of the Mediterranean - Part IV. *Riv. "La Conchiglia"*, 10(108–109), 4–5.

Schiró, G. (1978c) Naticidae (moon shells) of the Mediterranean - Part V. *Riv. "La Conchiglia"*, 10(110–111), 3–4.

Settepassi, F. (1972) Familia Naticidae=Naticoidea *in:* Settepassi (Ed.), *Atlante malacologico: i Molluschi marini viventi nel Mediterraneo - Part III*. INVAG, Roma, 1–34.

Strong, E.E. (2003) Refining molluscan characters: morphology, character coding and a phylogeny of the Caenogastropoda. *Zoological Journal of the Linnean Society*, 137(4), 447–554.

Swofford, D.L. (2003) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.* Sunderland, Massachusetts.

Terreni, G. (1980) Molluschi poco conosciuti dell´Arcipelago Toscano: 1e - Gasteropodi. *Bolletino Malacologico*, 16, 9–17.

Terreni, G. (1981) *Molluschi conchiferi del mare antistante la costa Toscana (Gastropoda, Scaphopoda, Amphineura, Bivalvia, Cephalopoda)*. Tip. Benvenuti & Cavaciocchi, Livorno, 106.

Thorson, G. (1946) Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planctonic larvae in the Sound. *Meddelelser fra Kommissionen for Danmarks Fiskeri- Og Havunder Soegelser. Serie: Plankton*, 4, 1–523.

Villa, R. (1985) La famiglia Naticidae Forbes, 1838 nel Mar Mediterraneo - Part I. *Argonauta (Roma)*, 1(6), 103–110, (December 1985).

Villa, R. (1986a) La famiglia Naticidae Forbes, 1838 nel Mar Mediterraneo - Part II. *Argonauta (Roma)*, 2(1–2), 134–136, (April 1986).

Villa, R. (1986b) La famiglia Naticidae Forbes, 1838 nel Mar Mediterraneo - Part III. *Argonauta (Roma)*, 2(3–4), 179–182, (August 1986).

Weinkauff (1867) *Die Conchylien des Mittelmeeres, ihre geographische und geologische Verbreitung*. Theodor Fischer, Cassel, 1–501.

Wenz, W. (1938–1944) Gastropoda, Teil I, Allgemeiner Teil und Prosobranchia *in:* O. H. Schindewolf (Ed.), *Handbuch der Paläozoologie*. Gebrueder Borntraeger, Berlin-Zehlendorf, 1639 [Naticidae: pp. 1017–1045, Oct. 1941; *fide* Kabat 1991].

Ziegelmeier, E. (1961) Zur Fortpflanzungsbiologie der Naticiden (Gastropoda: Prosobranchia). *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 8(1), 94–118.