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Systematics and phylogenetic species delimitation within *Polinices s.l.* (Caenogastropoda: Naticidae) based on molecular data and shell morphology

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Abstract Here, we present the first phylogenetic analysis of a group of species taxonomically assigned to *Polinices sensu latu* (Naticidae, Gastropoda) based on molecular data sets. *Polinices s.l.* represents a speciose group of the infaunal gastropod family Naticidae, including species that have often been assigned to subgenera of *Polinices* [e.g. *P.* (*Neverita*), *P.* (*Euspira*), *P.* (*Conuber*) and P. (*Mammilla*)] based on conchological data. The results of our molecular phylogenetic analysis confirm the validity of five genera, *Conuber*, *Polinices*, *Mammilla*, *Euspira* and *Neverita*, including four that have been used previously mainly as subgenera of *Polinices s.l.* Our results furthermore indicate a close relationship of members of the Polinicinae to *Sinum*—a genus traditionally placed in the naticid subfamily Sininae. We furthermore present conchological analyses to determine the validity of shell characters used traditionally in species designation in the genus *Polinices*. Our data reveal several characters (e.g. protoconch, operculum colour, parietal callus) to be informative, while many characters show a high degree of homoplasy (e.g. umbilicus, shell form). Among the species arranged in the genus *Polinices s.s.*, four conchologically very similar taxa often subsumed under the common Indo-Pacific species *P. mammilla* are separated distinctly in phylogenetic analyses. Despite their striking conchological similarities, none of these four taxa are related directly to each other. Additional conchological analyses of available name-bearing type specimens and type figures reveal the four "*mammilla*"-like white *Polinices* species to include true *P. mammilla* and three additional species, which could be assigned to *P. constanti* (replacement name for *P. dubius*), *P. jukesii* and possibly *P. tawhitirahia*, based on protoconch and operculum characteristics.

Keywords Polinices · Molecular systematics · Conchology · Polinicinae · Sininae · Naticinae · Barcoding

Introduction

Polinices sensu latu represents one of the most speciose genera within the infaunal caenogastropod family Naticidae, including species assigned taxonomically to *Polinices sensu strictu* (*s.s.*) (*Polinices* (*Polinices*)) or to species that were described as being members of subgeneric taxa of *Polinices*, such as *P.* (*Neverita*), *P.* (*Euspira*), *P.* (*Conuber*) and P. (*Mammilla*) (see Cernohorsky 1971; Marincovich 1977; Majima 1989). Members of *Polinices s.l.* are distributed widely, occurring predominantly in tropical waters of the Indo-Pacific region with only a few species living in the Atlantic Ocean, Mediterranean Sea, Caribbean Sea and the Eastern Pacific. Taxonomic assignment of *Polinices s.l.* species to the traditional subfamilial group Polinicinae is based on the presence of a corneous operculum (Marincovich 1977; Majima 1989; Kabat 1991).

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The genus *Polinices* Montfort, 1810 is based on the description of the purely white-shelled type species *Polinices albus* Montfort, 1810 (type locality: Ambon Island, Indonesia, *fide* Kabat 1990; see Supplement). Objective synonymy of this species with *Nerita mammilla* Linnaeus, 1758 was assured by the action of Kabat (1990), who designated the lectotype of *Nerita mammilla* as the neotype of *Polinices albus* (see Linnaeus 1758). Thus, Kabat prevented the well-established and broadly used generic level taxon *Polinices* to be discarded, should its type species, *Polinices albus,* for which no type material could be located (Kabat 1990), be considered a nomen dubium. The genus *Polinices* erected by Montfort was used later as the type genus for the subfamilial group Polinicinae Gray, 1847 (Montfort 1810). As molecular, anatomical, biogeographical, or ecological data are difficult to obtain for this species group, the characters most commonly used for species differentiation within the Polinicinae are the size and colour of the operculum, the arrangement of the funicle within the umbilicus, the shell form and colouration as well as the size and colouration of the protoconch (e.g. Risso 1826; Agassiz 1837; Chenu 1842; Récluz 1844; Philippi 1849; Tryon 1886; Garrard 1961; Cernohorsky 1971; Marincovich 1977; Majima 1989; Bandel 1999; Kabat 2000).

Conchologically, species assigned to *Polinices s.l.* are very similar to each other and are generally characterized by plain white or monochrome, glossy, ovate to pyriform-shaped shells; a brownish, corneous operculum; a medium-to-thick parietal callus; and a partly or completely filled umbilicus (Fig. 1). Based on intra-specific variation of these features and striking inter-specific similarities of Recent *Polinices s.l.* species, a large number of species with questionable taxonomic status have been described to date (see Supplement).

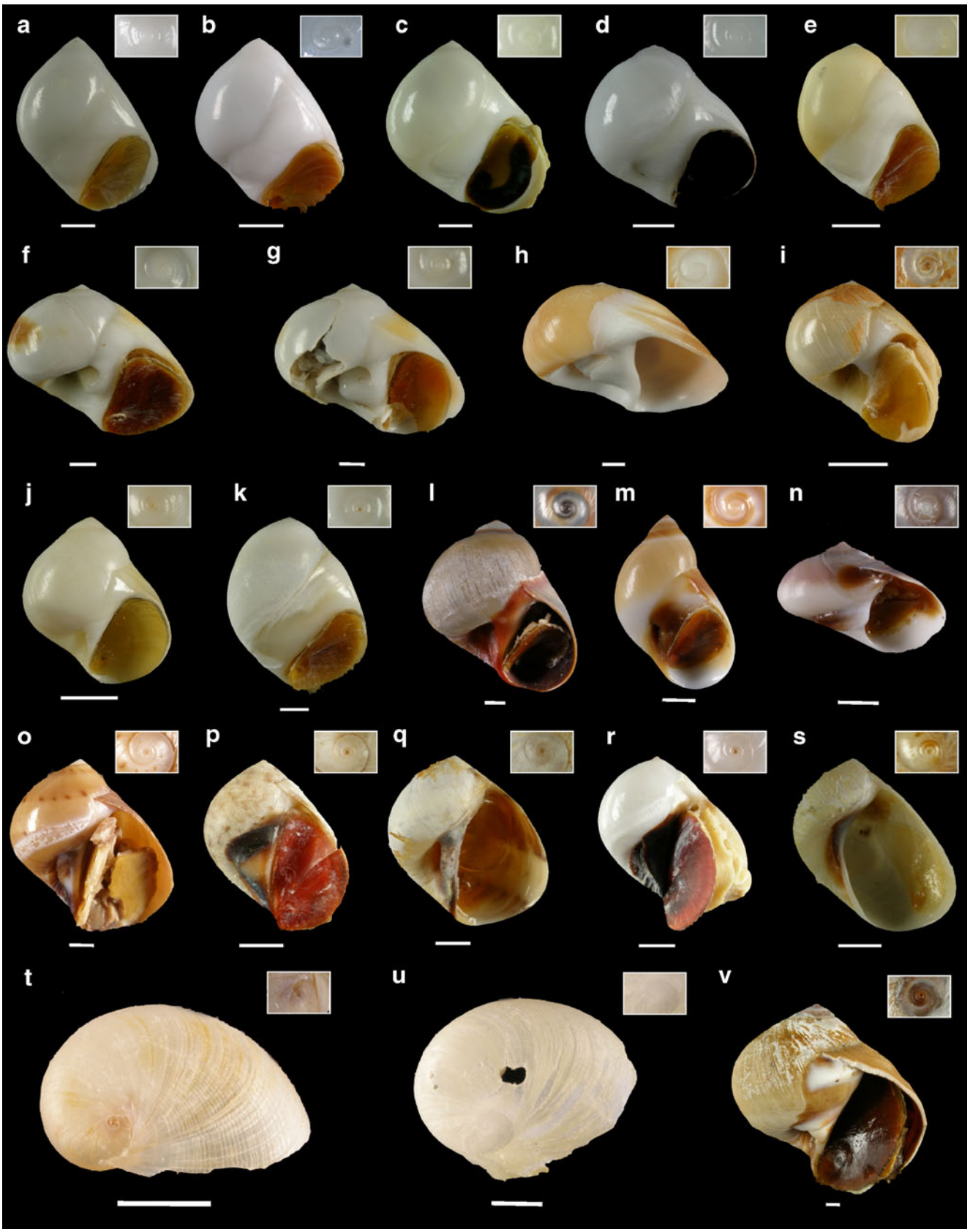
The problem of highly similar conchological features used for species identification, however, is not restricted to *Polinices s.l.* species, but is found in different (sub)generic lineages within the entire family Naticidae (Bandel 1999; Huelsken et al. 2011b). Differences in the shape and extent of the parietal callus, shell shape, thickness of the funicle and size and form of the umbilical cavity, observed in (sub)generic taxa within the Polinicinae are often limited to the degree of character expression only. Consequently, Cernohorsky stated, that "…umbilical and opercular characters are not always in agreement nor do they follow a pre-diagnosed generic pattern" (1971: 169). This statement concurs with analyses of Troschel (1856–1863) and Bandel (1984) who regarded members of the subfamilial groups Naticinae and Polinicinae to be congeneric based on similarities in the morphology of their radulae. Popenoe et al. (1987), amongst others, stated in their compilation of the late Cretaceous subfamilial naticid taxon Gyrodinae that the convergent development and inconstant characteristics of umbilical features complicate the classification within the entire family Naticidae.

Fig. 1 Species analysed in this study. **a** *Polinices* sp. 2 [#70-2, MNHN#IM-2009-5170]. **b** *Polinices* sp. 3 [#70-6, QM#MO80747]. **c** *Polinices flemingianus* (Récluz, 1844) [#141-1, MNHN#42645]. **d** *Polinices* sp.4 [#D6, QM#MO80750]. **e** *Polinices mellosus* (Hedley, 1924) [#59-4, MNHN#IM-2009-5167]. **f** *Polinices cumingianus* (Récluz, 1844) [AMS#C434459]. **g** *Polinices peselephanti* (Link, 1807) [AMS#C451672]. **h** *Polinices albumen* (Linnaeus, 1758) [SBD#026719]. **i** *Polinices mediopacificus* Kosuge, 1979 [MNHN#42646]. **j** *Polinices uber* (Valenciennes in Humboldt, 1832) [#30-1, MNHNIM-2009-5172]. **k** *Polinices* sp.1 [#51-1, MNHNIM-2009-5174]. **l** *Conuber sordidus* (Swainson, 1821) [AMS#EBU30442]. **m** *Conuber conicus* (Lamarck, 1822) [#80-2, $$]. **n** *Conuber incei* (Philippi, 1851) [#100-1, AMS#C399745]. **o** *Mammilla priamus* (Récluz, 1844) [#07-1, MNHNIM-2009-5179]. **p** *Mammilla simiae* (Deshayes in Deshayes & Edwards, 1838) [#77-1, MNHNIM-2009-5177]. **q** *Mammilla melanostomoides* (Quoy & Gaimard, 1832) [#87-1, MNHN42649]. **r** *Mammilla melanostoma* (Gmelin, 1791) [#25-1, MNHNIM-2009-5176]. **s** *Mammilla caprae* (Philippi, 1850) [#123-1, MNHNIM-2009-5178]. **t** *Sinum haliotoideum* (Linnaeus, 1758) [#97-1, AMS#C451594]. **u** *Sinum sanctijohannis* (Pilsbry & Lowe, 1932) [#35-1, MNHN#IM-2009-5162]. **v** *Euspira lewisii* (Gould, 1847) [#104-1, QM#MO80751]. Pictures of specimens analysed in earlier studies (*Euspira*, *Neverita*) can be found in Hülsken et al. (2006) and Huelsken et al. (2008). Enlarged images of the protoconchs are shown in the small inserts. *Bars* 0.5 cm

Not surprisingly, the generic classification within the Polinicinae has changed frequently during the last two centuries. Due to the lack of distinct and characteristic conchological features, members of the subfamilial taxon Polinicinae (*Polinices, Conuber* Finlay and Marwick 1937, *Euspira* Agassiz in Sowerby, 1837, *Mammilla* Schumacher, 1817, *Neverita* Risso, 1826) have been treated as subgenera of *Polinices* (e.g. Cernohorsky 1971; Marincovich 1977; Majima 1989) or have been considered to be closely related to the subfamilial taxon Sininae (e.g. the genus *Mammilla*; Cernohorsky 1971; Kabat 1996). These examples support scepticism in the application of conchological characters in cladistic analyses, because of their highly homoplasious nature (Kool 1993) caused by analogous adaptations to environmental constraints. This is particularly true for the infaunal Naticidae, all of which are burrowing species with a seemingly identical ecology and a predatory feeding behaviour that relies on drilling of the shells of their prey (Cernohorsky 1971; Huelsken 2011).

Yet, empty shells are often the only available information source with which to identify gastropod species. As scientific names are assigned formally to type specimens, which in gastropods most often are available only as empty shells, analysis of conchological characters is the boon and bane of taxonomic assignments: in most cases, species determination based on shells of type specimens allows reliable taxonomic assignment of recent and fossil species. In other cases, type lots are missing, shells are broken, the few available characters are homoplasious or available species descriptions are not informative enough for reliable species identification.

In the present study, we employ a multilocus molecular phylogenetic analysis to investigate the relationships within the genus *Polinices s.s.* and its association with the (sub) generic taxa *Conuber*, *Euspira*, *Mammilla*, *Neverita* and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Sinum that have been regarded traditionally as closely related. Analyses of conchological characters of molecularly characterized specimens serve to estimate the validity of traditionally used characters and their usage in type specimen assignment to *Polinices s.s.* species. Additionally, we provide species names and taxonomic descriptions for several plain white *Polinices s.s.* species, which can be separated from *P. mammilla* by phylogenetic analyses.

Materials and methods

Throughout the manuscript, the term '*Polinices*' is to be taken *sensu stricto* (*s.s*) unless mentioned otherwise. *Polinices sensu latu* (*s.l.*) refers to species assigned to *Polinices s.s.* and to species that have previously been assigned to subgenera of *Polinices* [e.g. *P.* (*Mammilla*), *P.* (*Euspira*)].

Material examined

Specimens (Fig. 1) analysed were collected by diving, snorkelling, and dredging from several spots around Lizard Island and Dingo Beach in Queensland, Australia or were on loan from the Australian Museum, Sydney, Australia (AMS), the Queensland Museum, Brisbane, Australia (QM) or the Muséum National d'Histoire Naturelle, Paris, France (MNHN) (Table 1). Additional specimens were obtained from 9 of 457 trawl samples taken during the Great Barrier Reef Seabed Biodiversity Project (see Pitcher et al. 2007). Collected specimens have been vouchered in the malacological collection at the Queensland Museum (QM) or have been taken from previous work (Hülsken et al. 2006; Huelsken et al. 2008). The criteria for an a priori definition of species and genera were based on previously published taxonomic descriptions (e.g. Röding 1798; Schumacher 1817; Swainson 1840; Récluz 1850; Philippi 1850; Garrard 1961; Cernohorsky 1971; Marincovich 1977; Majima 1989; Kabat 1991; Hülsken et al. 2006; Huelsken et al. 2008).

Collected animals were anaesthetised with 0.25 M $MgCl_2$, fixed in 75–85 % EtOH and subsequently stored in 94 % EtOH. Altogether, our data set is based on 87 specimens representing 32 naticid species in eight traditional (sub)genera from three traditional subfamilies, the Polinicinae (*Polinices*, *Conuber*, *Neverita*, *Mammilla*, *Euspira*, *Payraudeautia*), Sininae (*Sinum*) and Naticinae (*Tectonatica*) (Table 1). The genus *Tectonatica* was chosen as an internal outgroup to root the ingroup and to test the relationship of Polinicinae and Sininae.

We additionally selected several members of the caenogastropod families Strombidae [*Strombus dilatatus* (Swainson, 1821), *Strombus luhuanus* (Linnaeus, 1758)], Batillariidae [*Pyrazus ebeninus* (Bruguiere, 1792)], Calyptraeidae [*Bostrycapulus pritzkeri* (Collin, 2005)], Olividae [*Oliva amethystina* (Roeding, 1798)] and Cypraeidae [*Cypraea annulus* (Linnaeus, 1758)] for outgroup comparison (see Table 1).

Nucleic acid isolation and sequence analysis

Total DNA was extracted from ethanol/RNAlater (Qiagen, Hilden, Germany)-preserved tissue using a modified protocol of the DNeasy Extraction Kit (Qiagen) (Huelsken et al. 2011a) and stored in 0.1 mM Tris-EDTA pH7.4. A 447-bp fragment of the *COI* gene, 264 bp of the *H3* gene, 476 bp of the *16S* gene, 401 bp of the *18S* gene and 352 bp of the *28S* gene were amplified and sequenced from each specimen. Amplification reactions were performed with iProof polymerase (Bio-Rad Laboratories, Munich, Germany) on MJ Research thermocyclers (MJ Research, Watertown, MA). Amplification primers 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*, P398 (5′-cgtgttgatyctgccagt-3′) and P399 (5′- tctcaggctccytctccgg-3′) for the partial *18S* gene and P1017 (5′-acccsctgaayttaagcat-3′) and P1018 (5′- aactctctcmttcaragttc-3′) for the partial 28S gene fragment [primer sequences taken from Colgan et al. 2007 (3′end of 28S rRNA), Huelsken et al. 2008, 2011a].

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). Sequences of *Neverita*, *Euspira*, *Tectonatica* and *Payraudeautia* species had been obtained by us in the context of other studies (Hülsken et al. 2006; Huelsken et al. 2008). Pictures of these species can be found in the respective publications or under the Morphobank project ID#189 (O'Leary and Kaufman 2007).

Phylogenetic analyses

The phylogenetic trees (Figs. 2 and 3, Table 2) were calculated with *MrBayes* v3.1.2 (Ronquist and Huelsenbeck 2003), while the NeighborNet analysis (Fig. 4) was performed using *SplitsTree* v4.0 under the LogDet model (Huson 1998; Huson and Bryant 2006). Sequence distances have been demonstrated to represent evolutionary distances between species (Makowsky et al. 2010). Thus, genetic distances between taxa and clades were calculated using *PAUP*4.0b10* (Swofford 2003) (Tables 3 and 4). Sequences of all specimens analysed were uploaded to GenBank (accession numbers FJ263801–FJ263889, GQ328724–GQ328743 and FJ623464–FJ623465) and the concatenated alignment was deposited in TreeBASE (Sanderson et al. 1994).

For the Bayesian analysis, 15 x 10^6 generations were calculated saving every 1,000th tree. The first 3,000 trees were discarded as burn-in. In the phylogenetic analyses (single gene fragment; concatenated data set) protein-coding gene

Table 1 Specimens analyzed in this study, with specimen numbers, collection sites and museum voucher numbers

Species, author	Collection site	Voucher number/ reference
Outgroup taxa		
Bostrycapulus pritzkeri (Collin, 2005)	Edwards Beach, Balmoral, New South Wales, Australia	Colgan et al. 2007; AMS#C335468
Strombus luhuanus (Linnaeus, 1758)	Heron Island, Queensland, Australia	Colgan et al. 2007; AMS#C203214
	DB-TH225 Dingo Beach, Whitsunday Islands, Queensland, Australia	QM#MO80745
Cypraea annulus (Linnaeus, 1758)	Heron Island, Queensland, Australia	Colgan et al. 2007; AMS#C203215
Pyrazus ebeninus (Bruguiere, 1792)	SI-TH116 on muddy sand at low tide, Dunwich, North Stradbroke Island, Australia	QM#MO80742
Oliva amethystina (Röding, 1798)	DB-TH158 on muddy sand at low tide, Dingo Beach, Whitsunday Islands, Queensland, Australia	QM#MO80743
	DB-TH159 on muddy sand at low tide, Dingo Beach, Whitsunday Islands, Queensland, Australia	QM#MO80744
Strombus dilatatus (Swainson, 1821)	Seabed material, GPS −24.386282 152.593168, 25.0 m depth, Australia, SBD#035346	QM#MO80144
	Seabed material, GPS −24.127022 152.212747, 34.7 m depth, Australia, SBD#026638	QM#MO80145
	Seabed material, GPS −24.467679 152.969458, 27.1 m depth, Australia, SBD#026756	QM#MO80146
	Seabed material, GPS −24.127022 152.212747, 34.9 m depth, Australia, SBD#026638	QM#MO80147
	Seabed material, GPS −20.935000 150.455000, 39.9 m depth, Australia, SBD#023154	QM#MO80148
	Seabed material, GPS −24.386282 152.593168, 25.0 m depth, Australia, SBD#035346	QM#MO80149
	Seabed material, GPS −24.066716 152.134289, 31.9 m depth, Australia, SBD#035215	QM#MO80150
	Seabed material, GPS −21.089767 150.936033, 31.9 m depth, Australia, SBD#026848	QM#MO80151
Subfamily Naticinae, Guilding 1834		
Genus *Tectonatica* Sacco, 1890		
Tectonatica sagraiana (Orbigny, 1842)	#47-1, #47-2, #47-6, #47-8, #47-9 Campese Bay, Isola del Giglio, 7–10 m depth	Huelsken et al. 2008
Tectonatica cf. *rizzae* (Philippi, 1844)	C82, C126, C127, C131, egg masses, Pt. delle Secche, Isola del Giglio, 18 m depth	Huelsken et al. 2008
Subfamily Sininae Wenz, 1941		
Genus *Sinum* Röding, 1798		
Sinum haliotoideum (Linnaeus, 1758)	Swan Island, Shoalwater Bay, Great Barrier Reef	AMS#C451594
Sinum sanctijohannis (Pilsbry & Lowe, 1932)	#35-1 Baja California, Mexico	MNHN#IM-2009-5162
Subfamily Polinicinae Gray, 1847		
Genus *Euspira* Agassiz in J. Sowerby, 1837		
Euspira nitida (Donovan, 1804)	#114-5 Cala dell´Allume, Isola del Giglio, 9 m depth	MNHN#IM-2009-5163
Euspira catena (Da Costa, 1778)	#127-2 Island of Terschelling, Netherlands	MNHN#IM-2009-5164
Euspira fusca (de Blainville, 1825)	#126-1 off Olhão, Portugal, dredged in 380–400 m depth	MNHN#IM-2009-5165
Euspira intricata (Donovan, 1804)	#120-1 Cala dell´Allume, Isola del Giglio, 6 m depth	MNHN#IM-2009-5166
Genus *Polinices* Montfort, 1810		
Polinices albumen (Linnaeus, 1758)	North of Fraser Island, -24.485999 153.100564, 29.3 m depth, SBD#026719	QM#MO80152
Polinices cumingianus (Récluz, 1844)	#96-1 Fraser Island, Great Barrier Reef, Australia	AMS C434459

Table 1 (continued)

Species, author	Collection site	Voucher number/ reference
	#96-2 South Passage, Shark Bay, Western Australia, Australia	MNHN#IM-2009-5169
Polinices mellosus (Hedley, 1924)	#59-3 Casuarina Beach, Lizard Island, Australia, 2–3 m depth	MNHN#IM-2009-5167
	#59-4 Casuarina Beach, Lizard Island, Australia, 2–3 m depth	MNHN#IM-2009-5168
Polinices flemingianus (Récluz, 1844)	#141-1 Vanuatu: Santo Island, Aore Island	MNHN#42645
Polinices mediopacificus (Kosuge, 1979)	Bohol Sea, off Palimacan Island, Philippines	MNHN#42646
Polinices peselephanti (Link, 1807)	#99-1, #99-2, #99-3 Marble Island, Duke Group, Great Barrier Reef, Australia	AMS#C451672
Polinices sp. 1	#51-4 (IM-2009-5174), #51-5 (IM-2009-5175), #51-7, #51-10, #51-11, #51-13, #51-14, #51-15, #51-16, #51-17, #51-18, #51-19, #51-20, #51-21, #51-22, #51-64, #70-18 Casuarina Beach, Lizard Island, Australia, water depth 2–3 m	MNHN#IM-2009-5174; MNHN#IM-2009-5175
	#DB-TH143, #DB-TH144, #DB-TH145, Dingo Beach, Whitsunday Islands, Australia, water depth 0–1 m	QM#MO80740
	#VM2.1-#VM2.5, Santo Island, Vanuatu	MNHN#42647
	#M5.1 , 9°35.5′N 123°43.3′E/123°44.3′E Panglao Island, Doljo Point, Philippines, 0–2 m soft mixed intertidal platform, fringe mangrove, seagrass	MNHN#M8.1
	#51-24 Nabq National Park, Sinai, Egypt, water depth 0–3 m, coral sand	QM#MO80741
Polinices sp. 2	#70-1 (IM-2009-5170), #70-2 (IM-2009-5171), #70-3, #70-9, #70-14, #70-16, #70-17 Casuarina Beach, Lizard Island, Australia, water depth 2–3 m	MNHN#IM-2009-5170; MNHN#IM-2009-5171
	#70-8 Latalata Island, West of Halmahera, Indonesia	QM#MO80746
Polinices sp. 3	#70-6, #70-10 , #70-12 on coral sand, water depth 2–3 m, Casuarina Beach, Lizard Island, Australia	QM#MO80747–QM#MO80749
Polinices sp. 4	#D1, #D3, #D4, #D5, #D6 (QM#MO80750) Between Town Beach and Entrance Point, Broome, WA, Australia, on muddy sand close to shoreline	QM#MO80750
Polinices uber (Valenciennes in Humboldt, 1832)	#30-1 Cholla Bay, Puerto Penasco, Sonora, Mexico	MNHN#IM-2009-5172
	#30-3 Cholla Bay, Puerto Penasco, Sonora, Mexico	MNHN#IM-2009-5173
Genus *Mammilla* Schumacher, 1817		
Mammilla caprae (Philippi, 1850)	#123-1 Cedros Island, Costa Rica, dredged at 400 m depth	MNHN#IM-2009-5178
Mammilla melanostoma (Gmelin, 1791)	#25-4 Casuarina Beach, Lizard Island, Australia, water depth 1 m	MNHN#IM-2009-5176
Mammilla melanostomoides (Quoy & Gaimard, 1832)	#87-2 Santo Island, Segond Channel, Vanuatu	MNHN#42649
Mammilla priamus (Récluz, 1844)	#07-1 Philippines	MNHN#IM-2009-5179
Mammilla simiae (Deshayes in Deshayes & Edwards, 1838)	#77-1 Bernier Iland, Western Australia, Australia	MNHN#IM-2009-5177
Genus *Neverita* Risso, 1826		
Neverita aulacoglossa (Pilsbry & Vanatta, 1908)	#95-1, #95-2, #95-3 QLD, Australia, Great Barrier Reef, Fraser Island, offshore of Sandy Point, S of Mooan 25° 19.945′S, 153° 0.524′ E. On & in sand, LT, sandbank	AMS#C412600
Neverita delessertiana (Récluz in Chénu, 1843)	#19-1, #19-3, #19-5, #19-9 Clearwater, Florida	Hülsken et al. 2006
	#19-11 Cedar Key, Florida	Hülsken et al. 2006
Neverita didyma (Röding, 1798)	Taiwan	Collin 2003
Neverita duplicata (Say, 1822)	#21-2, #21-3, #21-4 Intertidal ocean, Little Talbot Island, Jacksonville, Florida	Hülsken et al. 2006
Neverita lewisii (Gould, 1847)	#104-1 Bainbridge Island, Tecoma, Pudget Sound, Washington, USA, under rocks and pebbles	QM#MO80751

Table 1 (continued)

Species, author	Collection site	Voucher number/ reference
Neverita josephinia (Risso, 1826)	#46-4, #46-6, #46-16 Giglio Campese, Campese Bay, depth: ca. 10 m, on sand ground, Isola del Giglio, Toscana, Italy, water depth 10–12 m	Huelsken et al. 2008
Neverita reclusiana (Deshayes, 1839)	#33-1 Cholla Bay, Puerto Penasco, Mexico	Huelsken et al. 2008
Genus *Conuber* Finlay & Marwick, 1837		
Conuber conicus (Lamarck, 1822)	Great Sandy Strait, SE of Urangan 25°19.980´S, 152°55.933´E; in & on muddy sand, on sandflat, QLD, Australia	AMS#C412917
Conuber incei (Philippi, 1853)	Crowdy Head, S beach 31°52.290´S 152°42.250´E, NSW, Australia, intertidal sand, surfing beach	AMS#C399745
	Lennox Head Beach, access via Pacific Pde/Ross St. junction 28°47.200´S 153°35.600´E, 0–0.5 m sand in surf zone	AMS#C414237
Conuber sordidus (Swainson, 1821)	Dingo Beach, Queensland, Australia, crawling on muddy sand at low tide	QM#MO80752
	Careel Bay, Pittwater, NSW	AMS# EBU30442; Colgan et al. 2006

fragments (*COI*, *H3*) were analysed using the model NY98 implemented in MrBayes (triplet code: metmt) to consider differences in omega variation across sites (neutral/purifying/positive selection of positions). Ribosomal gene fragments were calculated using a model predicted by MrModeltest (Nylander 2004). The GTR+G+I model was used for the *16S* gene fragment, while the HKY85 model was used for the *18S* and *28S* gene fragments. Ambiguously aligned parts of the rRNA sequences were excluded from the analysis. All gene fragments were analysed as being unlinked. Phylogenetic analyses were performed separately for each gene fragment as well as for a combined data set (Figs. 2, 3 and 4, see Supplementary Figs. S1, S2). Bootstrap analysis of the molecular data set in the network analysis using *SplitsTree v4.0* was performed with 1,000 replicates using the LogDet model.

Conchological analyses and studies of type material

In order to identify the validity of shell characters used in species identification and generic classifications of *Polinices* species, we analysed 27 conchological and one developmental character (Table 5, Supplementary Table S1). The characters were chosen based on their usage in species descriptions and the fact that they had been proposed to vary between the analysed species (see Tryon 1886; Murray 1966; Cernohorsky 1971; Marincovich 1977; Majima 1989; Kabat 1996; Aronowsky 2003). The character states were coded binarily and plotted on the phylogenetic tree (concatenated data set, Fig. 2) to calculate the consistency index (CI) and the retention index (RI) for each character using MacClade v4.06 (Maddison and Maddison 2006). All characters were equally weighted (see Supplementary Table S1).

In order to address quantitative variations of similar trait values within species and between closely related species, continuous characters (e.g. protoconch size, ratio of height to width, number of embryonic whorls) were coded as 'ordered' while all remaining characters were coded 'unordered' (see Wiens 2001). The analysis was performed for species taxonomically assigned to *Polinices s.s.* as well as for the entire data set (*Polinices s.l.*).

In the course of our molecular phylogenetic analyses, we came across a number of white *Polinices* species, which differed from *P. mammilla* but for which an unequivocal taxonomic assignment was difficult. We analysed a subset of 16 conchological key characters (characters A–P) for each of these species and compared those with characters determined by investigating existing type specimens in museum collections (Tables 6, 7). Unfortunately, many type specimens of white *Polinices* species are missing. In these cases, type figures or type descriptions were used to extract available information on shell features.

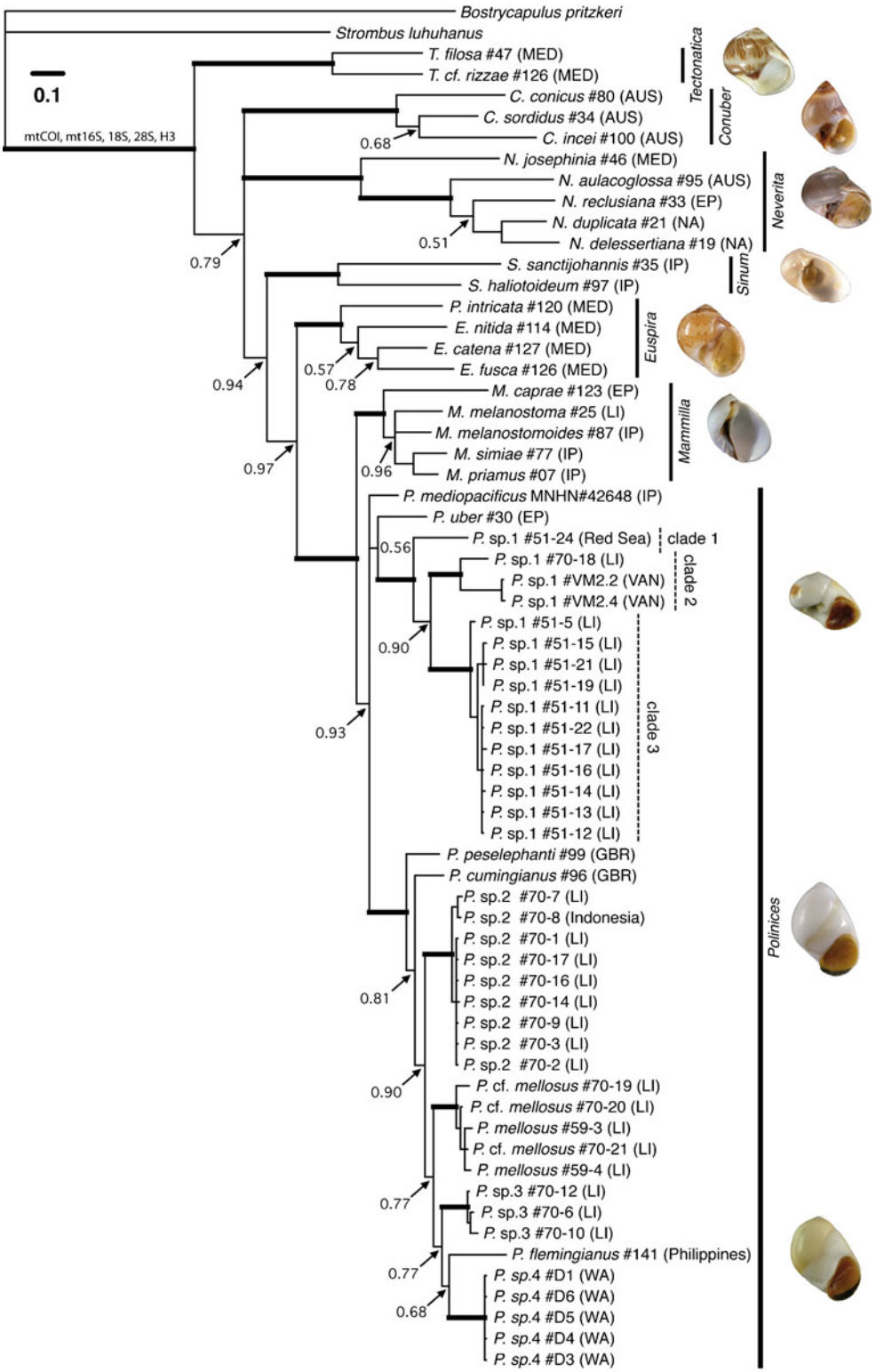

Fig. 2 Phylogram obtained through Bayesian inference based on the concatenated data set (*COI, 16S, 18S, 28S, H3*) for a reduced number of taxa. Posterior probabilities are indicated at the nodes. Branches supported by values>0.95 are indicated in *bold*. Polytomies are due to the cut-off value specified for the consensus tree (50 % used as the default value in MrBayes)

Shell height (h), shell width (w) and aperture height were measured from vertically positioned shells or from drawn and pictured shells (apex up and basal lip down; see Fig. 1). Further data was compiled by analyses of the shell form, shell colour, protoconch morphology, umbilicus morphology and operculum colouration (Tables 6, 7; Supplementary Table S1). Protoconch morphology was measured according to Solsona and Martinell (1999). The size of the first embryonic whorl (FEW) and the number of embryonic whorls (EW) were measured using a digital binocular. The data matrix was

Fig. 3 Phylogram obtained through Bayesian inference based on the *COI* gene fragment. Posterior probabilities are indicated at the nodes. Branches supported by values>0.95 are indicated in *bold*. Polytomies are due to the cut-off value specified for the consensus tree (50 % used as the default value in MrBayes)

Table 2 Arrangement of naticid taxa in the phylogenetic trees derived from different gene fragments. Bold taxa are supported by posterior probability> 0.95, capitalized taxa are arranged para- or polyphyletically

Gene fragment	Taxa arrangement
mtCOI	(SINUM(**Neverita**(**Tectonatica**(**Euspira**(**Conuber**(MAMMILLA, POLINICES))))))
mt16S rRNA	(MAMMILLA(Sinum, **Tectonatica**, **Euspira**, **Conuber**, **Neverita**)(Polinices))
ncH3	(Outgroup, NEVERITA(Tectonatica, CONUBER)(Euspira, CONUBER)Mammilla, Polinices)
nc28S rRNA	(Mammilla, POLINICES, **Conuber**)(Sinum, **Tectonatica**, EUSPIRA, **Neverita**)
nc18S rRNA	(Sinum(Tectonatica(**Conuber**(**Neverita**(MAMMILLA, POLINICES, Euspira)))))
ALL	(**Tectonatica**(**Conuber**, **Neverita**(**Sinum**(**Euspira**(**Mammilla**(**Polinices**))))))

uploaded to Morphobank project ID#189 (O'Leary and Kaufman 2007).

Results

Phylogenetic analyses

Partial sequences of two mitochondrial genes (*COI*, *16S*) and three nuclear genes (*28S*, *18S*, *H3*) were determined resulting in a concatenated alignment of 1,852 bp. In the phylograms, species were arranged into seven monophyletic groups, representing *Conuber*, *Euspira*, *Mammilla*, *Neverita*, *Polinices, Sinum* and *Tectonatica* (see Figs. 2, 3 and 4, Supplementary Figs. S1, S2). The assignment of the identified species to these monophyletic genera was similar in four single gene topologies (*COI*, *16S*, *28S*, *18S*) and the combined analysis (Table 2). However, the relationship of generic clades, especially *Mammilla* and *Euspira*, differed in the various tree analyses (Figs. S1, S2). The gene H3 showed low resolution, not all genera were recognized and analysis resulted in a comb-like topology (Fig. S1).

In the analysis of the concatenated data set (Fig. 2), *Tectonatica* presents the most basal naticid taxon followed

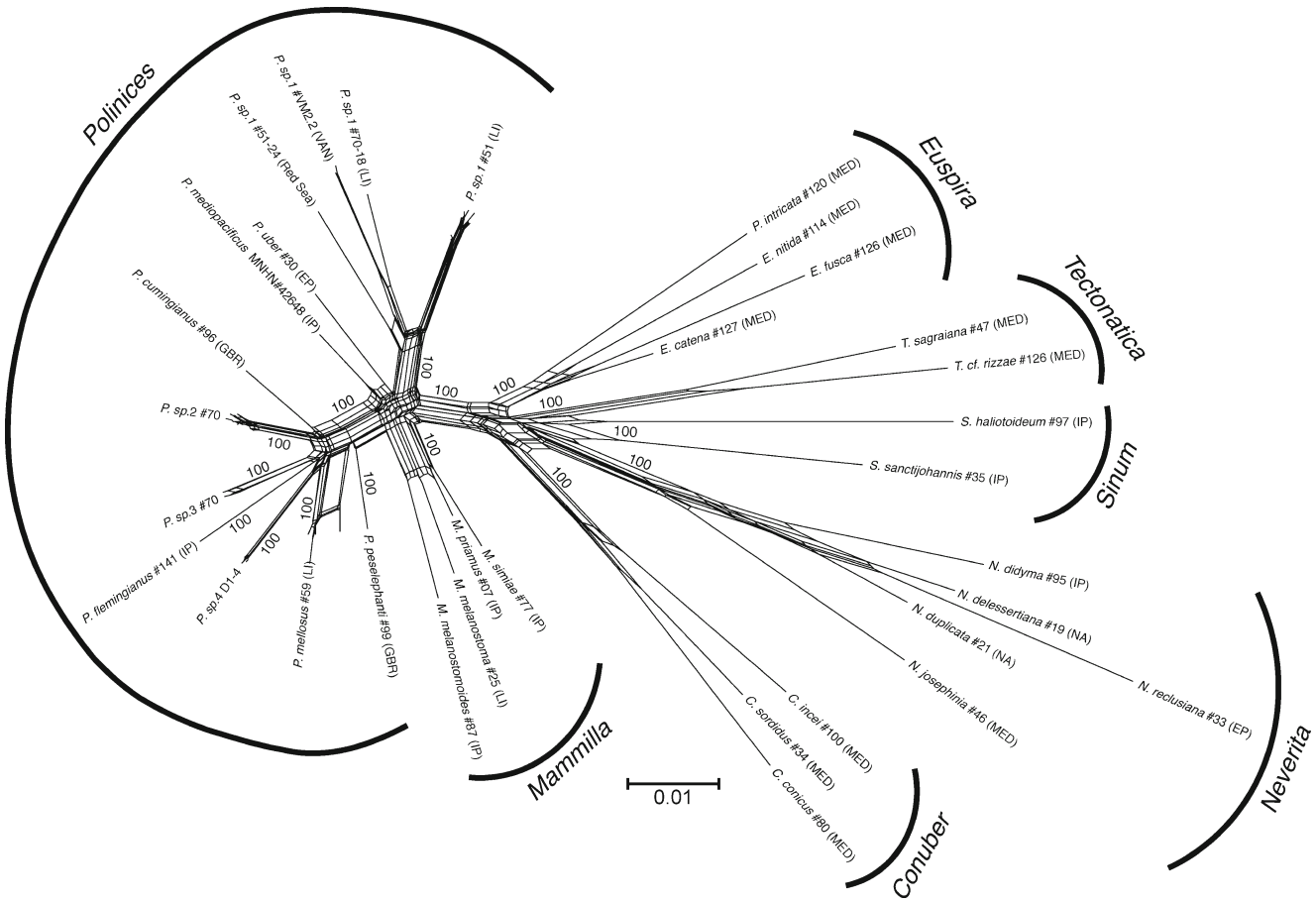

Fig. 4 NeighborNet network based on the concatenated data set (*COI, 16S, 18S, 28S, H3*). Bootstrap values are indicated

Table 3 Inter- (*normal*) and intra-specific (*bold*) genetic distances calculated for the analysed *Polinices* species based on the COI gene fragment (average ± standard deviation)

	P. alb (n = 1)	*P. cum* (*n* = 3)	*P. flem* (*n* = 1)	*P.* sp. 1 (*n* = 30)	*P. med* (*n* = 1)	*P. mel* (*n* = 5)	*P.* sp. 2 (*n* = 9)	*P.* sp. 3 (*n* = 3)	*P.* sp. 4 (*n* = 5)	*P. pes* (*n* = 3)	*P. ube* (*n* = 2)
P. albumen (*n* = 1)	–										
P. cumingianus (*n* = 3)	0.083 ± 0.000	**0.003 ± 0.003**									
P. flemingianus (*n* = 1)	0.071 ± 0.000	0.085 ± 0.000	–								
P. sp. 1 (*n* = 30)	0.111 ± 0.009	0.101 ± 0.008	0.106 ± 0.009	**0.089 ± 0.002**							
P. mediopacificus (*n* = 1)	0.089 ± –	0.081 ± 0.000	0.087 ± –	0.081 ± 0.004	–						
P. mellosus (*n* = 5)	0.065 ± 0.002	0.074 ± 0.002	0.064 ± 0.001	0.095 ± 0.010	0.085 ± 0.004	**0.003 ± 0.002**					
P. sp. 2 (*n* = 9)	0.082 ± 0.002	0.062 ± 0.004	0.075 ± 0.003	0.089 ± 0.012	0.075 ± 0.002	0.064 ± 0.002	**0.005 ± 0.006**				
P. sp. 3 (*n* = 3)	0.064 ± 0.006	0.071 ± 0.005	0.059 ± 0.004	0.092 ± 0.010	0.075 ± 0.004	0.058 ± 0.005	0.058 ± 0.005	**0.013 ± 0.003**			
P. sp. 4 (*n* = 5)	0.075 ± 0.001	0.082 ± 0.001	0.073 ± 0.001	0.111 ± 0.009	0.098 ± 0.001	0.071 ± 0.002	0.077 ± 0.003	0.062 ± 0.005	**0.001 ± 0.001**		
P. peselephanti (*n* = 3)	0.071 ± 0.001	0.0071 ± 0.001	0.076 ± 0.005	0.093 ± 0.011	0.062 ± 0.003	0.066 ± 0.002	0.067 ± 0.003	0.055 ± 0.005	0.079 ± 0.002	**0.001 ± 0.002**	
P. uber (*n* = 2)	0.087 ± 0.000	0.090 ± 0.001	0.092 ± 0.000	0.081 ± 0.009	0.062 ± 0.002	0.078 ± 0.001	0.074 ± 0.001	0.071 ± 0.004	0.099 ± 0.001	0.070 ± 0.003	**0.009 ± –**

Table 4 Inter- (normal) and intra-clade (bold) genetic distances calculated for clades 1–3 of *P.* sp. 1 based on the COI gene fragment (average genetic distance ± standard deviation)

	Clade 1	Clade 2	Clade 3
P. sp. 1 [clade 1]	–		
P. sp. 1 [clade 2]	0.089 ± 0.01	**0.077 ± 0.01**	
P. sp. 1 [clade 3]	0.086 ± 0.01	0.091 ± 0.01	**0.010 ± 0.02**

by a polytomy of *Conuber, Neverita* and a clade comprising *Sinum*, *Euspira*, *Mammilla* and *Polinices*. The sister taxa *Mammilla* and *Polinices* represent the most derived genera. The placement of *Sinum* as sister taxon to the clade *Euspira/Mammilla/Polinices* and even monophyly, is challenged in all the single gene analyses. Highest congruence with results obtained from the concatenated data set can be seen in the analysis of the *COI* gene (Table 2, Fig. 3).

The NeighborNet analysis of the concatenated gene fragments (Fig. 4) was congruent with the respective phylogenetic reconstructions. *Polinices* and *Mammilla* were well separated by distinct branches from all other monophyletic genera. There was a strong phylogenetic signal for the monophyly of *Mammilla,* but the taxon was nested within the *Polinices* species, rendering *Polinices* paraphyletic. Phylogenetic signal (recognizable in the long edges) for the monophyly of the genera *Conuber*, *Euspira*, *Neverita*, *Sinum* and *Tectonatica* was also high. However, a conflict was obvious in *Sinum*, represented here with only two species.

With few exceptions the grouping of species was identical in all phylogenetic reconstructions, placing species into supraspecific taxa according to their *a priori* taxonomic assignment (Figs. 2, 3 and 4, Supplementary Figs. S1, S2). However, our phylogenetic analyses revealed some unexpected species placements. First, individuals assigned to the Eastern Pacific species *Euspira lewisii* (Gould, 1847) grouped within the genus *Neverita* in the *COI* tree (Fig. 3). Second, our phylogenetic analyses revealed four conchologically similar, well-separated and highly supported plain-white species within *Polinices* (*P.* sp. 1 to *P.* sp. 4 in the following). *P.* sp. 1, *P.* sp. 2 and *P.* sp. 3 are very similar in shell structure and, at first glance, appear all to be referable to the common moon snail *P. mammilla* (Figs. 1, 5). They share a glossy, all-white shell, a closed-to-partly-open umbilicus and a honey-coloured, corneous operculum. The fourth all-white, glossy-shelled taxon (*P.* sp. 4) is distinguished only by an entirely black-coloured operculum.

Haplotype analyses of the mitochondrial cytochrome oxidase (*COI*) and *16S* gene fragments of 30 specimens of *P.* sp. 1 resulted in a strict separation of the specimens into three clades reflecting different localities (Fig. 3, Supplementary Fig. S1). The specimen in branch 1 was collected in Egypt (Nabq National Park, Sinai; #51–24), the specimens in clade 2 were collected in Indonesia (#M5.1), Lizard Island (Queensland,

Table 5 Results of the conchological analyses performed with MacClade v4.06 for the entire data set (*Polinices s.l.*) and for the reduced data set (*Polinices s.s.*) listed for each character. *Type* Type of coding (*o* ordered; *u*, unordered), *States* number of morphological states; *Steps* total number of steps in the phylogenetic tree, *CI* consistency index, *RI* retention index, *EW* embryonal whorls, *FEW* first embryonal whorl

			Polinices s.l.				*Polinices s.s.*			
	Character	Type	States	Steps	CI	RI	States	Steps	CI	RI
1	Protoconch color	u	2	6	0.17	0.81	2	1	1.00	1.00
2	No. of EW	o	8	24	0.28	0.81	7	9	0.78	0.97
3	Size of FEW	o	8	35	0.21	0.72	8	20	0.39	0.83
4	Shell color	u	3	9	0.22	0.56	3	4	0.50	0.50
5	Color pattern	u	2	6	0.17	0.58	2	2	0.50	0.00
6	Shell shape (ratio height to width)	o	4	12	0.25	0.53	3	6	0.33	0.00
7	Aperture height ratio	o	5	11	0.36	0.84	2	2	0.50	0.00
8	Suture	u	2	4	0.25	0.67	2	0	0.00	0.00
9	Subsutural wrinkels	u	2	1	1.00	1.00	1	0	0.00	0.00
10	Umbilicus	u	3	16	0.12	0.48	3	8	0.22	0.42
11	Sulcus	u	2	3	0.33	0.00	2	2	0.50	0.00
12	Funicle	u	2	4	0.25	0.57	1	0	0.00	0.00
13	Umbilical callus	u	2	1	1.00	1.00	1	0	0.00	0.00
14	Inner lip	u	2	5	0.20	0.69	1	0	0.00	0.00
15	Operculum surface	u	2	1	1.00	1.00	1	0	0.00	0.00
16	Operculum color	u	4	4	0.50	0.71	2	1	1.00	1.00
17	Operculum size	u	3	2	1.00	1.00	1	0	0.00	0.00
18	Shell solidity	u	2	4	0.25	0.25	1	0	0.00	0.00
19	Shell texture	u	2	3	0.33	0.33	1	0	0.00	0.00
20	Aperture size	u	2	4	0.25	0.75	1	0	0.00	0.00
21	Aperture shape	u	3	7	0.29	0.58	1	0	0.00	0.00
22	Parietal callus	u	2	1	1.00	1.00	1	0	0.00	0.00
23	Parietal callus thickness	u	2	4	0.25	0.63	1	0	0.00	0.00
24	Posterior apertural angle	u	2	5	0.20	0.73	1	0	0.00	0.00
25	Parietal callus merge	u	3	5	0.40	0.81	1	0	0.00	0.00
26	Parietal callus size	u	2	5	0.33	0.78	1	0	0.00	0.00
27	Whorl expansion	u	2	5	0.20	0.56	2	2	0.50	0.00
28	Egg mass structure	u	2	1	1.00	1.00	1	0	0.00	0.00

Australia; #70–18) and Vanuatu (VM2.1–2.5) and those in clade 3 at the Great Barrier Reef, Australia (Lizard Island, Whitsunday Islands).

Sequence distances of the mitochondrial COI gene fragments

The lowest genetic distance (uncorrected p-distance) was observed between species of *Polinices* and *Mammilla* (9.0 % ± 1.0). Other comparisons of species in distinct genera have genetic distances ranging from 13 % ± 2.0 to 16 % ± 2.0. Notably, these values did not reflect any subfamilial assignment of the species: intra- and inter-subfamilial distances are similar between genera of different subfamilies.

Within *Polinices*, species showed p-distances of 5.5 % ± 0.5 [*P. peselephanti* (Link, 1807) - *P.* sp. *3*) to 11.1 % ± 0.9 (*P.* sp. 1 - *P. albumen* (Linnaeus, 1758)]. *P.* sp. 1 had the largest genetic distance to the remaining *Polinices* species. The species is closely related to *P. mediopacificus* (Kosuge, 1979) (8.1 % ± 0.4) and *P. uber* (Valenciennes in Humboldt, 1832) (8.1 % ± 0.9) and had p-distances ranging from 8.9 % to 11.1 % to remaining *Polinices* species (Table 3). Intra-specifically, specimens of *P.* sp. 1 differed in 8.6 % ± 1.0 to 9.1 % ± 1.0 genetic distance (Table 4). Clade 2 within *P.* sp. 1 showed a high intraspecific average p-distance of 7.7 % ± 0.5 and member specimens comprised collecting sites with wide geographic distribution (Vanuatu–Philippines–UK). However, specimens from Vanuatu in clade 2 showed no genetic divergence at all (VM2.1–5). Similarly, the 21 specimens of *P.* sp.1 in clade3 from the Great Barrier Reef showed only 1.0 % ± 1.5 genetic distances (Whitsunday Islands– Lizard Island). By contrast, *P.*

Table 6 Morphological shell characters and their ranges, of *Polinices* molecularly analysed in this study. Numbers in *brackets* refer to character numbers used in the prior conchological analysis (see Table 5). *A* Protoconch colour: W, white; B, black; Br, brown. *B* Number of protoconch whorls. *C* Size of first whorl of protoconch; in μm. *D* Shell colour: W, white; Y, cream-yellowish; W(+C), white background, occasionally with faint brownish pattern; B, distinct brownish colour pattern. *E* Shell shape: ratio of shell height to shell width; *F* Shell solidity: M, thick and massive. *G* Operculum: C, corneous. *H* Operculum size: A, same size as aperture. *I* Operculum colour: B, black; H, honey-coloured; HB, honey-coloured with black streak; B, black. *J* Aperture size: 1, < 60 % of shell height; 2, 60-70 % of shell height; 3, 70-80 % of shell height. *K* Funicle: 1, funcicle prominent; 0, funicle not discernible. *L* Umbilical structure: 0, umbilicus open; 1, umbilicus fully closed, 2, umbilicus partly closed, leaving a distinct opening anteriorly. *M* Columellar shape: S, straight columella. *N* Transition of parietal callus to umbilical callus: 1, same width; 2, narrowing. *O* Parietal callus: T, thick. *P* Distribution: *IP* Indo-Pacific, *EP* Eastern Pacific

Species	Characters														
	A (1)	B (2)	C (3)	D (4)	E (6)	F (18)	G (15)	H (17)	I (16)	J (20)	K (12)	L (10)	M (14)	N (25)	O (23)	P
P. albumen	W	1.50–1.75	570 ± 30	O	0.71	M	C	A	H	0.70	1	1	S	1	T	IP
P. cumingianus	W	1.75	750 ± 70	W+C	1.04 ± 0.09	M	C	A	H	0.59 ± 0.02	1	0	S	2	T	IP
P. mellosus	W	1.25–1.45	690 ± 20	Y	1.20 ± 0.06	M	C	A	H	0.59 ± 0.08	0	1	S	1	T	IP
P. flemingianus	W	1.20	640	W	1.12	M	C	A	Hb	0.55	0	1/2	S	1	T	IP
P. mediopacificus	Br	2.50	560	W	1.02	M	C	A	H	0.65	0	0	S	1	T	IP
P. sp. 1	B	2.00–2.25	370 ± 67	W	1.29 ± 0.07	M	C	A	H	0.67 ± 0.07	0	0.7	S	1	T	IP
P. sp. 2	W	1.25–1.50	770 ± 60	W	1.27 ± 0.1	M	C	A	H	0.71 ± 0.06	0	1/2	S	1	T	IP
P. sp. 3	W	1.25–1.50	660 ± 60	W	1.28 ± 0.02	M	C	A	H	0.67 ± 0.04	0	1	S	1	T	IP
P. sp. 4	W	0.90–1.15	870 ± 70	W	1.09 ± 0.03	M	C	A	B	0.82 ± 0.01	0	1/2	S	1	T	IP
P. uber	Br	2.35–2.75	720 ± 10.0	W	1.26 ± 0.03	M	C	A	H	0.68 ± 0.04	0	2	S	1	T	EP
P. peselephanti	W	1.75	1,250	W+C	1.09 ± 0.02	M	C	A	H	0.60	1	0	S	2	T	IP

Table 7 Morphological shell characters and their range distribution, of *P.* sp. 1 through *P.* sp. 4 as well as possible name-bearing type specimens. Numbers in brackets refer to character numbers used in the preceding conchological analysis (see Table 5). *A* Protoconch colour: W, white; B, black; Br, brown. *B* Number of protoconch whorls. *C* Size of first whorl of protoconch; in µm. *D* Shell colour: W, white; Y, cream-yellowish; W(+C), white background, occasionally with faint brownish pattern; B, distinct brownish colour pattern. *E* Shell shape: ratio of shell height to shell width; F Shell solidity: M, thick and massive. *G* Operculum: C, corneous. *H* Operculum size: A, same size as aperture. *I* Operculum colour: B, black; H, honey-coloured; HB, honey-coloured with black streak; B, black. *J* Aperture size: 1, < 60 % of shell height; 2, 60-70 % of shell height; 3, 70-80 % of shell height. *K* Funicle: 1, funcicle prominent; 0, funicle not discernible. *L* Umbilical structure: 0, umbilicus open; 1, umbilicus fully closed, 2, umbilicus partly closed, leaving a distinct opening anteriorly. *M* Columellar shape: S, straight columella. *N* Transition of parietal callus to umbilical callus: 1, same width; 2, narrowing. *O* Parietal callus: T, thick. *P* Distribution: *IP* Indo-Pacific; *EP* Eastern Pacific

Species	Characters															
	A (1)	B (2)	C (3)	D (4)	E (6)	F (18)	G (15)	H (17)	I (16)	J (20)	K (12)	L (10)	M (14)	N (25)	O (23)	P
Polinices sp. 1	B	2.00–2.75	370 ± 67.0[a]	W	1.29 ± 0.07[a]	M	C	A	H	0.67 ± 0.07[a]	0	1/2	S	1	T	IP
Polinices sp. 2	W	1.25–1.50	770 ± 60.0[a]	W	1.27 ± 0.1[a]	M	C	A	H	0.72 ± 0.06[a]	0	1	S	1	T	IP
Polinices sp .3	W	1.25–1.50	660 ± 60.0[a]	W	1.28 ± 0.02[a]	M	C	A	H	0.65 ± 0.03[a]	0	1/2	S	1	T	IP
Polinices sp. 4	W	0.90–1.15	870 ± 70.0[a]	W	1.09 ± 0.03[a]	M	C	A	B	0.82 ± 0.01[a]	0	1	S	1	T	IP
Nerita mammilla Linnaeus, 1758	–	ind.	ind.[e]	W	1.26[b]	M	?	A	?	0.65[b]	0	1	S	1	T	?
Mammillaria tumida Swainson, 1840 [Mamma albula Chemnitz, 1781 [non-binomial]	–	ind.	ind.	W	1.28[b]	M	?	A	?	0.51[b]	0	1	S	1	T	?
Natica candidissima Le Guillou, 1842	?[d]	?	?	W	?	M	C	A	H	?	0	0	S	1	T	IP
Natica pyriformis Recluz, 1844	B	2.25–2.50[a]	410 ± 10.0[a]	W	1.14 ± 0.06[a]	M	?	A	?	0.61 ± 0.06[a]	0	1	S	1	T	IP
Natica dubia Récluz, 1844	W	1.15–1.25[a]	656 ± 97.0[a]	W	1.00 ± 0.06[a]	M	?	?	?	0.59 ± 0.02[a]	0	2	S	1	T	?
Natica cygnea Philippi, 1850	?	?	?	W	1.24[c]	M	?	?	?	0.61[c]	0	1	S	1	T	?
Natica virginea Philippi, 1850	?	?	?	W	1.21[c]	M	?	?	?	0.65[c]	0	2	S	1	T	?
Natica galactites Philippi, 1851	?	?	?	W	1.10[c]	M	?	?	?	0.68[c]	0	2	S	1	T	IP
Natica deiodosa Reeve, 1855	W	1.25–1.50[a]	740 ± 40.0[a]	Y	1.03 ± 0.14[a]	M	C	A	H	0.61 ± 0.07[a]	0	1/2	S	1	T	IP
Natica jukesii Reeve, 1855	W	1.50–1.75[a]	790 ± 50.0[a]	W	1.01 ± 0.04[a]	M	C	A	H	0.58 ± 0.05[a]	0	1/2	S	1	T	IP
Natica phytelephas Reeve 1855	W	2.00[b]	820[A]	W	1.01 ± 0.03[a]	M	?	?	?	0.66 ± 0.04[a]	0	0	S	1	T	IP
Natica vavaosi Reeve, 1855	?	?	?	W	1.16[B]	M	?	?	?	0.67[B]	0	2	S	1	T	IP
Polinices controversus Pritchard & Gatliff, 1913	W	1.75[b]	1.500[b]	W	0.90[b]	M	?	?	?	0.62[b]	1	2	S	2	T	IP
Polinices mellosus (Hedley, 1924)	W	1.25–1.50[a]	770 ± 10.0[a]	Y	1.05 ± 0.06[a]	M	C	A	B	0.58 ± 0.09[a]	0	1/2	S	1	T	IP
Polinices putealis Garrard, 1961	Br	1.75[b]	320[A]	W	1.12 ± 0.06[a]	M	?	?	?	0.59 ± 0.03[a]	0	0	S	1	T	AU
Polinices tawhitirahia Powell, 1965	W	1.75[b]	622[b]	W	1.02[b]	M	C	A	B	0.66[b]	0	1/2	S	1	T	NZ

[a] Average values for n ± 2 (sampled specimens of *P.* sp. 1–*P.* sp. 4; for types: holotype plus paratypes, or syntypes)

[b] Values from holotype only

[c] Values measured for figured type

[d] Data unknown

[e] Indeterminate (i.e., broken shell)

cumingianus (Récluz, 1844), *P. mellosus* (Hedley, 1924), *P. uber, P.* sp. 2, *P.* sp. 3 and *P.* sp. 4 had low intra-specific divergence ranging from 0.1 % ± 0.2 to 1.3 % ± 0.3 (Table 3), even between specimens of the same species collected from widely separated localities (e.g. *P.* sp. 2 collected from the Great Barrier Reef and Indonesia). Thus, the genetic divergence between the *P.* sp. 1 clades was similar or even higher than the divergence between other taxonomically distinct *Polinices* species (see Tables 3 and 4).

Conchological analysis

Our conchological analysis for the entire set of taxa (*Polinices s.l.*) revealed low CI and medium to high RI values for many shell characters (Table 5). Only five characters (9, 13, 15, 17, 22, 28) were identified with autapomorphic features, separating *Tectonatica* (9, 15), *Sinum* (13, 17, 22), *Conuber* (28) or *M. caprae* (17) from the remaining species. As they were the only members of the Naticinae in this study, only the *Tectonatica* species show subsutural wrinkles (9) and, obviously, a calcareous, white operculum (15). The shells of *Sinum* species differ from the remaining genera by the absence of an umbilical callus and a parietal callus (13, 22) and a strongly reduced operculum (17). *Conuber* is the only naticid genus whose members are known to produce gelatinous, sand-free egg masses (28) instead of a sand collar.

Other characters (colour of protoconch, number of embryonic whorls, aperture height/total height ratio, morphology of the suture, colouration of the operculum, aperture size and shape, thickness, shape, size and transition of the parietal callus) showed low to medium CI values ranging from 0.12 to 0.40 and medium to high RI values ranging from 0.25 to 0.84 (see Table 5).

Low CI and low/medium RI values were calculated for shell colour and colour pattern, funicle morphology, shell solidity, and shell texture (Table 5). Most of these characters showed overlapping states in particular in *Mammilla*, *Sinum* and *Neverita* species (e.g. depressed shell, spiral grooves) despite the fact that these groups were not directly related to each other in the phylogenetic reconstruction (see Figs. 2, 3 and 4).

When character analysis was applied to *Polinices s.s.* only, the results clearly demonstrated that only a few discrete shell characters differ between the species while many characters are identical or missing (Table 5). Only two characters (1, 16) were identified with autapomorphic states, separating *P.* sp. 1, *P. uber*, *P. mediopacificus* (1), *P. flemingianus* and *P.* sp. 4 (16) from the remaining species. Character 1 united *P.* sp. 1, *P. uber* and *P. mediopacificus*, which have brownish-to-black protoconchs. *P. flemingianus* features a black streak on its honey-coloured operculum while *P.* sp. 4 has an entirely black operculum (16). Of the remaining features, the number of embryonic whorls and the protoconch size showed low/medium CI and high RI values. Other characters, such as shell colour, colour pattern, umbilical morphology, ratio of total height to aperture height and whorl expansion showed low/medium CI and low RI values in *Polinices s.s.*

Protoconch colour, the size of the first embryonic whorl and number of protoconch whorls were observed to show little intra-specific variability (see Table 6). However, variations in protoconch whorl size ranged from 5 % to 25 % in most species (e.g. *P.* sp. 1, *P.* sp. 2, *P.* sp. 3, *P.* sp. 4, *P. albumen*) for which more than three specimens had been analysed (see Tables 6 and 7).

By contrast, shell colour, shell shape and umbilicus morphology were observed to vary strongly between adult and juvenile specimens. Adult specimens of *P.* sp. 1, *P.* sp. 2, *P.* sp. 3 and *P. mellosus* predominantly possessed a closed umbilicus and a pyriform shell shape (R[w/h]>1.1). Ratio of height to width (R[h/w]) and umbilical morphology, however, were observed to differ considerably within adults and between adult and juvenile specimens as juvenile specimens of *P. mellosus* and *P.* sp. 2 from Lizard Island have a globose shell (R[h/w]=0.9–1.1) and a partially open umbilicus with an anterior cleft-like opening (Supplementary Fig. S3).

P. mellosus and *P.* sp. 2 feature identical shells (Table 6) with the yellowish-cream shell colouration in *P. mellosus* as the only differentiating character. However, shell colouration in *P. mellosus* is less intense, covers parts of shells only or is missing entirely in juvenile specimens, thus impeding clear species identification (Supplementary Fig. S3). Similarly, it is known that *P. cumingianus* can feature a considerable range of colouration from faint brownish horizontal bands to an entirely brown shell and shows a large size range of the umbilical callus (Cernohorsky 1971). Despite the fact that those characters were described to differentiate *P. cumingianus* from *P. peselephanti*, both species can identified reliably only by differences in protoconch size (Table 6).

Identification of white *Polinices s.s.* species

Using the data from our conchological analyses, we were able to identify most of the phylogenetically determined *Polinices* species based on a subset of conchological characters or by at least one discrete conchological character typical to a certain species (characters A–P; Table 6). Thus, taxonomic assignment to valid species was possible for most *Polinices* species in the phylogenetic analyses, such as *P. albumen, P. cumingianus, P. flemingianus, P. mediopacificus, P. mellosus, P. peselephanti, P. uber* (Table 6). Our taxonomic assignments are in agreement with most previously published species descriptions (e.g. Marincovich 1977; Majima 1989; Kabat 2000).

The three "*mammilla*"-like taxa *P.* sp. 1, *P.* sp. 2 and *P.* sp. 3 as well as *P.* sp. 4 differed in colouration and size of the first

whorl of the protoconch ("first embryonic whorl", FEW), the total number of embryonic whorls of the protoconch (EW) and the colouration of the operculum. All *P.* sp. 1 specimens had a black protoconch with 2.25–2.75 EW and a FEW of 370 ± 67.0 μm, *P.* sp. 2 specimens showed a white protoconch with 1.25 EW and a FEW of 660 ± 60.0 μm and *P.* sp. 3 specimens had a slightly larger white protoconch with 1.25–1.50 EW and a FEW of 775 ± 60.0 μm. The protoconch morphologies of the latter two species are therefore virtually identical to those of *P. flemingianus* (1.25 EW, FEW = 640 μm), *P. mellosus* (1.25 EW, FEW = 690 ± 17.0 μm), *P. cumingianus* (1.75 EW, FEW = 700 μm) and *P. mediopacificus* (1.25 EW, FEW = 680 μm) and were even similar to *P.* sp. 4 (0.9–1.15 EW, FEW = 870 ± 70.0 μm) (Table 6).

However, *P.* sp. 2 and *P.* sp. 3 specimens clearly differed from *P. flemingianus* by the colouration of the operculum (brown with a black streak in *P. flemingianus*, honey-coloured in *P.* sp. 2 and *P.* sp. 3) and from *P. mellosus* by the shell colour (*P. mellosus*: cream-coloured to yellowish; *P.* sp. 2 and *P.* sp. 3: purely white). Due to identical shell and overlapping protoconch morphology, *P.* sp. 2 and *P.* sp. 3 could not be differentiated unambiguously from each other (Table 6) while *P.* sp. 4 could be differentiated from *P.* sp. 1, *P.* sp. 2, *P.* sp. 3 and *P. flemingianus* by its slightly larger white FEW (870 ± 70.0 μm), a slightly smaller EW (0.9–1.15) and its entirely black operculum.

Taxonomic considerations of *P.* sp. 1 through *P.* sp. 4

Our conchological analyses clearly demonstrated that species assigned to *Polinices s.s.* are very similar with regard to shell characters as they usually are characterized by plain white or monochrome glossy, ovate-shaped shells, overlapping protoconch features, a honey-coloured to black corneous operculum, a medium to thick parietal callus and a partly or completely filled umbilicus (see Fig. 1). Probably as a consequence of the intra-specific variation of these features and striking inter-specific similarities of recent *Polinices* species (e.g. partly and completely filled umbilici), a large number of species with questionable taxonomic status have been described to date: at least 55 plain white *Polinices* species have been proposed or described, including 21 from the Indo-Pacific region, 10 with unknown type locality and 24 from regions other than the Indo-Pacific (Supplementary Table S2). Many of these taxa are now regarded as junior synonyms of other *Polinices* species (Tryon 1886; Cernohorsky 1971; Marincovich 1977; Kabat 2000).

Our conchological analyses of type species revealed *P.* sp. 1 to be conspecific with *P. mammilla* based on the colouration and size of the protoconch, while *P.* sp. 2 and *P.* sp. 3 are considered to be referable to *P. jukesii* (Reeve, 1855) and *P. dubius* (Récluz, 1844), respectively. As *N. dubia* Recluz, 1844, however, is junior homonym of the fossil species *Natica dubia* Römer, 1836 we herewith introduce a replacement name for *Natica dubia* Recluz, 1844, *Polinices constanti* Huelsken and Hollmann, to maintain taxonomic stability.

However, we emphasise that the assignment of *P.* sp. 2 and *P.* sp. 3 to *P. jukesii* and *P. constanti* is preliminary as the existing types in the dry collection of the NHM (London) unfortunately cannot be analysed with molecular methods due to the lack of preserved tissue. *P.* sp. 4, by contrast, is similar to *P. tawhitirahia* Powell, 1965 based on the colouration of the operculum and the size of the protoconch. Detailed species descriptions and discussions of these four species follow below.

Family *Naticidae* Guilding, 1834
Subfamily *Polinicinae* Gray, 1847

Genus *Polinices* Montfort, 1810
Polinices mammilla (Linnaeus, 1758) [= *Polinices* sp. 1 in the preceding discussion]—Figs 1k, 5a

Nerita mammilla Linnaeus, 1758; Syst. Nat. ed. 10, pl. 52 [*fide* Kabat 1990]

+„Mamma albula Chemnitz, 1781“; Syst. Conch. Cab., 5: 280, pl. 189, Figs. 1928–31, (non binomial)

+*Albula mammilla* Röding, 1798; Mus. Bolten., p. 20, (ref. Chemnitz, op. cit., Figs. 1928–31)

+*Mammillaria tumida* Swainson, 1840; Treat. Malac. p. 345 (ref. Chemnitz, op. cit. Figs. 1928–31)

+*Natica pyriformis* Récluz, 1844; Proc. Zool. Soc. Lond. pt. 11: 211

+*Natica albula* Récluz, 1851; J. Conchyl. 2(2): 194 (ref. Rumphius), pl. 22 Fig. E)

+*Natica ponderosa* Philippi, 1849; Syst. Conch. Cab. 2nd ed. 2(1): 32 pl. 4, Figs. 9–10

+*Natica cygnea* Philippi, 1850; Syst. Conch. Cab. 2nd ed. 2(1): 80, pl. 12, fig. 6

Natica mammilla (Linnaeus, 1758); Reeve (1855), Conch. Icon., pl. 7, fig. 27

Natica mammilla (Linnaeus, 1758); Sowerby (1883), Thes. Conchyl., 5: 85, pl. 3, Figs. 28–30

Polinices (*Polinices*) *mammilla* (Linnaeus, 1758); Lass, Bern. P. Bish. (1943), Mas. Bull., 119: 210, pl. 36, Figs. 4–5

+*Polinices pyriformis* (Récluz, 1844); Habe & Kosuge (1967), Stand. book Jap. shells col., 3: 45, pl. 18, fig. 7

+*Polinices* (*Polinices*) *tumidus*, (Swainson, 1840); Cernohorsky (1971), Rec. Auckland. Inst. Mus., 8 (December) p.190, Figs. 49–50

P. mammilla (Linnaeus, 1758); Torigoe and Inaba (2011), sp. 93, pp. 37–38.

Description

Shell Shell up to 60 mm in height, ovate-pyriform to pyriform, glossy white, occasionally with light, ill-defined

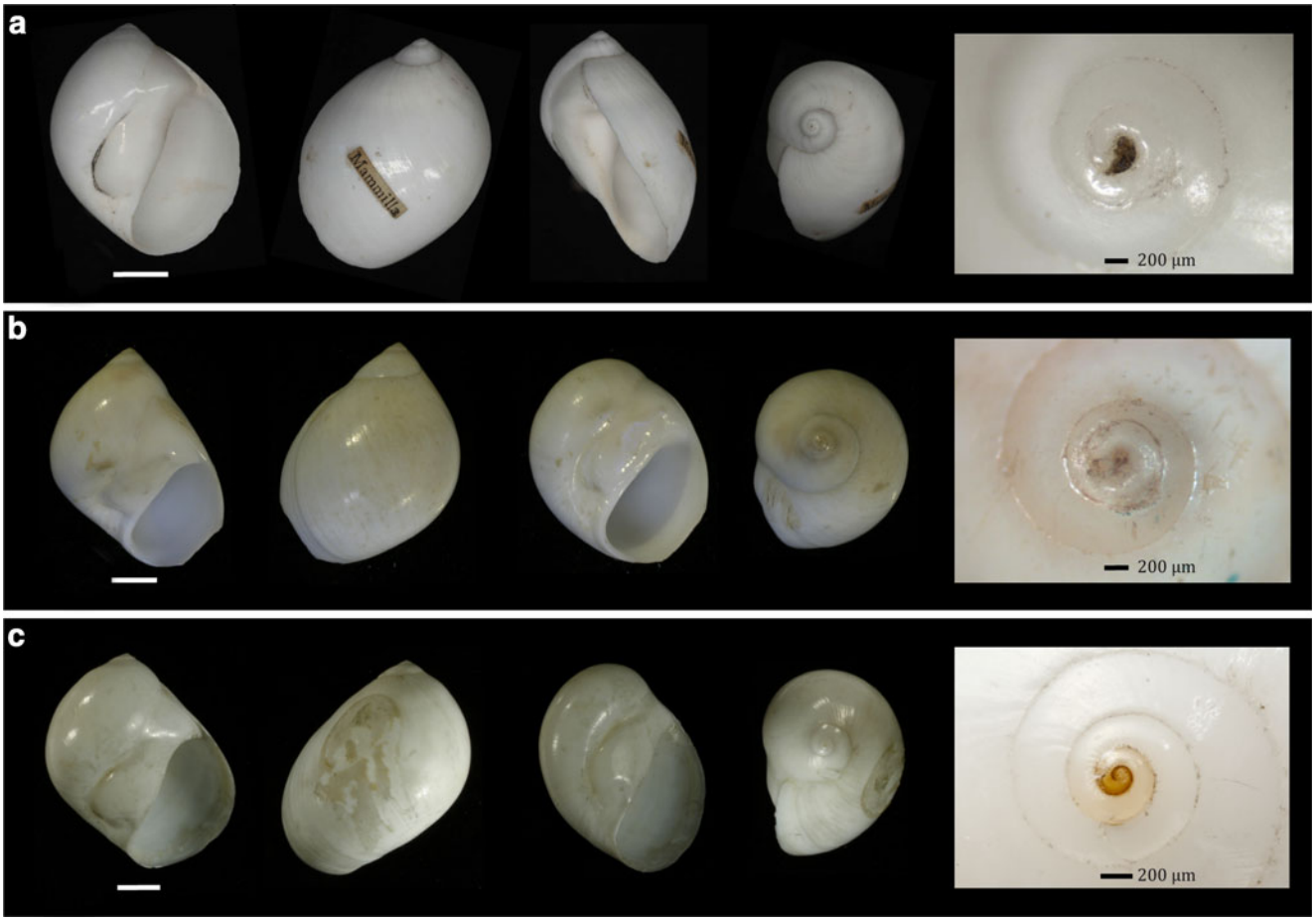

Fig. 5 Pictures of type specimens and protoconchs of **a** *Nerita mammilla* Linnaeus, 1758 [ZMUU#386] **b** Mamma albula Chemnitz, 1758 [non-binomial, ZMUC] and **c** *Natica pyriformis* Recluz, 1844 [BMNH#1991089.1]. For further information see Table 1. *Bars* 0.5 cm

brownish striae or brownish spots on the shoulder of the body-whorl, giving it a rusty appearance. Ratio of shell height to shell width 1.29 ± 0.07 in specimens analysed in this study (n=30) and 1.26 in the lectotype (n=1). Aperture wide and semi-ovate, ratio from aperture height to total height 0.67 ± 0.07 in analysed specimens (n=30) and 0.65 in the lectotype specimen (n=1). Umbilicus completely covered by a heavy callus in adult specimens; a small anterior umbilical groove may be present in juveniles but also in adult specimens. Parietal callus extends into umbilical callus without sulcus.

Protoconch Brownish to black, 2.00–2.25 whorls, size of first embryonic whorl 370 ± 67 µm in specimens analysed in this study (n=30). Protoconch in lectotype broken (see discussion below).

Operculum Corneous, light brown in colour.

Foot Propodium white and long (>2 times shell). Mesopodium white, overlapping the protoconch, leaving only a quarter of the shell surface visible.

Distribution Indo-west Pacific to Easter Island, Red Sea.

Differential diagnosis P. mammilla can distinctly be differentiated from any other Indo-west Pacific white *Polinices* species by its smaller and black protoconch with at least 2.00 embryonic whorls (EW=2.00–2.25, FEW= 370 ± 67 µm).

Material examined Type specimens of *Nerita mammilla* (ZMUU#769), *Mammillaria albula* [= Mamma albula, non-binomial] [ZMUC (Cernohorsky 1974)] and *Natica pyriformis* (BMNH#1991089.1-3, BMNH#1845.6.24.56-58, MHNG#2017, MHNG#2018). For molecularly and morphologically analysed specimens see Table 1.

Discussion Based on the black protoconch and the pyriform white shell, *P.* sp. 1 is identical to the description of a ZMUU specimen (#769) designated as lectotype for *Nerita mammilla* Linnaues, 1758 by Kabat (Kabat 1990). Conchological analyses of type material of *Nerita mammilla* and synonymized species, however, have revealed new

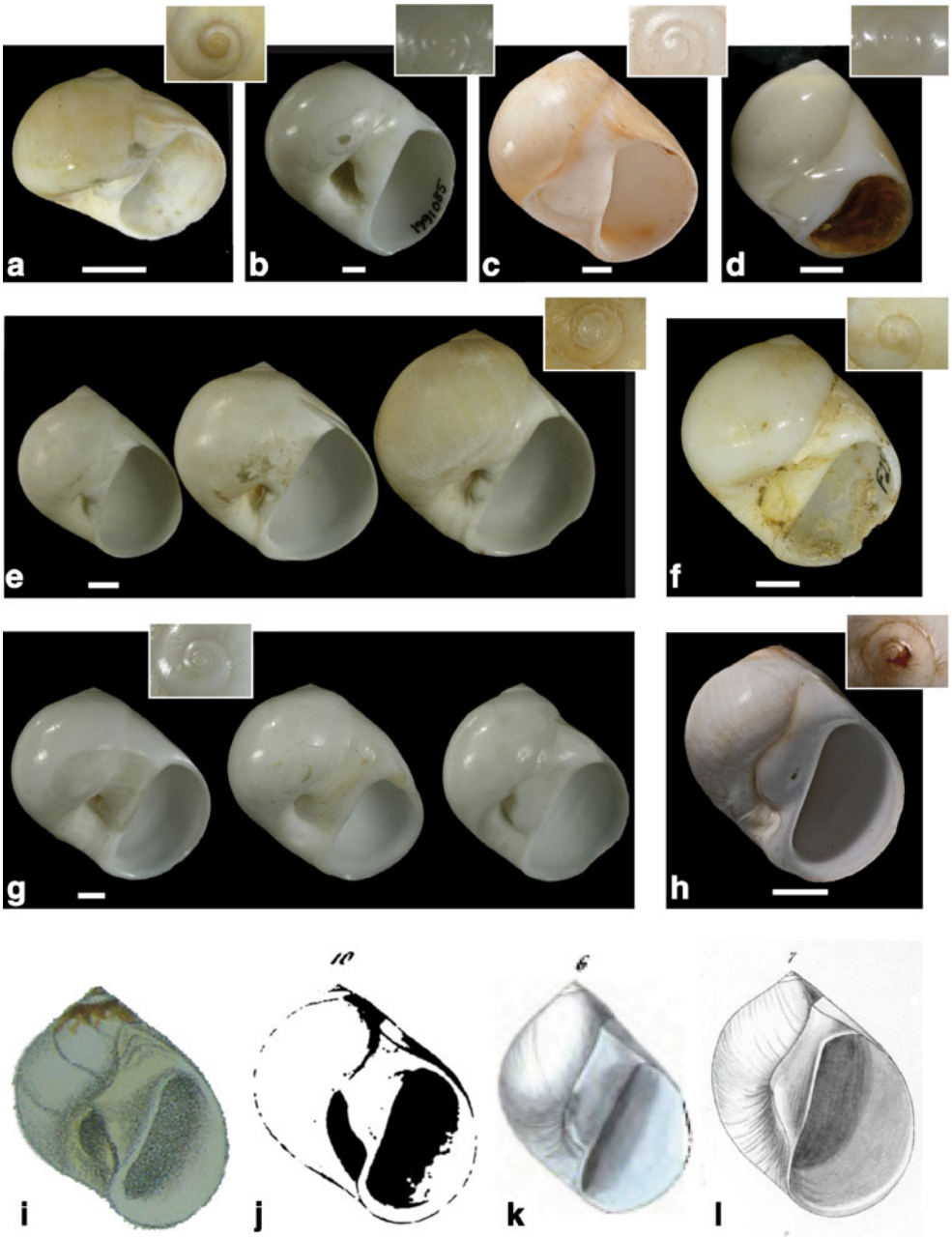

Fig. 6 Analysed type specimens or figured type specimens of taxa that could potentially represent *Polinices* sp. 2, *Polinices* sp. 3 or *Polinices* sp. 4. **a** *Natica controversa* Pritchard & Gatliff, 1913 [MV#F7695]. **b** *Natica dubia* Récluz, 1844 [BMNH#1991085] (= P. *constanti* Huelsken and Hollmann, herein; replacement name). **c** *Natica deiodosa* Reeve, 1855 [BMNH#1991069]. **d** *Uber mellosum* Hedley, 1924 [AMS#C20058]. **e** *Natica phytelephas* Reeve 1855 [BMNH#1991096]. **f** *Polinices putealis* Garrard, 1961 [AMS#C63344]. **g** *Natica jukesii* Reeve, 1855 [BMNH#1991067]. (**h**) *Polinices tawhitirahia* Powell, 1965 [Auckland Museum #71242]. **i** *Natica vavaosi* Reeve, 1855 [figured type]. **j** *Natica galactites* Philippi, 1851 [figured type]. **k** *Natica cygnea* Philippi, 1850 [figured type]. **l** *Natica virginea* Philippi, 1850 [figured type]. For further information see Table 1. *Bars* 0.5 cm

information that will be discussed in the following, in order to retain taxonomic stability in this important *Polinices* taxon.

The name *P. mammilla* has been used traditionally for white-shelled Indo-Pacific *Polinices* species with closed umbilicus (e.g. Majima 1989; Kabat 1990), which have a "…protoconch reddish to black" (Kabat 1990: p. 17). Although Kabat examined the type specimen (1990), he did not mention that the protoconch of the lectotype of *Nerita mammilla* Linnaeus, 1758 (ZMUU#386) is broken and filled with a blackish sand grain (Fig. 5a). It is therefore impossible to determine the exact proportions and the colouration of the protoconch of the lectotype of *Nerita mammilla* Linnaeus, 1758.

Motivated by the perceived uncertainty of the taxonomical validity of *Nerita mammilla Linnaeus*, 1758, the original collecting site of which is "Bahamas" in the Caribbean Sea, Cernohorsky (1971) suggested to use the next available name *Mammillaria tumida* Swainson, 1840 for the Indo-Pacific white *Polinices* species with a black protoconch (Fig. 5b, Table 7). Unfortunately, the protoconch of the syntype of *M. tumida* (= *Albula mammilla* Röding, 1798) at ZMUC (Cernohorsky 1971), which is based on Mamma albula Chemnitz,

1758 [non-binomial; in Martini and Chemnitz 1769–1829] and for which the type locality is unknown, is broken, too (see Fig. 5b). Therefore, even with this type specimen of a junior synonym it is impossible to determine the exact proportions and the colouration of the protoconch of *P. mammilla*.

The next available name for this taxon is *Natica pyriformis* Récluz, 1844 (our Fig. 5c). The three specimens in the collections of the NHM (London) (BMNH#1991089), "syntypes" according to Kabat et al. (1997), have been collected in the Philippines as is noted on the NHM collection label. In the original description, Australia is mentioned as an additional locality. Two of the syntypes have black protoconchs (BMNH#1991089.1; BMNH#1991089.3) of 410 μm ± 10 and 2.25–2.50 EW while the protoconch of the third syntype (BMNH#1991089.2) is broken (Table 7). The protoconchs of three additional "possible syntypes" (Kabat et al. 1997) at the NHM (London) (BMNH#1845.6.24.56–58) are blackish, while those of five further "possible syntypes" (Kabat et al. 1997) found at the MHNG (MHNG#2017, 2 specimens, MHNG#2018, 3 specimens) are white. Although the operculum is unknown in *N. pyriformis*, shell shape, protoconch morphology, type locality and morphology of the umbilicus is virtually identical between Recent *P. mammilla* and the specimen BMNH#1991089.1 of *N. pyriformis* and very similar to the other five specimens of *N. pyriformis* (syntypes BMNH#1991089.2 and BMNH#1991089.3; possible syntypes BMNH#1845.6.24.56-58) found at the NHM. In order to maintain taxonomic stability in this taxon, we designate the specimen No. BMNH#1991089.1 (black protoconch, FEW: 400 μm, EW 2.25; our Fig. 5c) as lectotype and the other five specimens at the NHM as paralectotypes (BMNH#1991089.2–3, BMNH#1845.6.24.56-58) of *N. pyriformis*.

While it can no longer be determined whether the neotype of *Nerita mammilla* or the type of *Natica tumida* originally had a black protoconch or not, the lectotype of *Natica pyriformis* clearly does so. As all three taxa are believed to be conspecific (*P. tumidus* and *P. pyriformis*: Cernohorsky 1971; Majima 1989; Kabat 1990; *P. mammilla* and *P. pyriformis*: Majima 1989; Kabat 1990; *P. mammilla* and *P. tumidus*: Majima 1989; Kabat 1990) and in order to retain taxonomic stability, we follow Kabat's concept of *P. mammilla* as the earliest name for the Indo-Pacific white-shelled, glossy *Polinices* specimens with a black protoconch, with P. *tumidus* and *P. pyriformis* being junior synonyms.

Polinices jukesii (Reeve, 1855) [= *Polinices* sp. 2 in the preceding discussion]—Figs. 1a, 6g

Natica jukesii Reeve, 1855; Gen. Natica, Conch. Icon. 9: sp. 84, pl. 19, Figs. 84a,b

Polinices jukesii (Reeve 1855); Torigoe and Inaba (2011), sp. 84, p. 33, Pl. 2, Fig. 11.

Description

Shell Shell morphologically similar to *P. mammilla* but smaller in maximum size with up to 34 mm in height (type specimens: 31–34 mm; specimens analysed in this study: 13–25 mm), pyriform to ovate in shape. Ratio of shell height to shell width 1.27 ± 0.01 in specimens analysed with molecular methods (n=12) and 1.01 ± 0.05 in type specimens (n=3; BMNH1991067.1-3). Aperture wide and semi-ovate, ratio from aperture height to total height 0.72 ± 0.06 in specimens analysed with molecular methods (n=12) and 0.58 ± 0.05 in type specimens (n=3). Umbilicus completely covered by a heavy callus in adult specimens; a small anterior umbilical groove may be present in juveniles. Parietal callus extending into umbilical callus without a sulcus.

Protoconch White, 1.25–1.50 whorls, size of first embryonic whorl 770 ± 60 μm (n=12) in specimens analysed in this study, 1.50–1.75 whorls, size of first embryonic whorl 790 ± 50 μm in type specimens (n=3).

Operculum Corneous, light brown in colour.

Foot Propodium white and long (>2 times shell). Mesopodium white and short, overlapping protoconch, leaving only a quarter of the shell surface visible.

Distribution Central Indo-Pacific, East-Australia

Differential diagnosis This species differs only in protoconch morphology slightly from *P. constanti* (*P. jukesii*: EW=1.25 – 1.50, FEW=770 ± 60 μm; *P. constanti*: EW= 1.25–1.50, FEW=660 ± 60 μm). As the shells of both species do not appear to have further differentiating characters, the identification of the species will be impossible in cases where protoconch features are identical. Similarly, the shells of *P. jukesii* are virtually identical to those of *P. flemingianus*. However, *P. flemingianus* can be unequivocally differentiated from *P. jukesii* by the black streak on its otherwise light brown operculum (Fig. 1, Table 6) and also has a slightly smaller protoconch (*P. jukesii*: FEW=770 ± 60 μm, EW= 1.25–1.50; *P. flemingianus*: FEW=640 μm, EW=1.25). When operculi are missing, species of *P. flemingianus* and *P. jukesii* can be separated from each other based only on molecular data.

Material examined Syntypes of *Natica jukesii,* BMNH 1991067.1-3, for additional specimens analysed in this study see Table 1.

Discussion see discussion of *P. constanti* [replacement name for *Polinices dubius* (Récluz, 1844)].

Polinices constanti Huelsken and Hollmann, herein (replacement name for *Polinices dubius* (Récluz, 1844) [= *Polinices* sp. 3 in the preceding discussion]—Figs 1b, 6b

Natica dubia Récluz, 1844 [non Römer, 1836]; Proc. Zool. Soc. London (for 1843) 11(130): 209–210

+*Polinices dubius* (Récluz, 1844); Torigoe and Inaba (2011), sp. 116, p. 44.

Description

Shell Shell morphologically identical to *P. mammilla*, *P. jukesii*, *P. flemingianus* and *P.* cf. *tawhitirahia* but the specimens analysed here were smaller in maximal and average size, with up to 32 mm in height (type specimens: 30–32 mm; specimens analysed in this study: 13–19 mm). The shell is pyriformly-ovate with a ratio of shell height to shell width of 1.28 ± 0.02 in specimens analysed with molecular methods (n=3) and 1.00 ± 0.06 in type specimens (n=2). Aperture wide and semi-ovate with a ratio of aperture height to total height of 0.65 ± 0.03 (n=3). Umbilicus completely covered by a heavy callus in adult specimens sometimes showing a small anterior umbilical groove. Smaller specimens investigated here predominantly exhibit an anterior umbilical groove. Parietal callus extending into umbilical callus without a sulcus.

Protoconch White, 1.25-1.50 embryonic whorls, size of first embryonic whorl 660 ± 60 μm in specimens molecularly analyzed in this study (n=3), EW=1.20, size of first embryonic whorl 656 ± 97 in type specimens (n=2).

Operculum Corneous, light brown in colour.

Foot Propodium white and long (> 2 times shell length). Mesopodium white and short, overlapping the protoconch, leaving only a quarter of the shell surface visible.

Distribution Central Indo-Pacific, East Australia.

Differential diagnosis The species is virtually identical to *P. jukesii* and can be differentiated only by the slight differences in protoconch features (see above). *P. flemingianus* can be differentiated from *P. constanti* only by its black streak on the operculum, as both species show identical protoconch features (*P. flemingianus*: EW=1.25, FEW=640 μm; *P. constanti*: EW=1.25–1.50, FEW=660 ± 60 μm). *P. mellosus* can only differentiated from *P. constanti* by its yellowish-cream colouration (*P. mellosus*: EW=1.25–1.45, FEW=690 ± 20 μm; *P. constanti*: EW=1.25–1.50, FEW=660 ± 60 μm) (Table 6 and 7). When operculi are missing, species of *P. flemingianus* and *P. constanti* can be separated from each other based only on molecular data.

Material examined Syntypes of *Natica dubia*, BMNH 1991085.1-2, for additional specimens analysed molecularly and morphologically, see Table 1.

Discussion While being identical to *P. mammilla* in most conchological characters, *P.* sp. 2 and *P.* sp. 3 both have significantly larger white protoconchs that are virtually identical among the two taxa. Based solely on shell features, assignment to the following species appears possible (original combinations given): *N. candidissima* Le Guillou, 1842, *N. dubia* Récluz, 1850, *N. cygnea* Philippi, 1850, *N. virginea* Philippi, 1850, *N. galactites* Philippi, 1851, *N. jukesii* Reeve, 1855, *N. phytelephas* Reeve, 1855, *N. vavaosi* Reeve, 1855, *N. deiodosa* Reeve, 1855, *N. controversa* Pritchard & Gatliff, 1913, *Uber mellosum* Hedley, 1924, *P. putealis* Garrard, 1961 and *P. tawhitirahia* Powell, 1965 (see Table 7).

Type specimens are available only for 8 out of those 13 taxa: *N. dubia*, *N. jukesii*, *N. phythelephas*, *N. deiodosa*, *N. controversa*, *U. mellosum*, *P. putealis* and *P. tawhitirahia*. Reliable species identification is possible only if operculum colouration as well as protoconch dimensions are known [see results about *P. flemingianus* and *P.* cf. *tawhitirahia* (= *P.* sp. 4) as an example of the importance of operculum colouration]. In our analysis we therefore focussed on those seven species out of the eight with available type material for which the operculum colour and protoconch dimensions are known: *N. dubia*, *N. jukesii*, *N. controversa*, *N. deiodosa*, *P. putealis*, *U. mellosum* and *P. tawhitirahia*, thus excluding *N. phytelephas* (Fig. 6).

Conchologically, *P. controversus* is identical to *P. peselephanti* based on the depressed shell shape, an open umbilicus, the presence of an umbilical callus and the large FEW, which reaches nearly 1,500 μm with 1.75 EW (*P. peselephanti*: 1.25 EW, FEW=1,200 μm). *P. putealis* is a deep sea species, found at >100 m depth off South-East Australia (type locality: Botany Bay, Sydney, NSW). It has a brownish protoconch, 1.75 EW and a FEW of 320 μm. *P. tawhitirahia* is the only naticid species reported to have an almost black operculum (Powell 1965) and might be conspecific with *P.* sp. 4. *P. mellosus* and *P. deidosus* can also be excluded from the list as both are of creme-yellowish shell colour. *P. mellosus* furthermore can be differentiated from *P.* sp. 2 and *P.* sp. 3 based on molecular results (see Figs. 2, 3 and 4, Table 3). These differences in shell morphology allow excluding these three species as candidate taxa for *P.* sp. 2 or *P.* sp. 3.

Thus, only *N. dubia* and *N. jukesii* remain as possible name-bearing types for the two unknown species, as they show similar shell morphology, operculum colour and protoconch characters as *P.* sp. 2 and *P.* sp. 3. Based on the observation that *P. jukesii* has a slightly larger protoconch than *P. dubius*, we conclude that *P. jukesii* is conspecific

with our *P.* sp. 2 while our *P.* sp. 3 is referrable to *P. dubius* (see Table 7). *N. dubia* Recluz, 1844, however, is a junior homonym of *Natica dubia* Römer, 1836 who used this name for a fossil naticid specimen. We therefore introduce *Polinices constanti* Huelsken and Hollmann, herein as a replacement name (nomen novum) for *Natica dubia* Recluz, 1844. Etymology: a patronym honoring Constant A. Récluz who described this species first in 1844 (as *Natica dubia*) and who made extensive contributions to naticid taxonomy.

However, we emphasize that the reference of the molecularly defined *P.* sp. 2 and *P.* sp. 3 to *P. jukesii* and *P. constanti,* respectively*,* cannot be verified by molecular analysis as the type material of *P. jukesii* and *P. constanti* kept at the NHM (London) does not include preserved tissue.

It is noteworthy that the ratio of height to width and the umbilical morphology differ between type specimens and specimens analysed in this study in *P. jukesii* and *P. constanti*. The specimens in both type lots are significantly larger than the specimens used in the molecular analyses, with differences ranging between 5 and 19 mm. As shown for *P. jukesii* and *P. mellosus*, the ratio of height to width may vary strongly between juvenile and adult *Polinices* specimens, ranging from 0.9 to 1.20. It is worth noting that the studied specimens of *P. mammilla* (n=30) also vary considerably in height/width ratio, from 1.09 to 1.50. The variability of this character is also reflected in the low statistical values (CI: 0.33) in our conchological analysis of *Polinices* species. This goes along with results from empty shell material of *P. mammilla* from Lizard Island showing a height to width ratio range of 0.8–1.5 (own observations of TH). Similarly, the umbilical morphology varied in *P. jukesii*, *P. mammilla*, *P. flemingianus* and *P. mellosus*, resulting in low statistical values (CI: 0.22, RI: 0.42) in our conchological analysis of *Polinices* species. At present, we therefore predict the shell shape (i.e. ratio of height to width) and the umbilical morphology of (Table 5, character 10) to be too variable to provide characters for reliable species identification in *P. jukesii* and *P. constanti*.

To our knowledge, no type material of species described earlier by Le Guillou and Philippi is available at this stage (*N. candidissima* Le Guillou, 1842, *N. cygnea* Philippi, 1850, *N. virginea* Philippi, 1850 and *N. galactites* Philippi, 1851). Should type material of Le Guillou and Philippi be found in the future, conchological analysis of the protoconchs and the operculi of such type specimens may change the taxonomic assignments for the species here referred to *P. jukesii* and *P. constanti*. As both species are virtually identical, synonymies of putative conspecific taxa are difficult or impossible to discuss. We therefore refrain from providing any synonymy for either of the two species. Such synonymies can be attempted only when protoconch or operculum features for the considered synonymous species become known, which could happen only if type specimens can be located in the future (see discussion).

Polinices cf. *tawhitirahia* Powell, 1965 [= *Polinices* sp. 4 in the preceding discussion]—Fig. 1e, 6h

P. tawhitirahia Powell, 1965, Rec. Auck. Inst. Mus., 6 (2), Figs. 22(1–3), p. 163

+*P. mellosum* (Hedley 1924), in Majima (1989), Figs. 18–19, p. 48 [not *mellosus* Hedley, 1924]

+*P. mellosum* (Hedley 1924), in Kabat (2000), Fig. 31, p. 72 [not *mellosus* Hedley, 1924]

+*P. pyriformis* (Récluz, 1844), in Okutani (2000), Fig. 29, pl. 126 [not *pyriformis* Récluz, 1844]

+*P. mellosus* (Hedley, 1924) Torigoe and Inaba (2011), sp. 94, p. 38, Pl. 2, Fig. 14. [not *mellosus* Hedley, 1924]

Description

Shell Shell morphologically similar to *P. mammilla*, *P. flemingianus*, *P. constanti* and *P. jukesii*: The shell is plain white, 13–20 mm in height, globose to slightly pyriform with a ratio of shell height to shell width of 1.09 ± 0.03 (n= 5). Aperture is wide and semi-ovate; the ratio of aperture height to total height is 0.82 ± 0.01 (n=5). Umbilicus is completely covered by a heavy callus. Parietal callus thick, filling posterior apertural angle. Parietal callus extending into umbilical callus without a sulcus.

Protoconch White, 0.9–1.15 whorls, size of first embryonic whorl 870 ± 70 μm in specimens analyzed in this study (n= 5); white, 1.75 whorls and 622 μm in the holotype.

Operculum Corneous and entirely black operculum.

Distribution New Zealand, West-Australia, Indonesia (Ambon).

Differential diagnosis This species can be differentiated from other plain white *Polinices* species by its entirely black operculum and its white protoconch with only 0.9–1.1 whorls and a large first embryonic whorl of 870 ± 60 μm.

Material examined For specimens analysed molecularly and morphologically see Table 1.

Discussion Shell characters are very similar to most of the other plain white *Polinices* species analysed in this study. However, in contrast to the honey-coloured corneous operculi of other white *Polinices* species, *P.* sp. 4 has an entirely black corneous operculum. Other distinguishing features are the larger size of the first protoconch whorl (FEW: 870 μm ± 70.0) and the low number of embryonic whorls (EW: 0.9–1.15). A glossy white *Polinices* species with a black operculum and a white protoconch has been described from New Zealand by Powell (1965) as *P. tawhitirahia* (his Fig. 7G). Interestingly, a white *Polinices* specimen with a

black operculum (Kabat 2000, his Fig. 31, p.72) was also found during the 1990 Rumphius Biohistorical Expedition to Ambon (Indonesia). Furthermore, specimens with shell characters identical to our material have also been pictured by Majima (1989; text: Figs. 18 and 19, on p. 48), by Okutani (2000; pl.126, fig. 20) and by Torigoe and Inaba (2011; pl.II, fig. 14). Kabat, Majima as well as Torigoe and Inaba erroneously named the species "*P. mellosum* (Hedley 1924)", while Okutani erroneously named it "*P. pyriformis* (Récluz, 1844)". Although the operculum of the type of *P. pyriformis* is unknown, that species has to be synonymized with *P. mammilla* based on protoconch morphology (300–400 μm) and protoconch colouration, as discussed above and thus must possess a honey-coloured operculum. Therefore, the specimen figured by Okutani as *P. pyriformis* with a black operculum cannot be *P. pyriformis* but instead is conspecific with our *P.* sp. 4.

Based on overlapping similarities in operculum and shell morphology we therefore conclude that *P.* sp. 4 can be assigned to P. *tawhitirahia* as differences in protoconch morphology of the holotype (NZ71242) of *P. tawhitirahia* and *P.* sp.4 (FEW=622 μm; EW=1.75 in *P. tawhitirahia* vs FEW=870 μm±70.0; EW=0.91.15 in *P.* sp.4) lie within the margin of morphological variability (5–25 %, see Results section) generally observed in white *Polinices* species. Supporting this conclusion, Kabat (2000) also synonymises his Ambon *Polinices* specimen with black operculum with *P. tawhitirahia* from New Zealand. The diverse collecting sites (North Western Australia/Ambon/Japan vs New Zealand) suggests that this species either has a very broad distribution range or, alternatively, the specimens investigated in this study represent an additional species with an entirely black operculum, in which case this opercular feature would occur in at least two different taxa of white *Polinices*. However, given the wide specimen distribution and the fact that most operculi are missing in museum specimens, we cannot exclude unequivocally that *P.* sp. 4 does not represent *P. tawhitirahia* (Table 7, Fig. 6).

Discussion

Our study represents the first approach to clarify the questionable species identifications and phylogenetic relationships within the conchologically rather uniform taxon *Polinices* and its predicted closely related taxa *Conuber*, *Euspira*, *Mammilla*, *Neverita* and *Sinum* (Cernohorsky 1971; Marincovich 1977; Kabat 1991, 1996). The monophyletic grouping and the high genetic divergence demonstrate clearly that *Conuber*, *Euspira*, *Mammilla* and *Neverita* indeed represent independent genera and not merely subgenera of *Polinices* (Figs. 2, 3 and 4, Supplementary Figs. S1, S2).

Beyond the clarification of supraspecific taxa relationships within *Polinices s.l.*, our phylogenetic analyses allow species differentiation within the group of conchologically uniform white *Polinices* species. This includes taxonomical evaluations and re-descriptions of the common *P. mammilla* (= *P.* sp. 1), the formerly synonymized species *P. jukesii* (= *P.* sp. 2) and *P. constanti* (= *P.* sp. 3) as well as *P.* cf. *tawhitirahia* (= *P.* sp. 4). Our analyses furthermore prove for the first time that many conchological characters traditionally used in the description and identification of species in *Polinices s.l.* are homoplasious and of low information value due to identical expression in distinct clades or intraspecific variability.

Discussion of conchological analyses

The low CI values indicate that most traditionally used shell features have a wide range of intra-specific variability or occur simultaneously in various *Polinices s.l.* genera, thus revealing strong homoplasy. However, some shell characters appeared to be informative with respect to the phylogenetic pattern of the taxa, which was revealed by the differences between high RI values and low CI values for the same characters (Table 5). Several characters listed here were identical (e.g. operculum surface, aperture size, colour of columellar callus) or showed intra-specific variability in all analysed *Polinices s.l.* species (e.g., umbilical structure). This pronounced uniformity of shell characters in *Polinices* clearly is the reason for problematic taxonomic assignments in this species group.

The apparent autapomorphic nature of some characters (e.g. subsutural wrinkels) can be an effect of insufficient taxon sampling. Other characters, however, seem to be helpful in discriminating between genera (e.g. operculum size and umbilical callus in the Sininae; egg mass structure in *Conuber*) or species (e.g. operculum size in *M. caprae*; protoconch colour in *P.* sp. 1, *P. uber*, *P. mediopacificus*; operculum colour in *P. flemingianus* and *P.* sp. 4). Characters with high CI/RI values include protoconch features (EW, FEW, colouration), the presence or absence of an umbilical callus, parietal callus features, operculum features and egg mass morphology. These characters are highly informative when used for species identification in a set of closely related *Polinices* species (see *Polinices*) and are even informative (but with lower CI/RI values) in discriminating *Polinices s.l.* species despite their intra-generic variability. However, characters such as shell shape, shell colour and general umbilical characters (anterior cleft) are less informative in discriminating either *Polinices s.l.* or *Polinices s.s.* species, owing to their intra-specific variability and convergent occurrences.

Our results therefore confirm predictions that developmental characters are highly informative in taxonomic

assignments of gastropods (Bouchet 1989). However, protoconch features coincide between closely related *Polinices s.l.* species (Table 6), indicating a need for additional discrete morphological characters for reliable species identification. Unfortunately, little is known about shell variability (shape and umbilicus) with regard to ontogeny. Given the fact that adult and juvenile specimens in *P. mellosus*, *P.* sp. 1 and *P.* sp. 2 show highly variable shell morphology and shell colouration, investigation on a larger scale than is presented here is needed.

Given our results, we cannot confirm Kool's sweeping criticism (1993) regarding the usefulness of shell character in phylogenetic and systematic analyses. Of course, shell characters in *Polinices* species (and probably in all naticids) are subject to evolutionary convergence due to analogous adaptations to environmental constraints based on identical predatory behaviour and their burrowing way of life (Bandel 1999). However, shell characters need to be analysed carefully before they can be rejected as uninformative (Vermeij and Carlson 2000). This is particularly important for the identification of species via DNA barcoding and phylogenetic approaches and for the subsequent assignment of type specimens of gastropods for which, in most cases, only empty shells are available. In conchologically homogeneous groups such as the moon snails, shell characters may not be informative enough to resolve taxa in phylogenetic and cladistics analyses, but can certainly be used in species identification when described and categorized accurately (see also Aronowsky 2003).

Phylogenetic and systematic considerations

In our study definitions of species and genera were based on the monophyletic arrangement of each of the taxa in the phylogenetic trees and the high genetic divergence (13 %–16 %) between the groups. Any species assignment thus follows a phylogenetic species concept.

Genus Polinices Montfort, 1810

Based on molecular data, the genus *Polinices* can be divided in two groups. One group comprises *P.* sp. 1 (= *P. mammilla*), *P. uber* and *P. mediopacificus*, while the second group contains *P. albumen*, *P. cumingianus*, *P. mellosus*, *P. flemingianus*, *P.* sp. 2 (= *P. jukesii*), *P.* sp. 3 (= *P. constanti*), *P.* sp. 4 (= *P.* cf. *tawhitirahia*) and *P. peselephanti* (Figs. 2, 3 and 4, Supplementary Figs. S1–S2). Species in the second *Polinices* group are related more closely to each other and showed lower intra-specific geographic resolution. This may be largely a consequence of limitations in taxon sampling, as most specimens were sampled in one region only or only one specimen per species was available for sequencing (e.g. *P. flemingianus*).

The patterns of genetic variation in the monophyletic *P. mammilla* (= *P.* sp. 1) between Egypt (clade 1), Indonesia, New Caledonia (clade 2) and the Great Barrier Reef (clades 2/3) correspond to the phylogeographic category II of Avise (2000) in which it is assumed that the different mitochondrial haplotypes originated either from hitherto unidentified sympatric species or from previously isolated lineages with restricted genetic connectivity (see Thomaz et al. 1996; Avise 2000). Thus, the mtDNAs appear to have diverged in allopatry, with a secondary admixture of populations in the northern Great Barrier Reef. This is supported by the fact that two genetically distinct lineages have been found on Lizard Island (clades 2/3). However, the specimens from Vanuatu and Lizard Island in clade 2 are also clearly separated from clade 3 by the slower evolving protein-coding histone *H3* gene fragment (see Supplementary Fig. S2). Thus, the apparent genetic divergences between the three *P. mammilla* lineages support a separation at species level. However, as all specimens are conchologically identical and grouped together in a single monophyletic taxon, more data will be needed to test whether these clades may indeed represent different species.

The phylogenetic separation of the morphologically virtually identical *P. constanti* and *P. jukesii* (Figs. 2, 3 and 4) could either indicate the existence of non-monophyletic species with two mitochondrial lineages caused by incomplete lineage sorting or hybridisation, or indicate the existence of two independent species (Davison 2000; Funk and Omland 2003; Meyer and Paulay 2005; Huelsken et al. 2011b). The genetic divergence between *P. jukesii* and *P. constanti* is identical to, or even higher than, values calculated for conchologically well-separated *Polinices* species in this group, such as *P. mellosus, P.* cf. *tawhitirahia* and *P. cumingianus* (Table 4). Additionally, virtually identical mitochondrial and nuclear sequences for *P. jukesii* were obtained from two independent and not directly connected localities in the Philippines and the Great Barrier Reef indicating strong genetic connectivity. The two taxa are furthermore strictly separated by the slower evolving protein-coding histone *H3* gene fragment, with *P. jukesii* sharing the same genetic information with *P. cumingianus*, *P. mediopacificus* and *P. peselephanti* in this gene fragment (see Supplementary Fig. S2). The molecular data in combination with slight differences in protoconch morphology therefore rather reject the hypotheses of hybridisation or incomplete lineage sorting in *P. jukesii* and *P. constanti* but support the idea that the two taxa are separated at the species level.

Genus Mammilla Schuhmacher, 1817

Considering the paraphyletic arrangement of *Polinices*, with *Mammilla* grouping within the former clade in some of the phylogenetic analyses (Figs. 2, 3 and 4), the question may

be posed whether *Mammilla* should be classified as a subgenus of *Polinices*. Despite a close genetic relationship between *Mammilla* and *Polinices*, both taxa are well separated by their distinct conchological characters (see Fig. 1). The phylogenetic trees show increasing resolution of *Mammilla* and *Polinices* from slow to fast evolving genes (Figs. 2, 3; Supplementary Figs. S1, S2), suggesting that *Mammilla* and *Polinices* represent two independent genera which were separated from each other more recently.

As mentioned above, the genus *Mammilla* in earlier classifications has been described occasionally as closely related to the Sininae. In his compilation of the Naticidae from Fiji, Cernohorsky (1971) stated that *Eunaticina* (subfamily Sininae) "… may represent an intermediate group between *Mammilla* and *Sinum*…". *Mammilla* and *Sinum* thus have been considered closely related taxa by Cernohorsky (1971), an idea that, amongst others, was later also taken up by Kabat (1996). Our morphological analyses indicate a high conchological concordance between members of these two taxa, such as the depressed shell shape, the thin shell, the shell texture, the widened aperture and the reduced operculum in some *Mammilla* species. As the molecular data presented here support a very close relationship between *Mammilla* and *Polinices* species, we conclude that the apparently similar shell characters in *Sinum* and *Mammilla* must have evolved independently at least twice within the Naticidae.

Conuber Finlay & Marwick, 1937

The genus *Conuber* is best suited to illustrate the extensive variability and high similarity of conchological features in species of the Polinicinae. Shell features characterizing valid *Conuber* species are also found in *Neverita* (compare *C. incei*) or *Polinices* (compare *C. conicus*, *C. sordidus*) (Fig. 1). Not surprisingly, *C. incei* has been assigned to the genus *Neverita* based on its depressed shell form, widened aperture and thin parietal callus (e.g. Hacking 1998). Based on the pyriform shell form, ratio of aperture height to total height and the morphology of parietal callus and columellar callus, *C. conicus* and *C. sordidus* were often assigned to the genus *Polinices* (e.g. Marincovich 1977; Booth 1995; Morton 2008). However, our phylogenetic analysis clearly demonstrated *Conuber* representing a distinct monophyletic taxon, thus contradicting the view of *Conuber* as a subgenus of *Polinices* as proposed by Finlay and Marwick in 1937 (p. 53).

The distinctive character defining the genus *Conuber* is the large, sausage-shaped gelatinous egg mass without sand grain incorporation, which differs from the typical sand collar found in all other naticids (Murray 1962, 1966; Booth 1995). This feature represents an autapomorphic character for *Conuber* (see also Riedel 2000) as it has not yet been found in any other naticid genus.

Neverita Risso, 1826

The genus *Neverita* was characterized by homogeneous conchological characters such as a depressed shell shape, an ovate aperture, a large parietal callus, a greatly enlarged body whorl and a typical umbilical area containing a large, distinctive funicle (see Cernohorsky 1971; Marincovich 1977; Majima 1989). Its placement as a subgeneric taxon within the genus *Polinices*, however, was based on the occurrence of several conchological characters that could be assigned to both taxa. For instance, *P. albumen*, *P. peselephanti* and *P. cumingianus* have "*Neverita*"-like depressed to slightly globose shells and thus have often been considered to belong to *Neverita,* either at the generic level or at the subgeneric level as in *Polinices* (*Neverita*) (e.g. Cernohorsky 1971; Majima 1989). The concept of *Neverita* as a subgenus of *Polinices* can now be rejected because the taxa *Neverita* and *Polinices* each form statistically well-supported monophyletic clades in our analyses. In consequence, similar or even identical shell characters have evolved separately in these two genera.

To our surprise, the widely known and well-investigated (e.g. Bernard 1967; Grey et al. 2007; Cook and Bendell-Young 2010) Northern Pacific species *E. lewisii* groups within *Neverita* as sister species to the Australian *N. aulacoglossa* in the *COI* tree. This confirms morphology-based cladistics with *E. lewisii* and *E. heros* grouping within *Neverita* (Aronowsky 2003). Although our phylogenetic placement is based only on sequence data obtained from the mitochondrial *COI* gene fragment, we conclude from the data sets of Aronowsky (2003) and this study that *E. lewisii* (and probably its sister species *E. heros*) should definitely be assigned to *Neverita* (see Table 1).

The existence of a separate (sub)genus *Glossaulax* Pilsbry, 1929 within *Neverita* (e.g. Marincovich 1977; Majima 1989) appears doubtful. The (sub)genus *Glossaulax* is defined by an umbilical callus that covers the umbilicus entirely and is divided into anterior and posterior lobes by a narrow transverse groove (Majima 1989). In the present study, the type species of the (sub)genus *Neverita* (*Glossaulax)*, *N.* (*G.*) *reclusiana*, is grouped together with its nominate sister species *N. (G.) didyma* but not with the Australian *N.* (*G.*) *aulacoglossa* (Figs. 2, 3 and 4). The species are in fact separated from each other by species which are distinctly assigned to *Neverita s.s.* (e.g. *N. delessertiana*, *N. duplicata*) (Figs. 2 and 3).

Our data, albeit somewhat preliminary, supports the proposed validity of *N. didyma* (Indo-Pacific) and *N. aulacoglossa* (Eastern Australia) as distinct species and reject their former synonymisation under the name *N. didyma* (Kabat 2000). However, more sequences for *N. didyma* (presented here by one sequence from Taiwan, AF550509; Strong 2003) are needed for clarification.

Genus Euspira Agassiz in J. Sowerby, 1837

According to the analyses presented here, *Euspira* is not a subgenus of *Polinices* (Marincovich 1977) but represents a valid genus related closely to *Conuber* and *Neverita*. Similar to earlier results, *Payraudeautia intricata* (Donovan, 1804) exhibits a high genetic similarity with and thus groups within, *Euspira* in all genetic analyses. Synonymisation of *Payraudeautia* with *Euspira* is therefore appropriate (see Table 1; Huelsken et al. 2008).

The genus *Euspira* Agassiz in Sowerby, 1837, is based on the fossil European species *Natica glaucinoides* Sowerby, 1812 from the Middle Eocene, by subsequent designation (Bucquoy et al. 1883). The genus is characterized by a globose to elongate-globose shell with a partly-to-fully open umbilicus, abutting to an impressed suture, a slender umbilical callus, convex whorls and a turreted spire (Bandel 1999). Species assigned to *Euspira* therefore show many shell characters (e.g. umbilical morphology, shell shape) that are identical to those in other naticid genera (e.g. *Natica*, *Tectonatica*). Understandably, Bandel (1999) criticized the application of these shell characters in the establishment of a separate genus, *Euspira,* in particular since neither operculum nor protoconch of the type species of *Euspira s.s*. is known. Thus, a conchological analysis of *N. glaucinoides* and other taxa assigned to *Euspira* is needed to re-evaluate the taxonomic validity of *Euspira*.

Genera Sinum Röding, 1798 and Tectonatica Sacco, 1890

The two *Sinum* species analysed in this study are sister taxa in the tree based on the concatenated data set and in the *18S* gene analysis (Fig. 2, Fig. S1). The separated placement of *Sinum* together with genera of the Polinicinae matches previous contentions that the Sininae more likely represent a genus within the Polinicinae (Finlay and Marwick 1937; Oyama 1969). However, the basal arrangement of Sinum species in both, the nuclear *18S* tree and the mitochondrial *COI* tree, favour the hypothesis for the Sininae being a true subfamilial taxon independent of Polinicinae. This view appears to be contradicted by the basal arrangement of *Tectonatica* in the tree based on the concatenated data set, which, however, cannot be observed in either of the single analyses. Phylogenetic analyses of the entire family including more species and genera not investigated yet are needed to clarify these taxonomic puzzles.

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References

Agassiz, L. (1837). In J. Sowerby (Ed.), *Großbritanniens Mineral-Conchologie oder ausgemahlte Abbildungen und Beschreibungen der Schaltier-Überreste, welche zu verschiedenen Zeiten und in verschiednen Tiefen der Erde erhalten worden sind*. Neuchatel: Buchdruckerei von Petitierre.

Aronowsky, A. (2003). *Evolutionary biology of naticid gastropods* (PhD thesis). Ann Arbor, Michigan: UMI Dissertation Services.

Avise, J. C. (2000). *Phylogeography. The history and formation of species*. Cambridge: Harvard University Press.

Bandel, K. (1984). The radulae of Caribbean and other Mesogastropoda and Neogastropoda. *Zoologische Verhandelingen, 214*, 1–188.

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.

Bernard, F. R. (1967). Studies on the biology of the naticid clam drill *Polinices lewisii* (Gould) (Gastropoda: Prosobranchia). *Fisheries Research Board of Canada Technical Report, 42*, 1–42.

Booth, D. T. (1995). Oxygen availablity and embryonic development in sand snail (Polinices sordidus) egg masses. *Journal of Experimental Biology, 198*, 241–247.

Bouchet, P. (1989). A review of poecilogony in gastropods. *Journal of Molluscan Studies, 55*, 67–78.

Bucquoy, E., Dautzenberg, P., & Dollfus, G. (1882–86). *Les mollusques marins du Roussillon. 1. Gastropodes.* Paris.

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

Cernohorsky, W. O. (1974). Type specimens of Mollusca in the University Zoological Museum, Copenhagen. *Records of the Auckland Institute and Museum, 11*, 143–192.

Chénu, J.-C. (1842–1853). *Illustrations conchyliologiques ou description et figures de toutes les coquilles connues vivantes et fossiles, classées suivant le systéme de Lamarck, modifié d´après les progrès de la science, et comprenant les genres nouveaux et les espèces récemment découvertes* (Vol. [Note: Pls. 2–4 of the genus *Natica* (pl. 1 was never published) are attributed to C.A. Récluz (fide Kabat et al. 1997)]). Paris: Fortin, Masson.

Colgan, D. J., Ponder, W. F., Beacham, E., & Macaranas, J. (2007). Molecular phylogenetics of Caenogastropoda (Gastropoda: Mollusca). *Molecular Phylogenetics and Evolution, 42*(3), 717–737. doi:10.1016/J.Ympev.2006.10.009.

Collin, R. (2003). Phylogenetic relationships among calyptraeid gastropods and their implications for the biogeography of marine speciation. *Systematic Biology, 52*, 618–640

Cook, N., & Bendell-Young, L. (2010). Determining the ecological role of *Euspira lewisii*: Part I: Feeding Ecology. *Journal of Shellfish Research, 29*(1), 223–232.

Davison, A. (2000). The inheritance of divergent mitochondria in the land snail, *Cepaea nemoralis*. *Journal of Molluscan Studies, 66*, 143–147.

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.

Funk, D. J., & Omland, K. E. (2003). Species-level paraphyly and polyphyly: Frequency, causes and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology Evolution and Systematics, 34*, 397–423. doi:10.1146/Annurev.Ecolsys.34.011802.132421.

Garrard, T. A. (1961). Mollusca collected by mv Challenge off the East Coast of Australia. *Journal of the Malacological Society of Australia, 1*(5), 2–37.

Grey, M., Lelievre, P. G., & Boulding, E. G. (2007). Selection for prey shell thickness by the naticid gastropod *Euspira lewisii* (Naticidae) on the bivalve *Protothaca staminea* (Veneridae). *Veliger, 48*(4), 317–322.

Hacking, N. (1998). Macrofaunal community structure of beaches in northern New South Wales, Australia. *Marine Freshwater Research, 49*, 47–53.

Huelsken, T. (2011). First evidence of drilling predation by *Conuber sordidus* (Swainson, 1821) (Gastropoda: Naticidae) on soldier crabs (Mictyridae). *Molluscan Research, 31*(2), 125–132.

Huelsken, T., Marek, C., Schreiber, S., Schmidt, I., & Hollmann, M. (2008). The Naticidae (Mollusca: Gastropoda) of Giglio Island (Tuscany, Italy): Shell characters, live animals and a molecular analysis of egg masses. *Zootaxa, 1770*, 1–40.

Huelsken, T., Schreiber, S., & Hollmann, M. (2011). COI amplification success from mucus-rich marine gastropods (Gastropoda: Naticidae) depends on DNA extraction method and preserving agent. *Mitteilungen der Deutschen Malakozoologischen Gesellschaft, 85* (July 2011), 17–26.

Huelsken, T., Wägele, H., Peters, B., Mather, A., & Hollmann, M. (2011). Molecular analysis of adults and egg masses reveals two independent lineages within the infaunal gastropod *Naticarius onca* (Röding, 1798) (Caenogastropoda: Naticidae). *Molluscan Research, 31*(3), 141–151.

Hülsken, T., Clemmensen, M., & Hollmann, M. (2006). *Neverita delessertiana* (Récluz in Chénu, 1843): a naticid species (Gastropoda: Caenogastropoda) distinct from *Neverita duplicata* (Say, 1822) based on molecular data, morphological characters and geographical distribution. *Zootaxa, 1257*, 1–25.

Huson, D. H. (1998). Splitstree: analyzing and visualizing evolutionary data. *Bioinformatics, 14*(1), 68–73.

Huson, D. H., & Bryant, D. (2006). Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution, 23*(2), 245–267.

Kabat, A. R. (1990). Species of Naticidae (Mollusca: Gastropoda) described by Linnaeus in the Systema Naturae (1758). *Zoological Journal of the Linnean Society, 100*(1), 1–25.

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* (7), 417–449.

Kabat, A. R. (1996). Biogeography of the genera of Naticidae (Gastropoda) in the Indo-Pacific. *American Malacological Bulletin, 12*(1/2), 29–35.

Kabat, A. R. (2000). Results of the Rumphius Biohistorical Expedition to Ambon. Part 10: Mollusca, Gastropoda, Naticidae. *Zoologische Mededelingen, 1*, 345–380.

Kabat, A. R., Finet, Y., & Way, K. (1997). Catalogue of the Naticidae (Mollusca: Gastropoda) described by C.A. Récluz, including the location of type specimens. *Apex, 12*, 15–26.

Kool, S. P. (1993). Phylogenetic analysis of the Rapaninae (Neogastropoda: Muricidae). *Malacologia, 35*, 155–259.

Linnaeus, C. (1758). *Systema Naturae per Regna tria Naturae, secundum classes ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I, Editio Decima, Reformata*. Holmiae: Laurentius Salvius.

Maddison, W. P., & Maddison, D. R. (2006). MacClade. (4.06 ed.). Sunderland, MA: Sinauer.

Majima, R. (1989). Cenozoic fossil Naticidae (Mollusca: Gastropoda) in Japan. *Bulletin of American Paleontology, 96*(331), 1–159. pls. 151–114.

Makowsky, R., Cox, C. L., Roelke, C., & Chippindale, P. T. (2010). Analyzing the relationship between sequence divergence and nodal support using Bayesian phylogenetic analyses. *Molecular Phylogenetics and Evolution, 57*(2), 485–494. doi:10.1016/J.Ympev.2010.05.009.

Marincovich, L. N. (1977). Cenozoic Naticidae (Mollusca: Gastropoda) of the Northeastern Pacific. *Bulletins of American Paleontology, 70*(294), 169–212.

Martini, F. H. W., & Chemnitz, J. H. (1769–1829). *Neues systematisches Conchylien-Cabinet.* Nürnberg, Vol. 5 [of 12], xxii + 324, pls. 160–198.

Meyer, C. P., & Paulay, G. (2005). DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology, 3*(12), 422.

Montfort, P. D. (1810). *Conchyliologie systematique et classification méthodique des coquilles …* (Vol. 2, Paris, F. Schoell).

Morton, B. (2008). Biology of the swash-riding Moon Snail *Polinices incei* (Gastropoda: Naticidae) predating the Pipi, *Donax deltoides* (Bivalvia: Donacidae), on wave-exposed sandy beaches of North Stradbroke Island, Queensland, Australia. *Memoirs of the Queensland Museum, 54*(1), 303–322.

Murray, F. V. (1962). Notes on the spawn and early life history of two species of *Conuber* Finlay & Marwick, 1937. *Journal of the Malacological Society of Australia, 6*, 49–58.

Murray, F. V. (1966). A brief account of the spawn of *Conuber incei* (Philippi, 1853). *Journal of the Malacological Society of Australia, 10*, 49–52.

Nylander, J. A. A. (2004). MrModeltest v2. Program distributed by the author.

MorphoBank 2.5: Web application for morphological phylogenetics and taxonomy (2007). http://www.morphobank.org.

Okutani, T. (2000). *Marine Mollusks in Japan*. Tokyo: Tokai University Press.

Oyama, K. (1969). Systematic revision of Japanese Naticidae. *Venus, The Japanese Journal of Malacology, 28*(2), 69–88. pl. 64–65.

Philippi, R. A. (1849–1853). Die Gattungen *Natica* und *Amaura*. In Martini, Chemnitz, Schubert G.H.v., J. A. Wagner, & H. C. Küster (Eds.), *Systematisches Conchylien-Cabinet von Martini und Chemnitz* (Vol. 2, pp. 164). Nürnberg: Bauer und Rapse.

Pitcher, C. R., Doherty, P., Arnold, P., Hooper, J., Gribble, N., Bartlett, C., et al. (2007). Seabed Biodiversity on the Continental Shelf of the Great Barrier Reef World Heritage Area. *AIMS/CSIRO/QM/QDPI Final Report to CRC Reef Research*.

Popenoe, W. P., Saul, L. R., & Susuki, T. (1987). Gyrodiform gastropods from the Pacific coast Cretaceous and Paleaocene. *Journal of Palaeontology, 61*(1), 70–100.

Powell, A. W. (1965). New Zealand molluscan systematics with descriptions of new species. 5. *Records of the Auckland Institute and Museum, 6*(2), 161–168.

Récluz, C.-A. (1844). Descriptions of new species of *Navicella*, *Neritina*, *Nerita* and *Natica*, in the cabinet of H. *Cuming. Proceedings of the Zoological Society of London, 11*(130), 197–214.

Récluz, C.-A. (1850). Description de Natices nouvelles. *Journal de Conchyliologie, 1*(4), 379–402.

Reeve, L. A. (1855). Monograph of the genus *Natica*. In *Conchologia Iconica. Or, Illustrations of the shells of molluscous animals* (Vol. 9, 30 pls.). London: Lovell Reeve.

Riedel, F. (2000). *Ursprung und Evolution der "höheren" Caenogastropoda* (Vol. Band E: Paläobiologie, Berliner Geowissenschaftlichen Abhandlungen, Vol. 32). Berlin.

Risso, A. (1826). *Histoire naturelle des principales productions de l´europe meridionale et particulierement de celles des environs de Nice et des Alpes maritimes*. Levrault.

Röding, P. F. (1798). *Museum Boltenianum, sive catalogus cimeliorum e tribus regnis naturae quae olim collegerat Joa. Fried. Bolten, M.D.p.d. Pars secunda, continens conchylia sive testacea univalvia, bivalvia & multivalvia*. Hamburg [Reprinted, 1906, Watford (England)]: Joachim Friedrich Bolten.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics, 19*(12), 1572–1574.

Sanderson, M. J., Donoghue, M. J., Piel, W., & T., E. (1994). TreeBASE: a prototype database of phylogenetic analyses and an interactive tool for browsing the phylogeny of life. *American Journal of Botany, 81*(6), 183.

Schuhmacher, C. F. (1817). *Essai d'un nouveau système des habitations des vers testacés avec XXII planches*. Copenhagen: Schultz.

Solsona, M., & Martinell, J. (1999). Protoconch as a taxonomic tool in Gastropoda systematics. *Application in the Pliocene Mediterranean Naticidae Geobios, 32*(3), 409–419.

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.

Swainson, W. (1840). *A Treatise on Malacology or the natural classification of shells and shellfish*. London: Spottiwoode.

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

Thomaz, D., Guiller, A., & Clarke, B. (1996). Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society of London Series B-Biological Sciences, 263*(1368), 363–368.

Torigoe K., & Inaba A. (2011). Revision on the classification of Recent Naticidae. *Bulletin of the Nishinomiya Shell Museum, 7*, 133 + 115 pp., 134 pls.

Troschel, F. H. (1856–1863). *Das Gebiss der schnecken zur Begründung einer natürlichen classification*. Berlin: Nicolaische Verlagsbuchhandlung.

Tryon, G. W. (1886). *Manual of Conchology, Series 1, Vol. 8: Naticidae, Calyptraeidae, Turritellidae, Vermetidae, Caecidae, Eulimidae, Turbonillidae, Pyramidellidae*. Philadelphia: Academy of Natural Science.

Vermeij, G. J., & Carlson, S. J. (2000). The muricid gastropod subfamily Rapaninae: phylogeny and ecological history. *Paleobiology, 26*(1), 19–46.

Wiens, J. J. (2001). Character analysis in morphological phylogenetics: problems and solutions. *Systematic Biology, 50*(5), 689–699.