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Revision of the genus complex *Gibbula*: an integrative approach to delineating the Eastern Mediterranean genera *Gibbula* Risso, 1826, *Steromphala* Gray, 1847, and *Phorcus* Risso, 1826 using DNA-barcoding and geometric morphometrics (Vetigastropoda, Trochoidea)

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Abstract The trochoid genus, *Gibbula*, is abundant and diverse in the Mediterranean Sea but problematic to identify and delineate. This is due to highly variable shell morphology, vague original descriptions, and missing or unspecific type material. In recent studies, COI barcoding yielded satisfactory results for species delineation. In the present study, a combination of geometric shell morphometric methods and COI barcoding was used to assess the most abundant species of the Eastern Mediterranean. All relevant identification characters were captured via standardised images of the shells in both lateral and ventral views. Agreeing with previous studies, *Gibbula* was recovered as paraphyletic in the molecular analysis and thus is restricted to the clade encompassing the type species *Gibbula magus* (Linnaeus, 1758). The geometric morphometric analyses and the barcoding approach clearly distinguish the remaining species into two groups: the genus *Steromphala* Gray, 1847 and the genus *Phorcus* Risso, 1826. Type material was used for the geometric morphometric analyses whenever possible. Based on re-examination of the original type descriptions, lectotypes were designated. The joint application of DNA-barcoding and geometric morphometrics not only effectively delineated the sister genera *Steromphala* and *Phorcus* but also delineated all analysed species in the *Gibbula*-*Steromphala*-*Phorcus* genus complex. The additional use of geometric morphometrics enables researchers to compare barcoded material with fossil specimens or dry collections in an objective way.

Keywords *Gibbula* · *Phorcus* · *Steromphala* · Barcoding · Geometric morphometrics · Species delineation

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Introduction

Gastropod taxonomy is traditionally shell-based. Methodology in recent years using genetic data has revealed cryptic species and questionable species delineation in several gastropod taxa (e.g. Delicado and Ramos 2012; Weigand et al. 2013). Therefore, species identification and delineation by shell morphology alone is often mistrusted if not supported by additional lines of evidence, such as DNA-barcoding or morphometric data. However, external characteristics (e.g. shell characters) remain the most straight forward way of identification of snails by scientists from related fields such as ecology or palaeontology and especially by amateurs. Reliable species identifications and delineations of even cryptic lineages not only are significant for taxonomic purposes but also impact the assessment of biodiversity, ecological niche differentiation, and conservation measurements (e.g. Bálint et al. 2011; Feckler et al. 2014). Thus, it is of paramount importance—particularly for common taxa—to re-evaluate the usefulness of shell morphology for species delineation and identification, based on independent molecular markers. Mediterranean trochid gastropods provide a suitable platform for re-evaluating earlier hazy species descriptions and delineations in a contemporary context.

The systematics and phylogenetic relationships of the trochoid families Trochidae and Turbinidae and the subfamily Cantharidinae are only partly resolved (Williams and Ozawa 2006; Williams et al. 2008, 2010). Although molecular data support the monophyly of the Cantharidinae (Williams et al. 2010; Uribe et al. 2016), relationships within the subfamily remain ambiguous.

One of the subfamily's most problematic groups contains *Gibbula* Risso, 1826 and *Phorcus* Risso, 1826. Problems with correctly identifying *Gibbula* and *Phorcus* species are main reasons for this unresolved status. An umbilicated shell, black and white striped epipodial tentacles, a non-calcified operculum, and a turbinate shell form currently characterise both genera. Species differ in spiral height, umbilicus size and shape, and overall shell form. Shell sculpture is diagnostic for a few species, and even coloration is sometimes used in the literature. However, all these characters show such a wide range of variation that reliable identification of species to *Gibbula* and *Phorcus* is difficult and depends on subjective, personal experience (e.g. Barco et al. 2013).

Among the reasons for the confusing status of some of these species are their vague original descriptions, mostly based on dry and empty shells. To exacerbate the situation, some of the type collections have been destroyed or lost (Table 1), making re-descriptions impossible. Other type collections contain many syntypes, some of which do not show consistent morphology. The vagueness of original descriptions leads to problems in recent literature used for identification, e.g. for the identification of *G. varia*, *G. rarilineata* and juvenile *G. divaricata* (Gofas et al. 2011; Nordsieck 1968), as well as *G. umbilicaris* and *G. nebulosa* (Barco et al. 2013; Oliverio pers. comm.).

In the comprehensive phylogenetic study by Donald et al. (2012), the monophyly of *Gibbula*, represented by *G. magus*, *G. fanulum*, *G. cineraria*, *G. pennanti*, *G. rarilineata*, *G. umbilicalis*, and *G. varia*, is disrupted by species of *Osilinus*, a junior synonym of *Phorcus* (Donald et al. 2012), and Williams et al. (2010) by *Jujubinus* (*See* Table 1 for taxon authorities). In the most recent molecular study based on six different genes (both mitochondrial and nuclear) by Uribe et al. (2016), the monophyly of *Gibbula* is disrupted by both *Phorcus* and *Jujubinus*. In the most comprehensive molecular study on the genus *Gibbula*, Barco et al. (2013) resolved the dilemma by using *Phorcus* species as outgroup and omitted other cantharidinid genera such as *Jujubinus*, whereby *Gibbula* was recovered monophyletic.

The morphological and phylogenetic problems in *Gibbula* and *Phorcus* (Philippi 1836-44, 1849; Thiele 1929-35; Nordsieck 1968) and their taxonomic implications were addressed by different authors (e.g. Williams et al. 2010, Uribe et al. 2016) introducing several subgenera (e.g. *Steromphala* Gray, 1847, *Phorcus* Risso, 1826, *Gibbulastra* Monterosato, 1884) which later were discarded or revised again. Risso's (1826) original species allocations to the two genera, *Gibbula* and *Phorcus*, were later re-established (Thiele 1929-35; Nordsieck 1968; Gofas and Jabaud 1997). Phylogenetic and systematic relationships remain unresolved.

Shell morphometrics

Morphometric methods have been successful in identifying certain species of gastropods via analysis of shell form (Kirchner et al. 2016). Since the classical morphometric measures like lengths and angles introduced by Thompson (1917) are insufficient to capture shape differences in the present taxa, geometric morphometric approaches utilising landmark-based algorithms are a valuable alternative. Mathematically, they are increasingly applied to assess form and shape as well as variation and intended as an objective means of comparison (Rohlf 1998). Landmarks and semi-landmarks offer the possibility to analyse objects without losing their geometrical form in the process (Bookstein 1991; Mitteroecker and Gunz 2009). Gastropod shells offer only few useful morphological landmarks as defined by Bookstein (1991). Thus, it is necessary to use semi-landmarks on outlines, which are processed later on in the sliding landmark algorithm, allowing for the landmark to slide on a tangent connection between neighbouring landmarks in order to optimise the Procrustes fit (Bookstein 1997; Mitteroecker and Gunz 2009). Cartesian coordinates gained from landmarks and semi-landmarks are aligned in a generalised Procrustes fit. Through a stepwise process, the original landmark configuration of each individual is scaled and rotated in a way to gain a group of landmark configurations showing the least possible difference. In this process, each resulting landmark configuration therefore, independent of size, orientation, and position, only comprises shape in the end (Bookstein 1996). The resulting Procrustes coordinates define the shape of an object in the Kendall shape space (Kendall 1981, 1984). Although its surface is curved, Procrustes distances can be approximated by Euclidean distances in the tangent space and can thus be analysed statistically through multivariate methods like principal components analyses (Mitteroecker and Gunz 2009). Geometric morphometrics, thus, offers the possibility of analysing and comparing complex shapes of shells of different sizes in a single sample (Mitteroecker and Gunz 2009).

Aims of the study

The genera *Gibbula* and *Phorcus* have undergone considerable changes in their 190 years of history. The rather vague morphological diagnosis of *Gibbula* and *Phorcus* left ample room for species assignment to these taxa (compare Nordsieck

Table 1 Authorities, type localities, and location of type material of *Gibbula* (s.s.), *Steromphala*, and *Phorcus* species most abundantly found in the Mediterranean

Species	Authority	Collection (Coll. numbers)	Type locality
Gibbula ardens	(Salis, 1793)	?	Naples
Gibbula fanulum	(Gmelin, 1791)	?	aquis sernabucensibus
Gibbula magus	(Linnaeus, 1758)	LSL+ZMUU (LSL.500+UPSZTY 720, 1131)	Mare Mediterraneo
Gibbula turbinoides	(Deshayes, 1835)	?	fossil, La Moree
Gibbula candei	(d'Orbigny, 1844)	?	?
Gibbula albida	(Gmelin, 1791)	?	?
Gibbula guttadauri	(Philippi, 1836)	?	Aci Castello, Sicily
Phorcus articulatus	(Lamarck, 1822)	MNHG (INVE 51532)	Mediterrané
Phorcus mutabilis	(Philippi, 1846)	?	Adriatic and Ionic Sea
Phorcus richardi	(Payraudeau, 1826)	MNHN (MNHN-IM-2000-28253)	Ile d'Corse
Phorcus turbinatus	(Born, 1778)	MNHW (NHMW 14002)	?
Phorcus lineatus	(da Costa, 1778)	? (sold in 1818, London)	British shores
Steromphala adansonii	(Payraudeau, 1826)	MNHN (MNHN-IM-2000-30071)	Golf d'Aiaccio
Steromphala adriatica	(Philippi, 1844)	?	Sicily
Steromphala divaricata	(Linnaeus, 1758)	LSL (LSL.503)	Mare Mediterraneo
Steromphala nebulosa	(Philippi, 1849)	?	Alexandria and Red Sea
Steromphala rarilineata	(Michaud, 1829)	MHNL	Mediterrané
Steromphala spratti	(Forbes, 1844)	?	Aegais
Steromphala umbilicaris	(Linnaeus, 1758)	LSL+ZMUU (LSL.504+UPSZTY 998, 1127, 1128, 1130)	Mare Mediterraneo
Steromphala varia	(Linnaeus, 1758)	LSL+ZMUU (LSL.501+UPSZTY 1136)	Mare Mediterraneo

Table 1 (continued)

Steromphala cineraria	(Linnaeus, 1758)	LSL+ZMUU (LSL.502+UPSZTY 1134, 1139)	Mare Mediterraneo
Steromphala nivosa	(A. Adams, 1853)	NHML (196898)	?
Steromphala pennanti	(Philippi, 1846)	?	English Coast
Steromphala umbilicalis	(da Costa, 1778)	? (sold in 1818, London)	British shores
Steromphala leucophaea	(Philippi, 1836)	?	l'Ognina, Sicily
Steromphala racketti	(Payraudeau, 1826)	MNHN (MNHN-IM-2000-28252)	Valinco
**Phorcus magaritaceus*	Risso 1826	MNHN (MNHN-IM-2000-28254, -2000-28255)	?
**Gibbula magus*	Risso 1826	MNHN (MNHN-IM-2012-36202, -2012-36203, -2012-36204)	?

Asterisks mark collections upon which genus descriptions were based. Species used in this study are shaded. (LSL Linnean Society of London Collection, MHNL Musée des Confluences Lyon, MNHG Museum of Natural History Geneva, MNHN Musée National d'Histoire Naturelle Paris, NHML Natural History Museum London, NHMW Natural History Museum Vienna, ZMUU Zoological Museum University of Uppsala)

1968). As broad taxon sampling in molecular phylogenetic and barcoding studies becomes more frequent, objective identification methods become more important as many of these projects rely on correct species designations based on well-acquired experience of both amateurs and scientists. The present study aims to re-evaluate the delimitation and the relationships of the genus complex *Gibbula* via an integrative approach based on DNA-barcoding, shell morphometrics, and shell characters using recently collected material with accurate locality data as well as type material of the described taxa. Furthermore, we aim to address questions regarding monophyly and internal systematics of *Gibbula* and *Phorcus* raised by recent molecular studies (Donald et al. 2012; Williams 2012; Uribe et al. 2016).

Material and methods

Sampling

Snails were collected by snorkelling off the north coast of Crete (Greece): west of Agia Pelagia (35° 24′ 21.8″ N 24° 50′ 07.7″ E), south coast: Martsalo valley (34° 55′ 48.0″ N 24° 46′ 14.0″ E), Dyticus bay (34° 55′ 46.2″ N 24° 54′ 45.9″ E), Lentas (34° 55′ 48.2″ N 24° 55′ 24.4″ E), Rovinj (Croatia, 45° 07′ 03.7″ N 13° 36′ 59.4″ E), and Lebanon (south of Tyre, 33° 15′ 38.0″ N 35° 12′ 31.2″ E) at 0.3–3 m depth (Fig. 1). From Croatia, 30 specimens were collected from a rocky shore neighbouring a zone of macro algae. Marsalto Valley (30 specimens) represents a sheltered habitat with a solid rock ground, whereas Lentas and Dyticus Bay (two specimens each) have coarse gravel. Another 26 specimens from the Agia Pelagia area (very exposed, coarse gravel) and six specimens from Lebanon (artificial coastal protection boulders) were included (Table 2). The animals were anaesthetized with isotonic $MgCl_2$ solution and then frozen at − 80 °C or preserved in 96% ethanol. Shells and tissues were separated and individualised. The shells were kept under dry conditions. DNA extractions (Tissue DNA Mini Kit PeqGOLD, VWR, Germany) from foot tissue and PCR reactions with COI primers LCO and HCO (Folmer et al. 1994) followed standard protocols. PCR reactions were cleaned up with the Cycle-Pure Kit (PeqGOLD, VWR, Germany). Sequencing was carried out on standard ABI Sequencers (VBC Biotech, Vienna, Austria). Shells, tissue, and DNA extractions are kept for further studies at the Department of Integrative Zoology, University of Vienna.

In addition to these new samples, the type material for the following species was examined: *Phorcus articulatus* (Lamarck 1822), *Ph. richardi* (Payraudeau 1826), *Ph. turbinatus* (von Born, 1778), *Steromphala divaricata*

Fig. 1 Sampling sites for Mediterranean specimens of *Steromphala* and *Phorcus* species

(Linnaeus, 1758), *St. umbilicaris* (Linnaeus, 1758), *St. varia* (Linnaeus, 1758), *St. adansonii* (Payraudeau 1826), and *St. cineraria* (Linnaeus, 1758). However, as *Steromphala cineraria* does not occur in the Mediterranean (Gofas et al. 2011) and is clearly distinguished by its spiral ridges, it was excluded from the morphometric analyses.

The Linnean type material poses several problems. Although all species described by Linnaeus (1758) are represented in the collection of the Linnean Society London, it is not clear how many of them were used for the description or may have been added to the collection by later researchers and curators. Linneaus did not report numbers of specimens or individual type localities. Vague localities, such as "Mare Mediterraneo," provide no geographical clue for lectotype designations. We hope to demonstrate here that the chosen approach is suitable to link DNA-free types with extant specimens.

Photography

Shells were stabilised on a piece of clay and photographed in standardised lateral and ventral positions under identical optical settings with a Nikon SMZ 1500 stereomicroscope (Nikon Corp., Japan) (see Fig. 2). The shells in the Linnean Society London were photographed by one of the authors (SA). All other images of type material were provided by curators of type collections and taken under similar conditions. The lateral views are stacked in Adobe Photoshop (Adobe, Inc.) from up to 12 images at different focal depths. The lateral view shows the shell with the apex, the attachment point of the aperture on the previous whorl, and the umbilicus centre connected by an imaginary horizontal line. The ventral view is oriented in a way that the apex-umbilicus line is vertical.

Morphometric analysis

The images were landmarked in tps Dig2 2.16 (Rohlf 2010). The lateral view shows a set of 44 landmarks, including 29 semi-landmarks. The fixed landmarks in the lateral view (Fig. 2a) are the following: 1 = apex, 2 = leftmost point visible on the suture between last and second last whorl, 3 = attachment point of the aperture lip, 4 = rightmost point visible on the suture between ultimate and penultimate whorl, 5 = crossing point of aperture

Table 2 Sampling sites and numbers of individuals collected

Sampling site	# of Individuals analysed	Coordinates
Rovinj, Croatia	30	45° 07′ 03.7″ N 13° 36′ 59.4″ E
Agia Pelagia, Crete, Greece	26	35° 24′ 21.8″ N 24° 50′ 07.7″ E
Martsalo Valley, Crete, Greece	30	34° 55′ 48.0″ N 24° 46′ 14.0″ E
Lentas Bay, Crete, Greece	2	34° 55′ 48.2″ N 24° 55′ 24.4″ E
Dyticus Bay, Crete, Greece	2	34° 55′ 46.2″ N 24° 54′ 45.9″ E
Lebanon	6	33° 15′ 38.0″ N 35° 12′ 31.2″ E

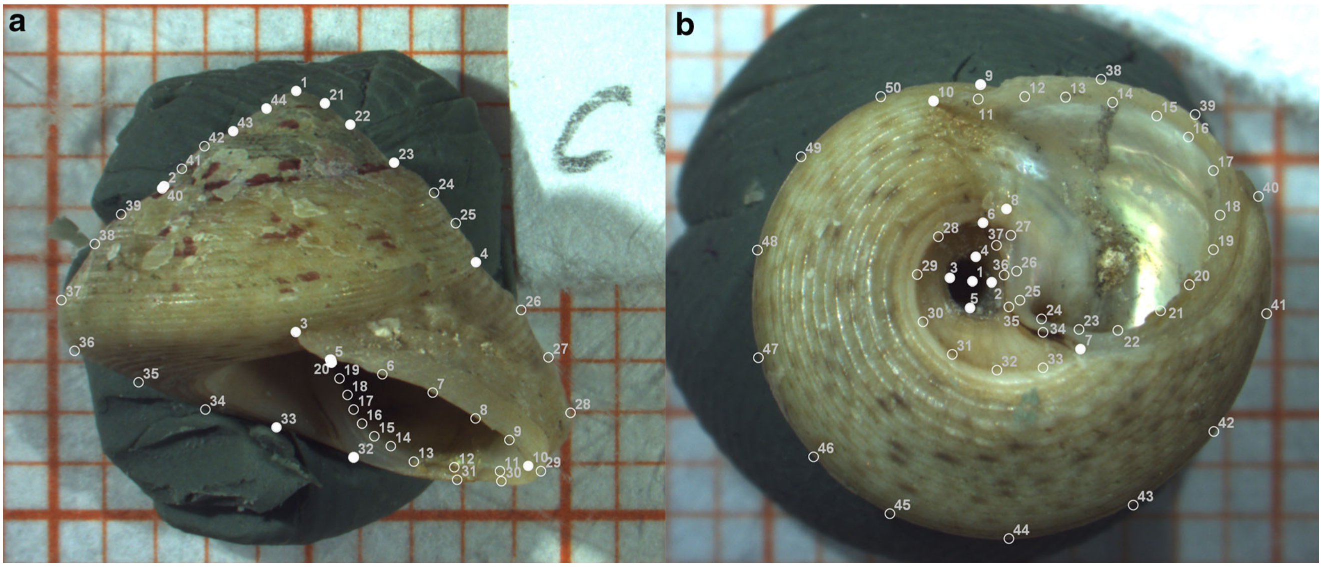

Fig. 2 Landmark templates for representative **a** lateral and **b** ventral standardised views. Filled circles show fixed landmarks. Empty circles show semi-landmarks, processed as sliding landmarks

lip and columella, 10 = leftmost point on the aperture lip, 20 = last visible point of the columella, 21–23 = leftmost points on the sutures, 32 = lowest point of the columellar fold, 33 = centre of the umbilicus, 40 = shoulder of the last whorl, and 43–44 = rightmost points of the sutures. The ventral view includes a set of 50 landmarks, 40 of which are semi-landmarks. The fixed landmarks in the lateral view (Fig. 2b) are defined as following: 1 = centre of the umbilicus, 2 = umbilicus edge closest to the

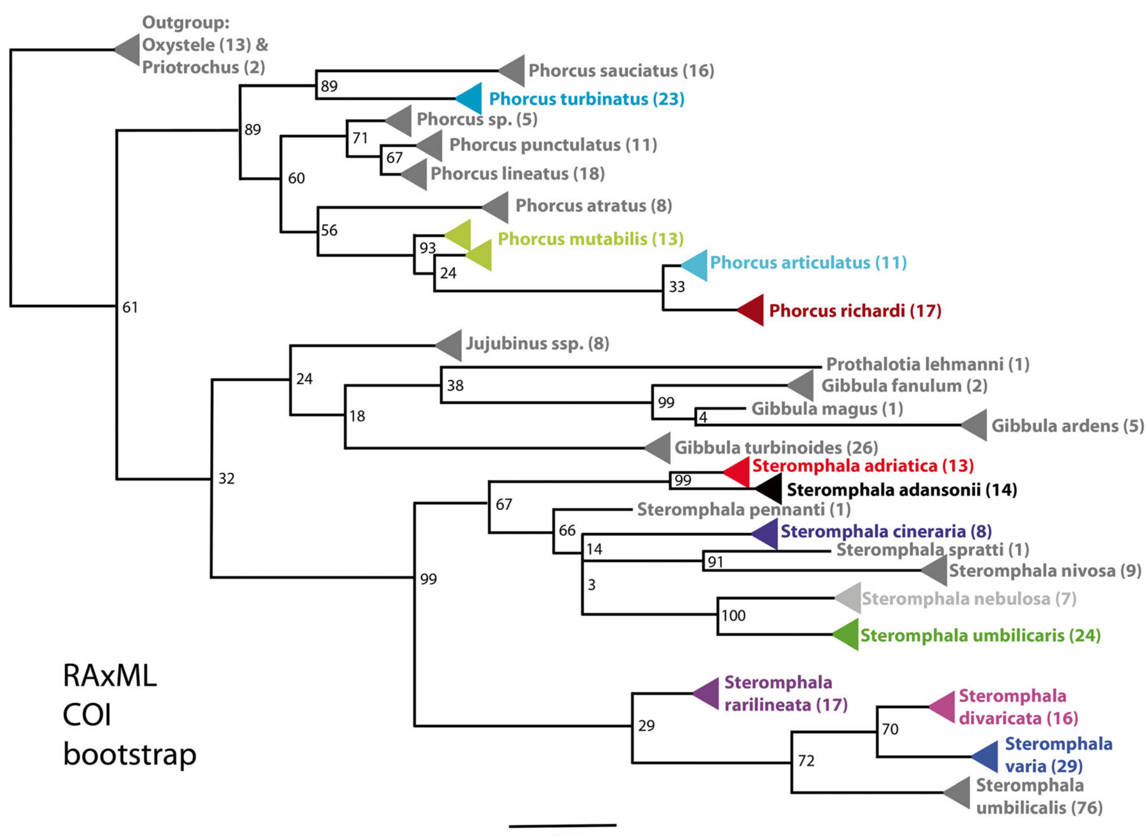

Fig. 3 RAxML based phylogenetic reconstruction (GTRGAMMA model and 1000 rapid bootstrap replicates). COI barcodes were used to identify species and reconstruct relationships between species and genera. Number of individuals per species is in parentheses. Bootstrap values are given at nodes. Species evaluated in morphometric analysis are in corresponding colours (compare Table 4)

Table 3 List of specimens used in the phylogenetic analyses

Species	Voucher ID	Accession number COI	Reference
Gibbula ardens (Salis, 1793)	1075.4-5, 10-11	JQ839302-JQ839303, JQ839309- JQ839310	Barco et al. 2013
Gibbula ardens (Salis, 1793)	1097.2	JQ839306	Barco et al. 2013
Gibbula fanulum (Gmelin, 1791)	1088	JQ839325	Barco et al. 2013
Gibbula fanulum (Gmelin, 1791)	GFAN.KRC.1	GQ232363	Williams et al. 2010
Gibbula magus (Linnaeus, 1758)	GMAG.FAR.1	GQ232364	Williams et al. 2010
Gibbula turbinoides (Deshayes, 1835)	1080.1–17, 19–22, 24–28	JQ839351-JQ839376	Barco et al. 2013
Jujubinus exasperatus (Pennant, 1777)	JEXA.KRC.1	GQ232368	Williams et al. 2010
Jujubinus montagui (Wood, 1828)	KR084531	KR084531	Barco et al. 2016
Jujubinus montagui (Wood, 1828)	KR084831	KR084831	Barco et al. 2016
Jujubinus striatus (Linnaeus, 1758)	CSTR.CAL.1	GQ232361	Williams et al. 2010
Jujubinus striatus (Linnaeus, 1758)	ech50	KJ183017	Cowart et al. Unpublished
Jujubinus striatus (Linnaeus, 1758)	KR084400	KR084400	Barco et al. 2016
Oxystele impervia (Menke, 1843)	South Africa	DQ061093	Donald, Kennedy and Spencer 2005
Oxystele sinensis (Gmelin, 1791)	OsHB18	JX303353	Muteveri et al. Unpublished
Oxystele sinensis (Gmelin, 1791)	OsHH03	JX303372	Muteveri et al. Unpublished
Oxystele sinensis (Gmelin, 1791)	OsKH01	JX303354	Muteveri et al. Unpublished
Oxystele sinensis (Gmelin, 1791)	OsPA23	JX303371	Muteveri et al. Unpublished
Oxystele tabularis (Krauss, 1848)	South Africa	DQ061090	Donald, Kennedey and Spencer 2005
Oxystele tigrina (Dillwyn, 1817)	OtHH01	JX303448	Muteveri et al. Unpublished
Oxystele tigrina (Dillwyn, 1817)	OtKH24	JX303442	Muteveri et al. Unpublished
Oxystele tigrina (Dillwyn, 1817)	OtPA09	JX303447	Muteveri et al. Unpublished
Oxystele tigrina (Dillwyn, 1817)	OtPE18	JX303443	Muteveri et al. Unpublished
Oxystele variegata (Anton, 1838)	OvCA23	JX303471	Muteveri et al. Unpublished
Oxystele variegata (Anton, 1838)	OvHB01	JX303472	Muteveri et al. Unpublished
Oxystele variegata (Anton, 1838)	OvKH17	JX303485	Muteveri et al. Unpublished
Oxystele variegata (Anton, 1838)	OvPE05	JX303486	Muteveri et al. Unpublished
Phorcus articulatus (Lamarck 1822)	1 1Spain	JN686286	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	1a 1Tunisia	JN686283	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	1d 2Spain	JN686279	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	1 s 1Spain	JN686280	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	2 1Spain	JN686287	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	2a 2Tunisia	JN686284	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	3 Spain	JN686288	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	3a Tunisia	JN686285	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	5 1Spain	JN686289	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	6 2Tunisia	JN686290	Donald et al. 2012
Phorcus articulatus (Lamarck 1822)	Lib13_Lebanon	KY364988	this study
Phorcus atratus (Wood, 1828)	1 Spain	JN686291	Donald et al. 2012
Phorcus atratus (Wood, 1828)	1 Spain	JN686293	Donald et al. 2012
Phorcus atratus (Wood, 1828)	1 Spain	JN686295	Donald et al. 2012
Phorcus atratus (Wood, 1828)	1 Spain	JN686297	Donald et al. 2012
Phorcus atratus (Wood, 1828)	2 Spain	JN686294	Donald et al. 2012
Phorcus atratus (Wood, 1828)	2 Spain	JN686298	Donald et al. 2012
Phorcus atratus (Wood, 1828)	3 Spain	JN686292	Donald et al. 2012
Phorcus atratus (Wood, 1828)	3 Spain	JN686296	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 France	JN686331	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 Morocco	JN686325	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 Morocco	JN686333	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 Portugal	JN686317	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 Spain	JN686324	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 United Kingdom	JN686321	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	1 United Kingdom	JN686328	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	2 Morocco	JN686326	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	2 Portugal	JN686318	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	2 Portugal	JN686319	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	2 United Kingdom	JN686322	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	2 United Kingdom	JN686329	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	3 France	JN686332	Donald et al. 2012

Table 3 (continued)

Species	Voucher ID	Accession number COI	Reference
Phorcus lineatus (da Costa, 1778)	3 Morocco	JN686327	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	3 Portugal	JN686320	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	3 United Kingdom	JN686323	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	3 United Kingdom	JN686330	Donald et al. 2012
Phorcus lineatus (da Costa, 1778)	Figueras55	JN241978	Prado-Sanchez et al. Unpublished
Phorcus mutabilis (Philippi, 1846)	1 Italy	JN686364	Donald et al. 2012
Phorcus mutabilis (Philippi, 1846)	1 Turkey	JN686339	Donald et al. 2012
Phorcus mutabilis (Philippi, 1846)	1278.2, 4, 7	JX887430, JX887455, JX887446	Barco et al. 2013
Phorcus mutabilis (Philippi, 1846)	2 Italy	JN686365	Donald et al. 2012
Phorcus mutabilis (Philippi, 1846)	C12_Croatia	KY364918	this study
Phorcus mutabilis (Philippi, 1846)	C13_Croatia	KY364919	this study
Phorcus mutabilis (Philippi, 1846)	C14_Croatia	KY364920	this study
Phorcus mutabilis (Philippi, 1846)	C15_Croatia	KY364921	this study
Phorcus mutabilis (Philippi, 1846)	N02_NCrete	KY364934	this study
Phorcus mutabilis (Philippi, 1846)	N08_NCrete	KY364940	this study
Phorcus mutabilis (Philippi, 1846)	S24_SCrete	KY364978	this study
Phorcus punctulatus (Lamarck 1822)	1 Senegal	JN686357	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	10 Senegal	JN686350	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	11 Senegal	JN686351	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	12 Senegal	JN686352	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	2 Senegal	JN686358	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	3 Senegal	JN686359	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	4 Senegal	JN686360	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	5 Senegal	JN686353	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	6 Senegal	JN686354	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	8 Senegal	JN686355	Donald et al. 2012
Phorcus punctulatus (Lamarck 1822)	9 Senegal	JN686356	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	1 Croatia	JN686281	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	1 Spain, Almuñecar	JN686361	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	1 Spain, Cabo Raja	JN686362	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	1278.1, 3, 5, 8	JX887443, JX887433, JX887457, JX887444	Barco et al. 2013
Phorcus richardi (Payraudeau 1826)	2 Croatia	JN686282	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	2 Spain	JN686363	Donald et al. 2012
Phorcus richardi (Payraudeau 1826)	D01_SCrete	KY364929	This study
Phorcus richardi (Payraudeau 1826)	D02_SCrete	KY364930	This study
Phorcus richardi (Payraudeau 1826)	L01_SCrete	KY364931	This study
Phorcus richardi (Payraudeau 1826)	L02_SCrete	KY364932	This study
Phorcus richardi (Payraudeau 1826)	N01_NCrete	KY364933	This study
Phorcus richardi (Payraudeau 1826)	N03_NCrete	KY364935	This study
Phorcus richardi (Payraudeau 1826)	N04_NCrete	KY364936	This study
Phorcus richardi (Payraudeau 1826)	N05_NCrete	KY364937	This study
Phorcus richardi (Payraudeau 1826)	N06_NCrete	KY364938	This study
Phorcus richardi (Payraudeau 1826)	N07_NCrete	KY364939	This study
Phorcus richardi (Payraudeau 1826)	N09_NCrete	KY364941	This study
Phorcus richardi (Payraudeau 1826)	N10_NCrete	KY364942	This study
Phorcus richardi (Payraudeau 1826)	N11_NCrete	KY364943	This study
Phorcus richardi (Payraudeau 1826)	N12_NCrete	KY364944	This study
Phorcus richardi (Payraudeau 1826)	N13_NCrete	KY364945	This study
Phorcus richardi (Payraudeau 1826)	N14_NCrete	KY364946	This study
Phorcus richardi (Payraudeau 1826)	N15_NCrete	KY364947	This study
Phorcus richardi (Payraudeau 1826)	N16_NCrete	KY364948	This study
Phorcus richardi (Payraudeau 1826)	N17_NCrete	KY364949	This study
Phorcus richardi (Payraudeau 1826)	N18_NCrete	KY364950	This study
Phorcus richardi (Payraudeau 1826)	N19_NCrete	KY364951	This study
Phorcus richardi (Payraudeau 1826)	N20_NCrete	KY364952	This study
Phorcus richardi (Payraudeau 1826)	N21_NCrete	KY364953	This study
Phorcus richardi (Payraudeau 1826)	N22_NCrete	KY364954	This study
Phorcus richardi (Payraudeau 1826)	N23_NCrete	KY364955	This study
Phorcus richardi (Payraudeau 1826)	N24_NCrete	KY364956	This study
Phorcus richardi (Payraudeau 1826)	S02_SCrete	KY364958	This study
Phorcus richardi (Payraudeau 1826)	S03_SCrete	KY364959	This study

Table 3 (continued)

Species	Voucher ID	Accession number COI	Reference
Phorcus richardi (Payraudeau 1826)	S04_SCrete	KY364960	This study
Phorcus richardi (Payraudeau 1826)	S06_SCrete	KY364962	This study
Phorcus richardi (Payraudeau 1826)	S07_SCrete	KY364963	This study
Phorcus richardi (Payraudeau 1826)	S08_SCrete	KY364964	This study
Phorcus richardi (Payraudeau 1826)	S09_SCrete	KY364965	This study
Phorcus richardi (Payraudeau 1826)	S13_SCrete	KY364968	This study
Phorcus richardi (Payraudeau 1826)	S14_SCrete	KY364969	This study
Phorcus richardi (Payraudeau 1826)	S15_SCrete	KY364970	This study
Phorcus richardi (Payraudeau 1826)	S16_SCrete	KY364971	This study
Phorcus richardi (Payraudeau 1826)	S19_SCrete	KY364973	This study
Phorcus richardi (Payraudeau 1826)	S20_SCrete	KY364974	This study
Phorcus richardi (Payraudeau 1826)	S21_SCrete	KY364975	This study
Phorcus richardi (Payraudeau 1826)	S27_SCrete	KY364980	This study
Phorcus richardi (Payraudeau 1826)	S29_SCrete	KY364982	This study
Phorcus richardi (Payraudeau 1826)	S30_SCrete	KY364983	This study
Phorcus sauciatus (Koch, 1845)	1 Morocco	JN686307	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Morocco	JN686312	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Portugal	JN686300	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Spain	JN686299	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Spain	JN686305	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Spain	JN686309	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	1 Spain	JN686314	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	2 Morocco	JN686308	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	2 Portugal	JN686306	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	2 Spain	JN686301	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	2 Spain	JN686303	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	2 Spain	JN686310	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	3 Morocco	JN686313	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	3 Spain	JN686302	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	3 Spain	JN686304	Donald et al. 2012
Phorcus sauciatus (Koch, 1845)	3 Spain	JN686311	Donald et al. 2012
Phorcus sp	1 Cape Verde	JN686334	Donald et al. 2012
Phorcus sp	1 Cape Verde	JN686337	Donald et al. 2012
Phorcus sp	2 Cape Verde	JN686338	Donald et al. 2012
Phorcus sp	3 Cape Verde	JN686336	Donald et al. 2012
Phorcus sp	2 Cape Verde	JN686335	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	1 Croatia	JN686344	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	1 Cyprus	JN686341	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	1 Italy	JN686348	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	1 Spain O.turbinatus1 3496	JN686340	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	1 Spain O.turbinatus2	JN686343	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	2 Spain	JN686346	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	2 Turkey	JN686345	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	283,993,123	GQ434018	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	3 Cyprus	JN686342	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	3 Spain	JN686347	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	3Italy	JN686349	Donald et al. 2012
Phorcus turbinatus (Born, 1778)	Lib02_Lebanon	KY364985	this study
Phorcus turbinatus (Born, 1778)	Lib03_Lebanon	KY364986	this study
Phorcus turbinatus (Born, 1778)	Lib04_Lebanon	KY364987	this study
Phorcus turbinatus (Born, 1778)	N26_NCrete	KY364989	this study
Phorcus turbinatus (Born, 1778)	N27_NCrete	KY364992	this study
Phorcus turbinatus (Born, 1778)	NHMUK20080408	GQ434019	Williams et al. 2010
Priotrochus kotschyi (Philippi 1849)	1 Bahrain	JN686315	Donald et al. 2012
Priotrochus kotschyi (Philippi 1849)	3 Bahrain	JN686316	Donald et al. 2012
Prothalotia lehmanni (Menke, 1843)	PLEH.DNS.1	EU530123	Williams, Karube and Ozawa 2008
Steromphala adansonii (Payraudeau 1826)	1072.2-11	JQ839291-JQ839300	Barco et al. 2013
Steromphala adansonii (Payraudeau 1826)	1075.2	JQ839290	Barco et al. 2013
Steromphala adansonii (Payraudeau 1826)	1284.3, 4	JX887439, JX887458	Barco et al. 2013
Steromphala adansonii (Payraudeau 1826)	C16_Croatia	KY364922	This study
Steromphala adriatica (Philippi, 1844)	S05_SCrete	KY364961	This study

Table 3 (continued)

Species	Voucher ID	Accession number COI	Reference
Steromphala adriatica (Philippi, 1844)	S11_SCrete	KY364966	This study
Steromphala adriatica (Philippi, 1844)	S12_SCrete	KY364967	This study
Steromphala adriatica (Philippi, 1844)	S18_SCrete	KY364972	This study
Steromphala adriatica (Philippi, 1844)	S22_SCrete	KY364976	This study
Steromphala adriatica (Philippi, 1844)	S23_SCrete	KY364977	This study
Steromphala adriatica (Philippi, 1844)	S25_SCrete	KY364979	This study
Steromphala adriatica (Philippi, 1844)	S28_SCrete	KY364981	This study
Steromphala adriatica[a] (Philippi, 1844)	1260	JX887463	Barco et al. 2013
Steromphala adriatica[a] (Philippi, 1844)	1277.4, 6	JX887465, JX887452	Barco et al. 2013
Steromphala adriatica[a] (Philippi, 1844)	1286.1, 2, 4, 5	JX887435, JX887453, JX887431, KC417497	Barco et al. 2013
Steromphala adriatica[b] (Philippi, 1844)	146335560	EF541179	Samadi & Steiner unpublished
Steromphala cineraria (Linnaeus, 1758)	ech49	KJ183016	Cowart et al. Unpublished
Steromphala cineraria (Linnaeus, 1758)	GCIN.WEM.1	AM049339	Williams and Ozawa 2006
Steromphala cineraria (Linnaeus, 1758)	MT09438	KR084411	Barco et al. 2016
Steromphala cineraria (Linnaeus, 1758)	MT09439	KR084951	Barco et al. 2016
Steromphala cineraria (Linnaeus, 1758)	MT09464	KR084500	Barco et al. 2016
Steromphala cineraria (Linnaeus, 1758)	MT09467	KR084441	Barco et al. 2016
Steromphala cineraria (Linnaeus, 1758)	MT09471	KR084415	Barco et al. 2016
Steromphala divaricata (Linnaeus, 1758)	1076.1, 3, 4, 7, 11	JQ839319, JQ839321-JQ839324	Barco et al. 2013
Steromphala divaricata (Linnaeus, 1758)	1087.1-7	JQ839313-JQ839318, JQ839320	Barco et al. 2013
Steromphala divaricata (Linnaeus, 1758)	BAU1253 Italy	KC417500	Barco et al. 2013
Steromphala divaricata (Linnaeus, 1758)	C17_Croatia	KY364923	This study
Steromphala divaricata (Linnaeus, 1758)	C20_Croatia	KY364924	This study
Steromphala divaricata (Linnaeus, 1758)	C21_Croatia	KY364925	This study
Steromphala divaricata (Linnaeus, 1758)	C22_Croatia	KY364926	This study
Steromphala divaricata (Linnaeus, 1758)	C24_Croatia	KY364928	This study
Steromphala nebulosa (Philippi, 1848)	1086.5, 7 1098.2	JQ8393228, JQ8393230, JQ839327	Barco et al. 2013
Steromphala nebulosa (Philippi, 1848)	C35_Croatia	KY364991	this study
Steromphala nivosa (Adams, 1853)	1018.1-3, 1078.1-6	JQ839332-JQ839340	Barco et al. 2013
Steromphala pennanti (Pennanti, 1846)	GPEN.RSF.1	GQ232365	Williams et al. 2010
Steromphala rarilineata (Michaud, 1829)	1076.8	JQ839344	Barco et al. 2013
Steromphala rarilineata (Michaud, 1829)	1079.1-8	JQ839341-JQ839343, JQ839345 -JQ839349	Barco et al. 2013
Steromphala rarilineata (Michaud, 1829)	1279.1-3, 5, 6	JX887451, JX887466, JX887467, JX887471	Barco et al. 2013
Steromphala rarilineata (Michaud, 1829)	1287.6	JX887450	Barco et al. 2013
Steromphala rarilineata (Michaud, 1829)	C23_Croatia	KY364927	this study
Steromphala rarilineata (Michaud, 1829)	GRAR.KRC.1	GQ232366	Williams et al. 2010
Steromphala rarilineata (Michaud, 1829)	S01_SCrete	KY364957	this study
Steromphala spratti (Forbes, 1844)	1108	JQ839350	Barco et al. 2013
Steromphala umbilicalis (da Costa, 1778)	1 UK	JN686273	Donald et al. 2012
Steromphala umbilicalis (da Costa, 1778)	1A10	KP064694	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A11	KP064695	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A12	KP064696	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A14	KP064697	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A15	KP064698	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A18	KP064699	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A19	KP064700	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A22	KP064701	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A23	KP064702	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A29	KP064703	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A5	KP064704	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1A7	KP064705	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1G7	KP064706	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	1G8	KP064707	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	2 Portugal	JN686275	Donald et al. 2012
Steromphala umbilicalis (da Costa, 1778)	2 Spain	JN686277	Donald et al. 2012
Steromphala umbilicalis (da Costa, 1778)	2 UK	JN686274	Donald et al. 2012

Table 3 (continued)

Species	Voucher ID	Accession number COI	Reference
Steromphala umbilicalis (da Costa, 1778)	3 Portugal	JN686276	Donald et al. 2012
Steromphala umbilicalis (da Costa, 1778)	3 Spain	JN686278	Donald et al. 2012
Steromphala umbilicalis (da Costa, 1778)	3A19	KP064708	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	3A52	KP064709	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5A12	KP064710	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5A5	KP064711	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5A8	KP064712	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5A9	KP064713	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G106	KP064714	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G107	KP064715	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G110	KP064716	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G111	KP064717	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G116	KP064718	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G149	KP064719	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G150	KP064720	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G156	KP064721	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G166	KP064722	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G40	KP064723	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G53	KP064724	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G56	KP064725	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G65	KP064726	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G69	KP064727	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G77	KP064728	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G95	KP064729	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	5G97	KP064730	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	6A10	KP064731	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	6A14	KP064732	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	6A4	KP064733	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	6G11	KP064734	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	6G8	KP064735	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	7G11	KP064736	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	7G12	KP064737	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	7G19	KP064738	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A1	KP064739	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A12	KP064740	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A15	KP064741	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A17	KP064742	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A20	KP064743	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A24	KP064744	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A3	KP064745	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8A30	KP064746	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G1	KP064747	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G13	KP064748	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G16	KP064749	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G17	KP064750	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G20	KP064751	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G23	KP064752	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G26	KP064753	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G36	KP064754	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G5	KP064755	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	8G8	KP064756	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	GN7	KJ818224	Muñoz-Colmenero et al. 2015
Steromphala umbilicalis (da Costa, 1778)	GUMB.WEM.1	GQ232367	Williams et al. 2010
Steromphala umbilicalis (da Costa, 1778)	SPedro13, 2, 4, 5, 6	JN241975, JN241973, JN241976, JN241977, JN241974	Prado-Sanchez et al. Unpublished
Steromphala umbilicaris (Linnaeus, 1758)	1083.2-8	JQ839383-JQ839389	Barco et al. 2013
Steromphala umbilicaris (Linnaeus, 1758)	1084.1-5	JQ839378-JQ839381, JQ839390	Barco et al. 2013

Table 3 (continued)

Species	Voucher ID	Accession number COI	Reference
Steromphala umbilicaris (Linnaeus, 1758)	1086.2, 3	JQ839382, JQ839377	Barco et al. 2013
Steromphala umbilicaris (Linnaeus, 1758)	1275.1, 2, 6, 8-11	JX887442, JX887447, JX887461, JX887468-JX887470, JX887472	Barco et al. 2013
Steromphala umbilicaris (Linnaeus, 1758)	1286.6	JX887428	Barco et al. 2013
Steromphala umbilicaris (Linnaeus, 1758)	C26_Croatia	KY364993	this study
Steromphala umbilicaris (Linnaeus, 1758)	C27_Croatia	KY364990	this study
Steromphala umbilicaris (Linnaeus, 1758)	C34_Croatia	KY364994	this study
Steromphala varia (Linnaeus, 1758)	1257	KC417496	Barco et al. 2013
Steromphala varia (Linnaeus, 1758)	1081.1, 3	JQ839395, JQ839391	Barco et al. 2013
Steromphala varia (Linnaeus, 1758)	1082.3-5	JQ839392- JQ839394	Barco et al. 2013
Steromphala varia (Linnaeus, 1758)	1262.1-2	JX887438, JX887429	Barco et al. 2013
Steromphala varia (Linnaeus, 1758)	1276.1-7, 9, 11-13, 16-18	JX887427, JX887432, JX887434, JX887437, JX887440, JX887441, JX887448, JX887449, JX887454, JX887456, JX887459, JX887460, JX887462, JX887464	Barco et al. 2013
Steromphala varia (Linnaeus, 1758)	C01_Croatia	KY364908	This study
Steromphala varia (Linnaeus, 1758)	C02_Croatia	KY364909	This study
Steromphala varia (Linnaeus, 1758)	C03_Croatia	KY364910	This study
Steromphala varia (Linnaeus, 1758)	C04_Croatia	KY364911	This study
Steromphala varia (Linnaeus, 1758)	C05_Croatia	KY364912	This study
Steromphala varia (Linnaeus, 1758)	C06_Croatia	KY364913	This study
Steromphala varia (Linnaeus, 1758)	C07_Croatia	KY364914	This study
Steromphala varia (Linnaeus, 1758)	C08_Croatia	KY364915	This study
Steromphala varia (Linnaeus, 1758)	C09_Croatia	KY364916	This study
Steromphala varia (Linnaeus, 1758)	C10_Croatia	KY364917	This study
Steromphala varia (Linnaeus, 1758)	Lib01_Lebanon	KY364984	This study

[a] Genbank entry as *Gibbula adansonii*

[b] Genbank entry as *Gibbula varia*

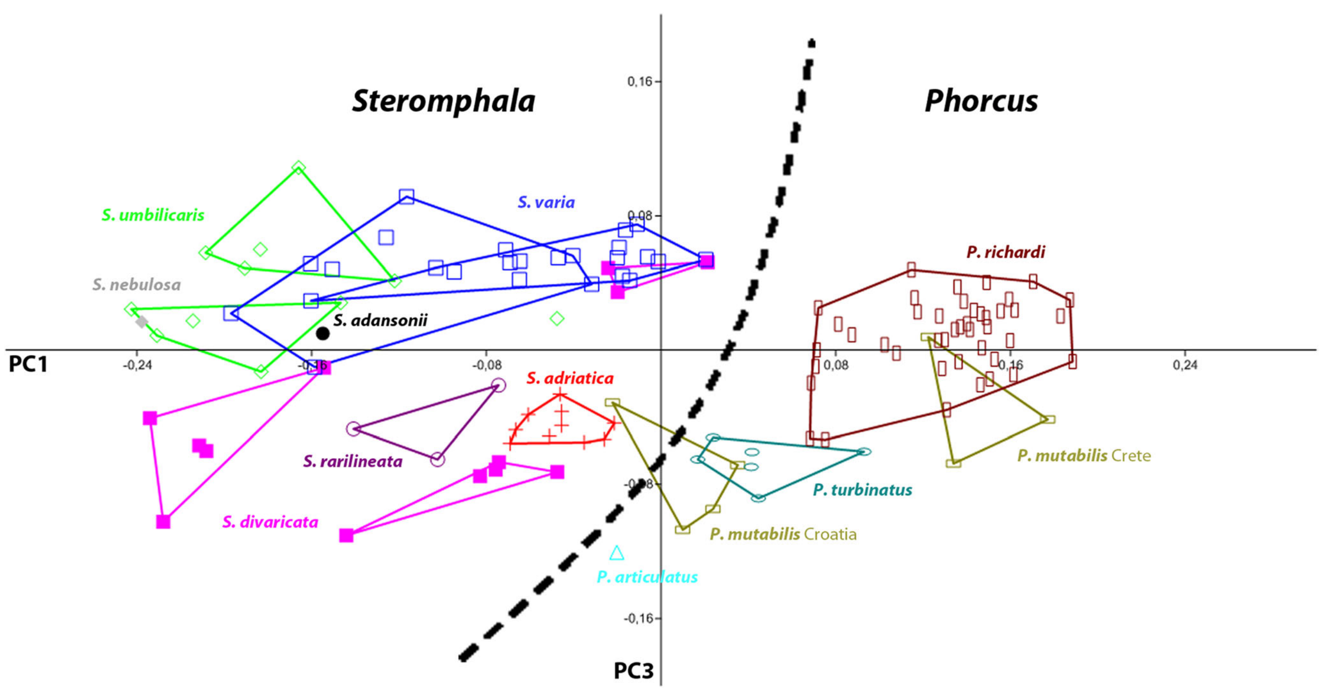

Fig. 4 Plot of first and third principal component of combined lateral and ventral landmark data from *Phorcus* and *Steromphala* individuals. A morphological separation of the two genera becomes apparent

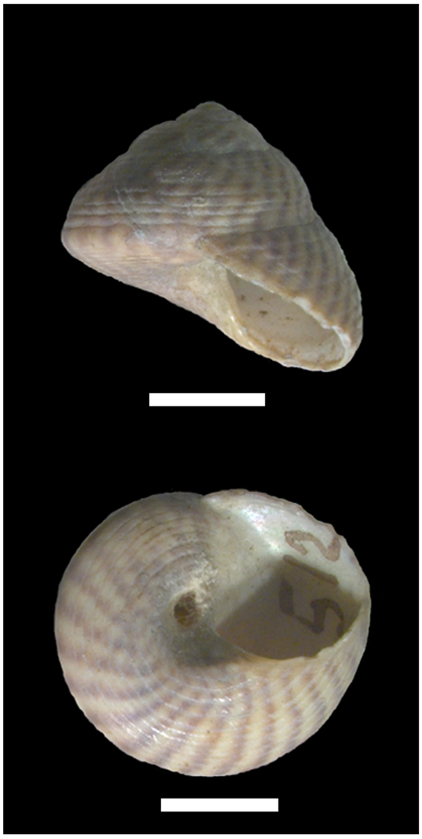

Fig. 5 Lectotype of *Steromphala cineraria* (Linnaeus, 1758). Located at LSL (LSL.502). Scale bar 5 mm

columella, 3 = umbilicus edge facing LM2, 4 = umbilicus edge closest to basis of columella end, 5 = umbilicus edge facing LM4, 6 = basis of the columella at umbilicus side, 7 = uppermost point of umbilicus atrium, 8 = basis of columella at aperture side, 9 = crossing point of aperture lip and shell edge, and 10 = attachment point of the aperture lip. After an initial round of setting landmarks on all pictures, all authors checked the complete data set for missed landmarks and correct positioning.

Semi-landmarks were defined as sliding landmarks in tps util (Rohlf 2009). The generalised Procrustes fit was carried out in tps relw (Rohlf 2005). Aligned landmark sets were checked for outliers in an XY-plot. The ventral and lateral coordinate sets were concatenated for a combined analysis. The aligned specimen coordinates were processed via a principal components analysis in PAST 2.12 (Hammer et al., 2001). First three principal components were chosen based on scree plots and separation in scatter plots.

COI sequences were checked for contamination by BLAST searches and then aligned with available sequences of this group in ClustalX. Sequences of *Priotrochus* and *Oxystele* served as outgroups (Table 3). Maximum Likelihood analyses were made in raxmlGUI (Silvestro and Michalak 2011) using the GTRGAMMA model with ML + 1000 rapid bootstrap replicates (*See* Online Resources for the reduced alignment in relaxed PHYLIP format and the ML-tree in pdf format).

Results

In the phylogenetic reconstruction based on the COI gene, the genera *Jujubinus* and *Prothalotia* dissolve the monophyly of *Gibbula*. The clade of the *Gibbula* type species, *G. magus*, includes the species *G. ardens* and *G. fanulum*. The type species *Steromphala cineraria*, *St. adriatica*, *St. adansonii*, *St. pennanti*,

Table 4 Species names assigned to specimen shells according to phylogenetic reconstruction based on COI sequences

Specimen ID	Species	Colour	Collection site
Lib13	*Phorcus articulatus*	Turquoise	Lebanon
C12-C15, N02, N08, S24	*Phorcus mutabilis*	Olive	Croatia, Crete north, Crete south
D01–D02, L01–L02, N01, N03–N07, N09–N24, S02–S04, S06–S09, S13–S16, S19–S21, S27, S29–S30	*Phorcus richardi*	Maroon	Crete north, Crete south
N26–N27, Lib02–Lib05*	*Phorcus turbinatus*	Petrol	Crete north, Lebanon
C16	*Steromphala adansonii*	Black	Croatia
S05, S11–S12, S18, S22–S23, S25, S26*, S28	*Steromphala adriatica*	Red	Crete south
Linne26–Linne35	*Steromphala cineraria*	Dark blue	
C17, C20–C22, C24, Linne36–Linne43	*Steromphala divaricata*	Pink	Croatia,
C35	*Steromphala nebulosa*	Grey	Croatia
C23, S01, S10*	*Steromphala rarilineata*	Violet	Croatia, Crete south
C18*, C26–C27, C33*, C34, Linne20–Linne25	*Steromphala umbilicaris*	Green	Croatia
C01–C10, Lib01, Linne06–Linne19	*Steromphala varia*	Blue	Croatia, Lebanon, –

Specimens marked with * were identified by shell characters only. Colour codes assigned to species as used in morphometric analyses. Specimens were coded for collection sites with running numbers: C Croatia, N Crete north, S Crete south, Lib Lebanon, D Crete south Dyticus, L Crete south Lentas, Linne type collection LSL

Table 5 Variance explained by the first three axes of principal component analyses for combined lateral and ventral landmark sets of *Steromphala*, *Phorcus*, and both

	PC1	PC2	PC3
Steromphala + *Phorcus*	49.37%	13.64%	7.57%
Steromphala	32.04%	21.86%	13.45%
Phorcus	30.17%	21.07%	7.96%

St. spratti, *St. nivosa*, *St. nebulosa*, *St. umbilicaris*, *St. rarilineata*, *St. umbilicalis*, *St. divaricata*, and *St. varia* are nested in a second clade, *Steromphala* Gray, 1847. The genus *Phorcus* is monophyletic. The genus clades *Phorcus*, *Gibbula*, and *Steromphala* show robust bootstrap support (Fig. 3).

The individuals comprising this study were assigned to 11 nominal species (Fig. 3). These species allocations (Table 4) were used in the geometric morphometric analyses.

One specimen is placed in *Steromphala adansonii*. Eight individuals are classified as *St. adriatica*. Five specimens clustered within *St. divaricata* and two into *St. rarilineata*. Eleven individuals were barcoded as *St. varia*. *Steromphala umbilicaris* was assigned to three specimens. One individual was identified as *St. nebulosa*. Additionally, four shells were morphologically identified as *St. adriatica*, *St. rarilineata*, and *St. umbilicaris* without molecular data (compare Table 4). A total of 35 specimens were assigned to the genus *Steromphala*.

The remaining individuals split into seven *Phorcus mutabilis* and 43 *Ph. richardi*. *Phorcus articulatus* is represented by a single individual from Lebanon. Six specimens were identified as *Ph. turbinatus*, including one morphologically identified individual. The genus *Phorcus* was assigned to 57 individuals in this study.

The Linnean type specimens used for morphometric analyses were treated as separate species groups.

The combined data of lateral and ventral views of all taxa yield a first principal component axis (PC) explaining 49.37% of the variance in shell shape. The second and third PC explain 13.64 and 7.57%, respectively (Table 5). In total, the first three PCs cover 70.58% of variance. Subsequent PCs provided little further information and no separation of taxa and were, thus, disregarded. In this analysis, a morphological separation of *Steromphala* and *Phorcus* is achieved (Fig. 4). To improve the resolution of the species within these two genera, separate principal component analyses were carried out for the *Phorcus* and *Steromphala* data sets. The strictly Atlantic *St. cineraria* was excluded (Fig. 5).

The principal component analysis of the combined lateral and ventral landmark set of the genus *Steromphala* shows the first PC with 32.04%, the second PC with 21.86%, and the third PC with 13.45% variance (Table 5), covering 67.35% of total shell shape variance.

The *Steromphala* analysis separates *Steromphala umbilicaris* from the other species. The type material and the recent specimens cluster together. The single specimen of *St. nebulosa* nests within this cluster (Fig. 6a). The single specimen of *St. adansonii* always separates from all other species (Fig. 7a). *Steromphala adriatica*, *St. divaricata*, and *St. varia* cluster with some overlap. *S. adriatica* is separated on the third PC (Fig. 8). Likewise, *St. rarilineata* nests within *St. divaricata* on the first two PCs but is set apart on the third PC (Fig. 9a). Three individuals of the type material of *St. divaricata* were re-identified as *St. varia* according to the characteristic umbilical atrium and cluster with the other *St. varia* specimens (Figs. 10a and 11a). All *Steromphala* species can be recovered as separate groups in the first three principal components, with the exception of *St. divaricata*, which overlaps slightly with *St. varia* in the first two principal components and with *St. adriatica* in the third principal component (Figs. 6, 7, 8, 9, 10 and 11).

In the principal component analysis for the genus *Phorcus*, the first PC explains 30.17%, the second PC explains 21.07%, and the third PC explains 7.96% of variance (Table 5), covering 59.2% of total in shell shape variance.

The species of the genus *Phorcus* are mostly recovered as separate clusters in the first three principal components. *Phorcus richardi* is the most abundant species in the sample and shows some variance in shell shape but can be recovered as a single species (Fig. 12). Only a single specimen from Crete, genetically unambiguously assigned to *Phorcus mutabilis*, shows a shell shape more similar to *Ph. richardi* (Fig. 13). *Phorcus mutabilis* from Crete is inseparable from *Ph. richardi* on the first and second principal component axes but shows some separation on the third. *Phorcus mutabilis* from Croatia forms a distinct cluster without overlap with the Crete group (Fig. 13). The cluster of *Phorcus turbinatus* is well separated from all other species in the first two principal components (Fig. 14). The single specimen of *Ph. articulatus* is separated from all other species (Fig. 15).

Discussion

Methods for species delineation

Geometric morphometrics offer a tool for species delineation by objective, mathematical characterisation of shell shape. The ventral views show important characteristics of the umbilicus and its atrium where the lateral view captures the shape of the shell whorls. The combination of both views into a single data set offers the possibility to combine characteristics that cannot be captured in a single photo.

In general, the applicability of geometric morphometrics to species delineation in trochoid gastropods depends mainly on the standardising of views. Small rotations in the lateral view and tilting in ventral orientation bias results. Lateral and ventral views with 44 and 50 landmarks, respectively, appear to be sufficient to capture the most important features of the shell. However, in the case of *Steromphala* and *Phorcus*, shell morphometrics does not always serve species delineation without additional, non-morphometric characters, like shell sculpture in *Ph. mutabilis* specimens or very distinct and stable colouration in *St. adriatica*.

Systematics: genus delineation and species diagnosis

The molecular tree (Fig. 3) recovers a monophyletic *Phorcus* clade, but a paraphyletic situation for species traditionally placed in *Gibbula*, which agrees with earlier studies (Donald et al. 2012; Uribe et al. 2016; Williams et al. 2010; Williams 2012). We therefore propose the genus-group name *Steromphala* Gray, 1847 with the type species *St. cineraria* (Linnaeus, 1758) for this monophyletic clade and restrict the use of the name *Gibbula* to the group of its type species, *G. magus* (Linneaus, 1758).

Geometric shell morphometrics of the Mediterranean taxa serves well in separating *Phorcus* from *Steromphala*, supporting the recognition of *Phorcus* as a valid genus by Gofas and Jabaud (1997). The only exception is a Croatian specimen that genetically belongs to *Ph. mutabilis* with a shell morphometrically resembling *St. adriatica* (Fig. 4). A closer inspection of the shell itself shows a clear distinction in shell size and colouration, as well as umbilical atrium morphology, which enable easy differentiation between these species.

Genus *Steromphala* Gray, 1847

p. 146, as junior synonym of *Gibbula*

Type species: *Steromphala cineraria* (Linneaus, 1758), by original designation

Steromphala is defined by a subtriangular shell shape and a concave base. Fine ridges and striae are present, but prominent nodes or cusps such as in *Gibbula magus* are not developed. The upper and lower aperture attachment points are separated by a quarter turn of the whorl or less ($\alpha \leq 90°$). The umbilicus is open and may be set off the shell base by an umbilical atrium. The only species closing the umbilicus with age is *St. divaricata*.

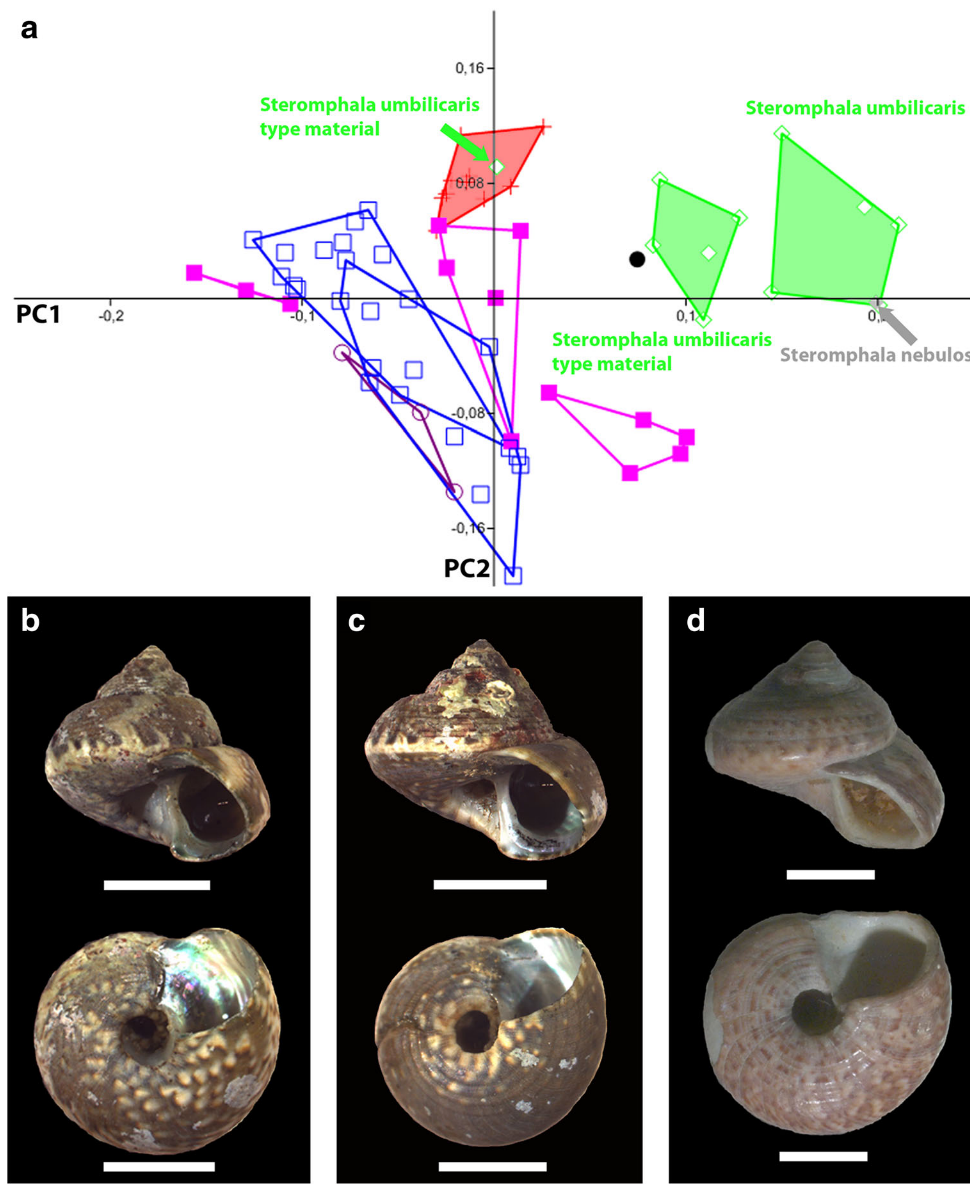

Fig. 6 *Steromphala umbilicaris* (Linnaeus, 1758) and *Steromphala nebulosa* (Philippi, 1848). **a** PCA plot of PC1 vs. PC2 of genus *Steromphala*. *Steromphala umbilicaris* (green) separates well from all other species. There is no overlap between specimens from this study and the type material. One individual (Linné 24) nests within *St. adriatica*. *Steromphala nebulosa* (grey) nests at the edge of *St. umbilicaris*. **b** Representative specimen of *St. umbilicaris* from this study. **c** Representative specimen of *St. nebulosa* from this study. d: Designated lectotype for *St. umbilicaris* located at LSL (LSL.504). Scale bars 5 mm

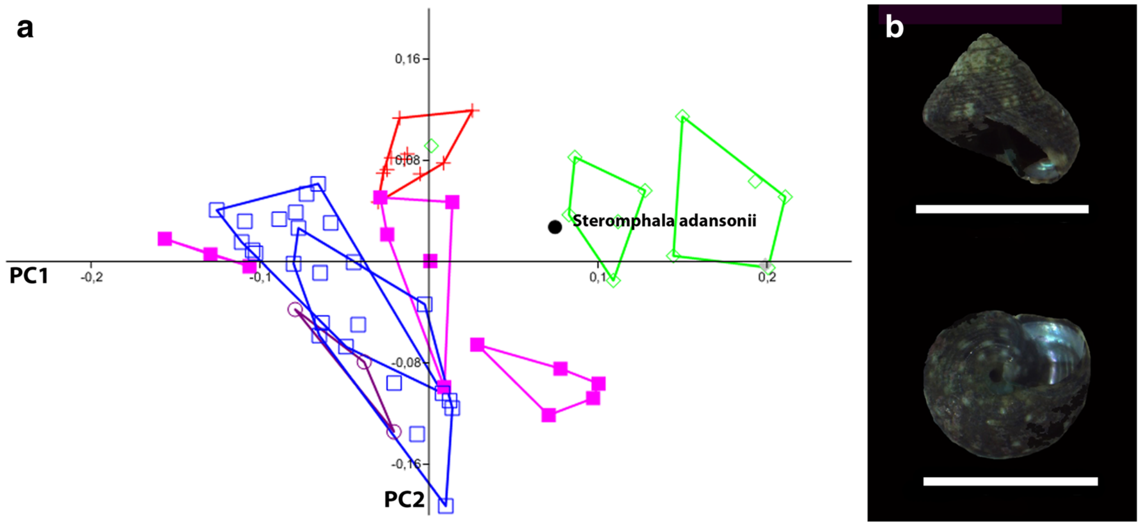

Fig. 7 *Steromphala adansonii* (Payraudeau 1826). **a** PCA plot of PC1 vs. PC2 of genus *Steromphala*. Single specimen of *Steromphala adansonii* (black) clusters near *St. umbilicaris*. **b** Representative specimen of *St. adansonii* from this study. Scale bar 5 mm

Steromphala cineraria (Linnaeus, 1758)

Original combination: *Trochus cinerarius* Linnaeus, 1758: p 758, n 512.

Type material: LSL.502 (Linnean Society of London, UK), ten specimens.

We here designate the specimen "Linné 35" (this shell is marked "512," corresponding to the number given in the original description), as lectotype (Fig. 5). The remaining nine type specimens are paralectotypes.

Steromphala cineraria was originally described as an umbilicated shell with rounded whorls. Its overall shape is triangular. The sculpture consists of distinct spiral ridges and the small umbilical atrium has a sharp edge. The base is concave with a narrow and round umbilicus.

Steromphala umbilicaris (Linnaeus, 1758)

Original combination: *Trochus umbilicaris* Linnaeus, 1758: p 758, n 514

Type material: LSL.504 (Linnean Society of London, UK), six specimens.

We here designate the specimen "Linné 23" (Fig. 6d) as lectotype. The remaining five specimens are paralectotypes. (The identification of specimen "Linné 24" as *St. umbilicaris* is questionable.)

The original description of this species refers to an open and deep cylindrical umbilicus, white in colour. The whorls

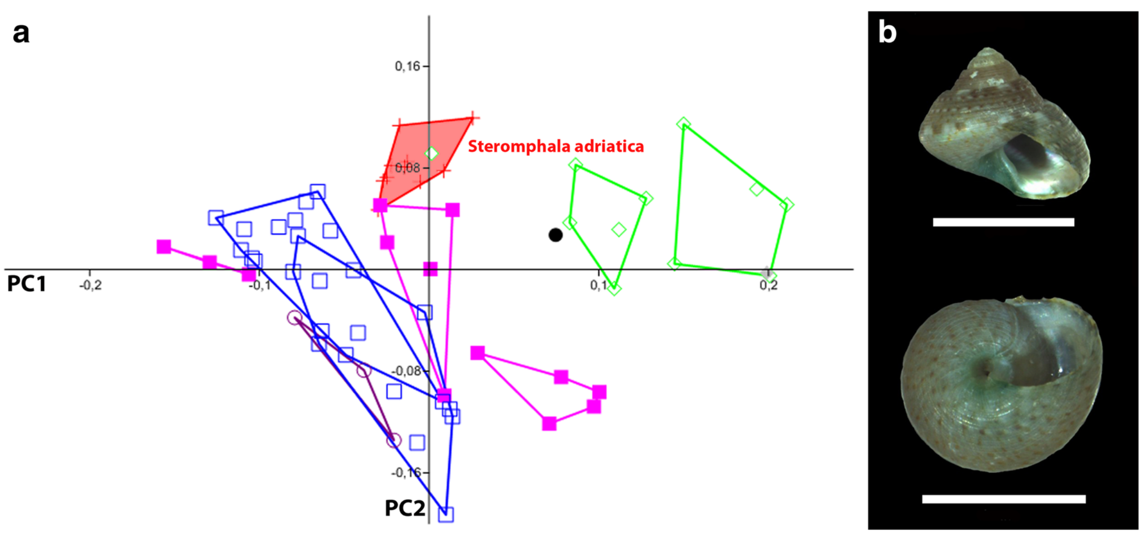

Fig. 8 *Steromphala adriatica* (Philippi, 1844). **a** PCA plot of PC1 vs PC2 of genus *Steromphala. Steromphala adriatica* (red) is separated from all but *St. divaricata*. One individual of *Steromphala umbilicaris* (Linné 24) nests within *St. adriatica*. **b** Representative individual of *St. adriatica* from this study. Scale bar 5 mm

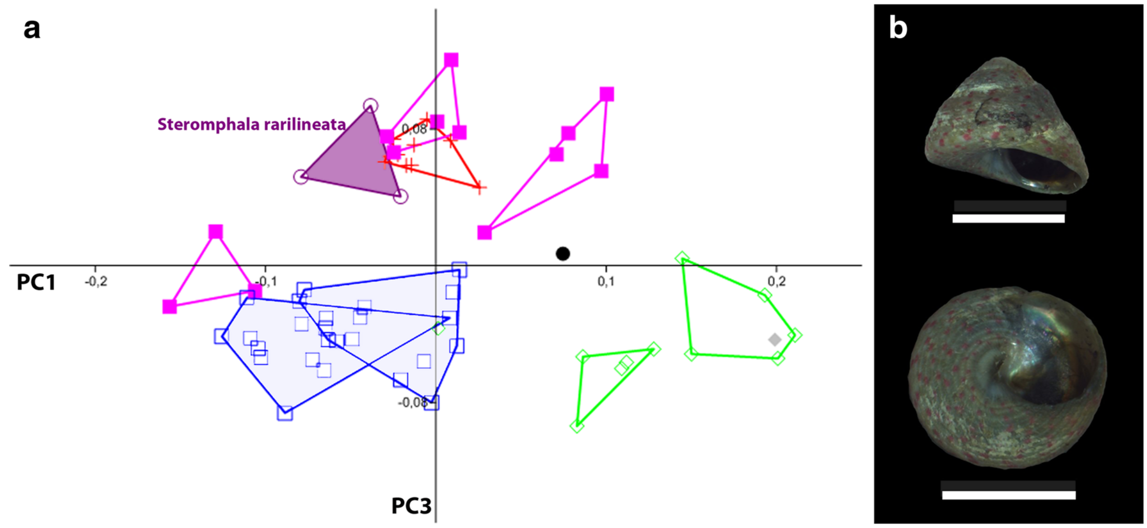

Fig. 9 *Steromphala rarilineata* (Michaud, 1829). **a** PCA plot of PC1 vs. PC3 from genus *Steromphala*. *Steromphala rarilineata* (violet) is separated but very near *St. adriatica* and *St. divaricata*. **b** Representative specimen of *St. rarilineata* from this study. Scale bar 5 mm

are conic and convex with a shallow suture. The individuals collected for this study show a matching deep and cylindrical umbilicus, but the whorls are shouldered and separated by an incised suture (e.g. Fig. 6b). The type material consists of six shells with a wide variety of shapes, none of which resembles our specimens, and only one shows convex whorls as described by Linnaeus. We therefore designate this individual as lectotype (Linnè 23, Fig. 6d). *Steromphala umbilicaris* sometimes shows a slightly convex shell base. The umbilicus is deep and round. Its columella base is angular. The shell diameter is bigger than the shell height. The aperture is small, with the attachment points only separated by approximately an eighth of a shell turn ($\alpha \approx 45°$).

The wide range of variability in shell shape resembles that of *Steromphala divaricata*. As there is no information on the type locality, future investigations may address potential geographic differentiation of shell shape in these species.

Steromphala nebulosa (Philippi, 1849)

Original combination: *Trochus nebulosus* Philippi, 1849: p 109–110, n 32

Type material not located.

DNA-barcoding suggests the distinction of the sister-species *Steromphala nebulosa* and *St. umbilicaris* (Barco et al. 2013). The molecular analysis in this study further strengthens the argument for the validity of both species. The split between *St. umbilicaris* and *St. nebulosa* is well supported (bootstrap

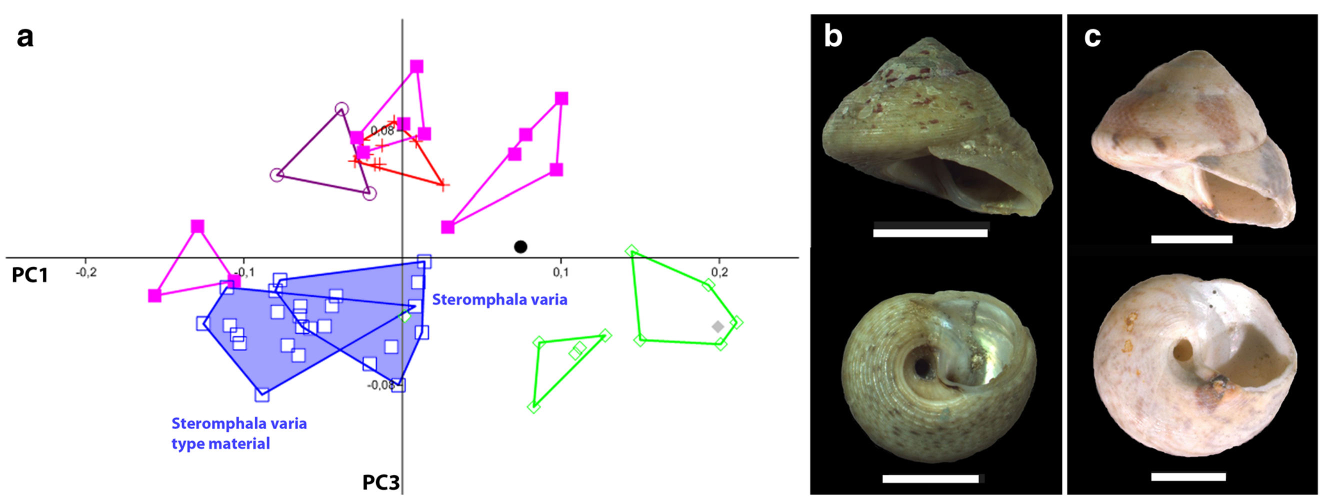

Fig. 10 *Steromphala varia* (Linnaeus, 1758). **a** PCA plot of PC1 vs. PC3 of genus *Steromphala*. *Steromphala varia* (blue) separates from all other species. Specimens from this study overlap with the type material. **b** Representative specimen of *St. varia* from this study. **c** Lectotype designated for *St. varia* (LSL.501). Scale bars 5 mm

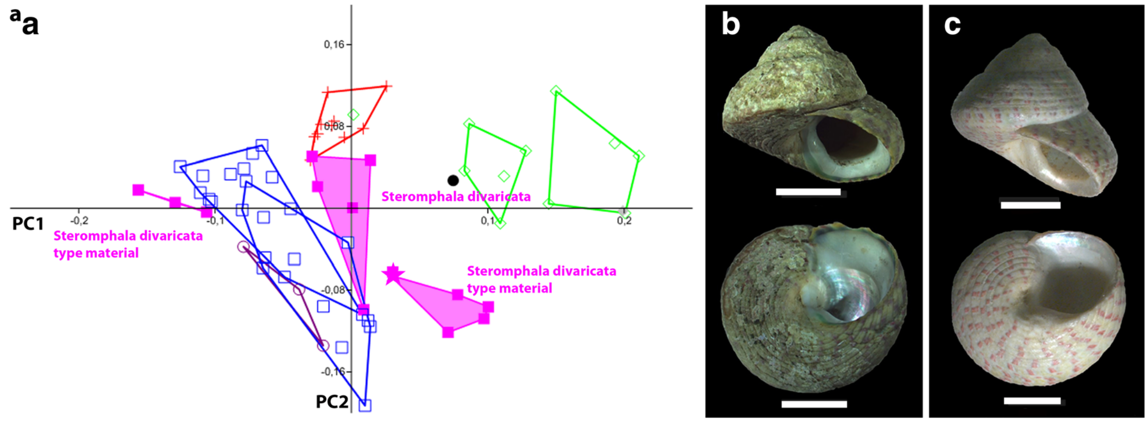

Fig. 11 *Steromphala divaricata* (Linnaeus, 1758). **a** PCA plot of PC1 vs. PC2 from genus *Steromphala*. *Steromphala divaricata* (pink) cannot be recovered as a completely separated group as it shows a small overlap with *St. varia*. Type material and material from this study do not overlap. Three individuals (Linné 41–43) of the type material (pink group on the left) were re-identified as *St. varia* (compare Fig. 8). **b** Representative specimen of *St. divaricata* from this study. **c** Lectotype (pink star) designated for *St. divaricata* (LSL.503) by Anistratenko and Anistratenko (2001). Scale bars 5 mm

value = 100). Specimens of both species were sampled from Italy and Croatia, and the tree does not show a geographical bias, corroborating the separation of the two taxa. However, the specimens barcoded for each species in this study cannot be distinguished morphologically. The single specimen identified as *St. nebulosa* by COI barcoding following Barco et al. (2013) nests within *St. umbilicaris* in the morphometric analyses (Figs. 6a and 9a). A re-examination of the shell itself did not yield any distinguishing characters.

The original description of *St. nebulosa* reports an umbilicated conical shell, reddish with white marks; whorls are convex, with the last one being slightly angular. Philippi (1849) mentioned six sulci on the whorls and four striae at the base. Although this description is more detailed than that of *Steromphala umbilicaris*, it fails to mention diagnostic differences. As none of the collected specimens (Fig. 6c) overlap with the available type material in the geometric morphometric analysis or matches one of the original descriptions sufficiently to differentiate between the two species, morphological definitions of *St. umbilicaris* and *St. nebulosa* remain questionable. From a conchological point of view, these two species have to be defined as cryptic sister species. Further morphological investigations, e.g. radula comparison, and a larger molecular sample are necessary to determine if they are truly cryptic.

Steromphala adansonii (Payraudeau 1826)

Original combination: *Trochus adansonii* Payraudeau 1826: p127, n 267, Pl. 6, Figs. 7 and 8.

Type material: MNHN-IM-2000-30071 (Muséum national d'Histoire naturelle, Paris, France), 3 syntypes.

The original description of *Steromphala adansonii* focuses on the colouration of the shell. Payraudeau (1826) describes

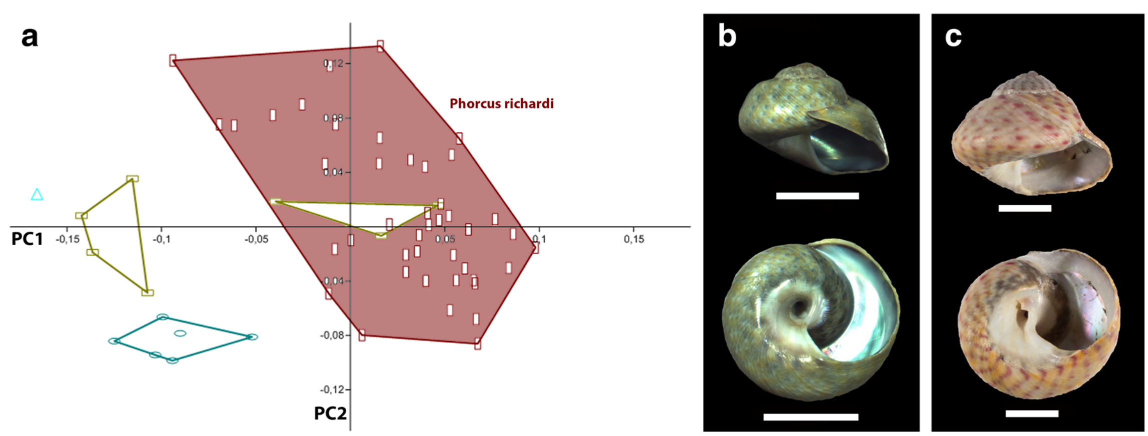

Fig. 12 *Phorcus richardi* (Payraudeau 1826). **a** PCA plot of PC1 vs. PC2 of genus *Phorcus*. *Phorcus richardi* (maroon) is the most abundant species in the current sample. It separates well from *Ph. articulatus* and *Ph. turbinatus*. One group of *Ph. mutabilis* is not distinguishable from *Ph. richardi* in the first two principle components. **b** Representative specimen of *Ph. richardi* from this study. **c** One syntype of *Ph. richardi*. Located at MNHN (MNHN-IM-2000-28253). Scale bars 5 mm

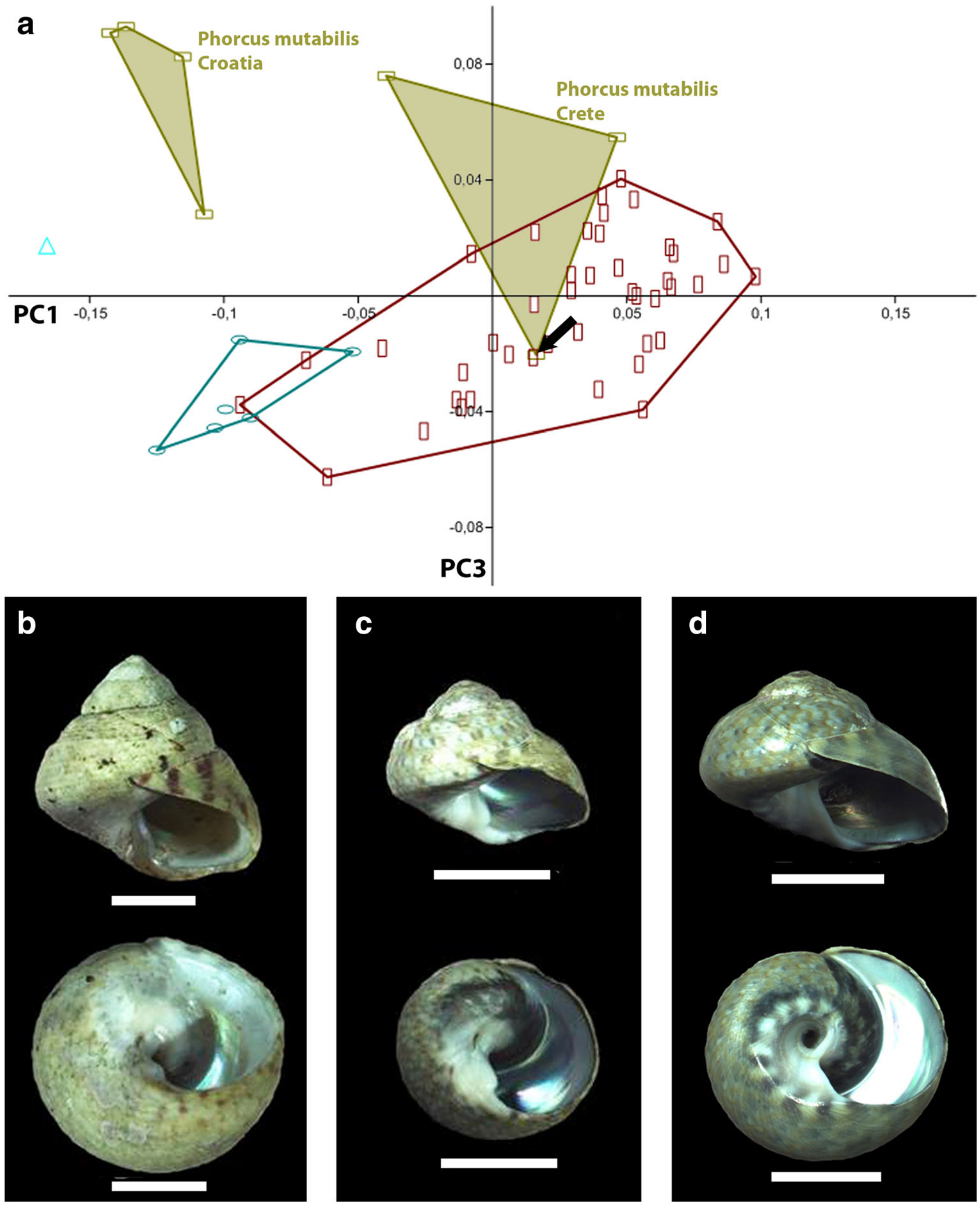

Fig. 13 *Phorcus mutabilis* (Philippi, 1846). **a** PCA plot of PC1 vs. PC3 from genus *Phorcus*. *Phorcus mutabilis* (olive) splits into two morphotypes: one from Croatia and one from Crete. **b** Representative specimen of *Ph. mutabilis* (Croatia) from this study. **c** Representative specimen of *Ph. mutabilis* (Crete) from this study. **d** Specimen (black arrow) showing *Ph. richardi* morphology, but *Ph. mutabilis* COI barcode. From this study. Scale bars 5 mm

them as golden-coloured with white marks and flames and a sculpture of multiple spiral bands. Nordsieck (1968) notes the base of the columella being folded over and sometimes partly obscuring the otherwise open and rounded umbilicus.

The shell shape of *Steromphala adansonii* is similar to that of *St. umbilicaris*, but of much smaller size (Fig. 7). Morphologically, it resembles *St. adriatica*. The single specimen in this study (Fig. 7b), however, differs by a steeper angle in the aperture lip, a wider umbilicus, and lacks the shoulder in the last whorl.

Steromphala adriatica (Philippi, 1844)

Original combination: *Trochus adriaticus* Philippi, 1836–44: p 153, n 21, Tab. XXV, Fig. 10

Type material not located.

Originally described as a variation of *Steromphala adansonii*, it differs from this species by the angular suture of the bigger last whorl. Philippi (1844) mentions a node on the columellar side of the aperture, but this is not observed in the present specimens. Morphometric and genetic data confirm the separation of the sister species, *St. adriatica* and *St. adansonii*.

Steromphala adriatica shows a distinct shape in lateral and ventral views. It is characterised by the larger last whorl, an acute apex, as well as a narrow, round, and conical umbilicus without distinct atrium. The whorls are slightly shouldered. Fresh shells of this species always show green colouration around the umbilicus, which tends to fade in dry and preserved shells. The sculpture consists of several spiral lines, sometimes marked in colour or white bands (Fig. 6).

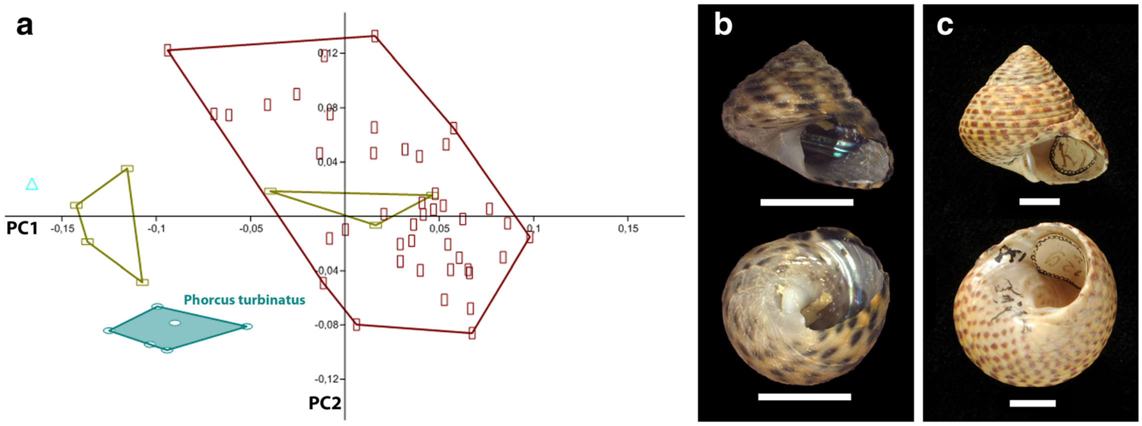

Fig. 14 *Phorcus turbinatus* (Born, 1778). **a** PCA plot of PC1 vs. PC2 for genus *Phorcus*. *Phorcus turbinatus* (petrol) separates from all other species. **b** Representative specimen of *Ph. turbinatus* from this study. **c** One syntype of *Ph. turbinatus* (NHMW 14002). Scale bars 5 mm

Steromphala rarilineata (Michaud, 1829)

Original combination: *Trochus rarilineatus* Michaud, 1829: p 266–267, n 8, Fig. 12

Type material: lot °45011450 (Collection Malacologie, Musée des Confluences, Lyon, France), three syntypes (compare Boyer and Audibert 2007). Type material was not re-examined for this study.

Although this species cannot be distinguished from *St. varia* in the first two principal components (Fig. 8a), it is recovered in the third (Fig. 9a). Compared to *St. varia*, it differs in the shape of the umbilicus and the absence of an umbilical atrium (Fig. 9b). A distinction between *St. rarilineata* and juveniles of *St. divaricata* is very difficult. In agreement with the original description of this species, the typical shell characters include angular whorls with indistinct suture and multiple curved lines of reddish-purple dots on the fine shell sculpture. The colouration pattern is very common but unreliable as a diagnostic feature, because it is shared with *St. divaricata* and disappears in dry material or shells stored in ethanol.

Steromphala rarilineata is defined by an overall triangular shape. Its diameter and height are nearly the same. The base is slightly concave. The aperture lip is strongly tilted and attaches exactly on the angular lower edge of the last whorl, leaving the shell with no distinct suture (Fig. 9b).

Steromphala varia (Linnaeus, 1758)

Original combination: *Trochus varius* Linnaeus, 1758: p 758, n 511

Type material: LSL.501 (Linnean Society of London, UK) 14 specimens.

We here designate the specimen “Linné 7” as lectotype (Fig. 10c). The 13 remaining specimens are paralectotypes.

Steromphala varia is originally described as a shell with an overall convex shape and an open umbilicus. Linnaeus did not mention the diagnostic umbilical atrium. The collected specimens correspond well with the type material.

Steromphala varia is the only species with a well-defined, sharp-edged umbilical atrium and a wide and round umbilicus (compare Gofas et al. 2011) (Fig. 10). Furthermore, *St. varia* shows several spiral ridges and a more or less visible suture. The shell base is slightly concave. The aperture is tilted and the lip attaches just below the angle of the last whorl.

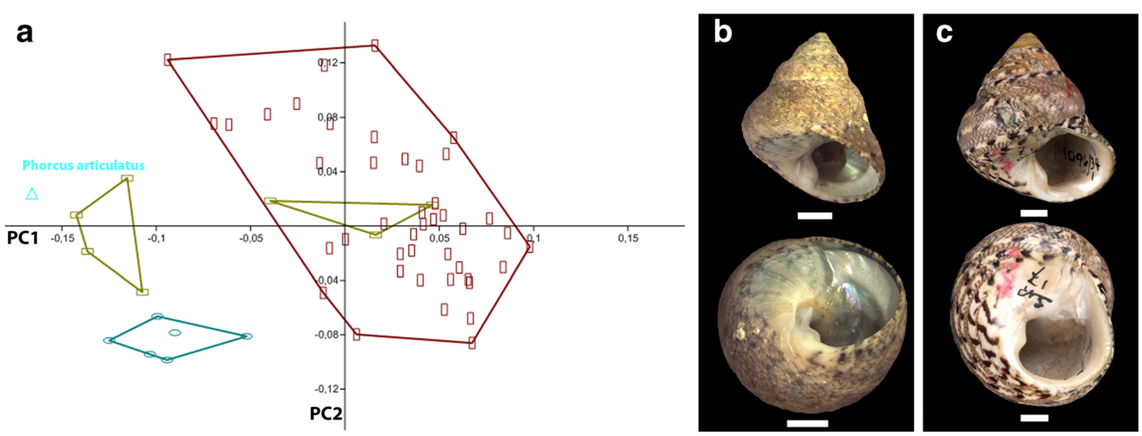

Fig. 15 *Phorcus articulatus* (Lamarck 1822). **a** PCA plot of PC1 vs. PC2 for genus *Phorcus*. Single specimen of *Phorcus articulatus* separates from all other species. **b** Representative specimen of *Ph. articulatus* from this study. **c** One syntype of *Ph. articulatus* (INVE 51532). Scale bars 5 mm

Steromphala divaricata (Linnaeus, 1758)

Original combination: *Trochus divaricatus* Linnaeus, 1758: p 758, n 513

Type material: LSL.503 (Linnean Society of London, UK), eight specimens.

Lectotype (Fig. 11c) and seven paralectotypes (Anistratenko and Anistratenko 2001, English translation: Anistratenko 2005)

The great variability in shell shape makes *Steromphala divaricata* difficult to identify (Fig. 11a). The original species description mentions an ovate shell with a mostly closed umbilicus and deep suture. The set-off last whorl, however, is not always developed as already observed by Anistratenko and Anistratenko (2001) (Anistratenko 2005). The specimens examined here vary from angular whorls (Fig. 11b) reminiscent of *St. varia* and *St. rarilineata*, to convex whorls as mentioned in the original description. But regardless of the variation in the lateral aspect and the overall size of the shell, all individuals showed a slit-like umbilicus and no distinct umbilical atrium. Future studies may shed light on potential geographic variation and ecophenotypic plasticity.

The examination of the type material revealed three of the current paralectotypes (Linné 41- Linné 43) belonging to *St. varia* and need to be excluded from the type material of *St. divaricata* (Figs. 10a and 11a).

A shell with a larger last whorl characterises *Steromphala divaricata*. The aperture lip attaches below the edge of the last whorl, which can be round or angular, and leaves the shell with a distinct suture. The aperture is small and round. The shell base is flat. The umbilicus is slit like or completely closed by the columellar fold. There is no umbilical atrium.

Genus *Phorcus* Risso, 1826

Type species: *Phorcus margaritaceus* Risso, 1826 (by subsequent designation), accepted as *Phorcus richardi* (Payraudeau 1826)

Phorcus is characterised by a subtriangular shell shape with whorls more rounded than in *Steromphala* and a convex base. The shells are either smooth or show coarser spiral striae than in *Steromphala*. The umbilicus narrows with age. In large species, it can be closed completely by a columellar fold. The upper and lower aperture attachment points are separated by at least a quarter turn of the whorl ($\alpha \geq 90°$).

Phorcus richardi (Payraudeau 1826)

Original combination: *Monodonta richardi* Payraudeau 1826: p 138, n278, Pl VII, Figs. 1 and 2.

Type material: MNHN-IM-2000-28253 (Muséum National d'Histoire naturelle Paris, France), two syntypes.

The shell of *Phorcus richardi* is depressed with a bloated last whorl as originally described (Payraudeau 1826). It appears rather thin-walled, and the inside of the rhomboid aperture always shows a layer of bluish nacre (Gofas et al. 2011; Nordsieck 1968; Payraudeau 1826) (Fig. 12). The type material matches the original descriptions well and corresponds to the collected specimens (Fig. 12).

Phorcus richardi is characterised by a distinct shell shape and an open, large, round umbilicus surrounded by a rounded, white umbilical atrium edged by a black and white pattern, which is unique for this species (compare Gofas et al. 2011; Nordsieck 1968). The shell is wider than high. The attachment points of the aperture are separated by nearly a half turn of the shell ($\alpha \approx 180°$). The aperture lip attaches above the whorl edge with a slight shoulder and suture.

Phorcus mutabilis (Philippi, 1846)

Original combination: *Trochus mutabilis* Philippi, 1846: p 166, n 201, Pl 26, Figs. 18–22

Type material not located.

The original description by Philippi (1846) distinguishes *Phorcus mutabilis* from *Ph. turbinatus* by the denticle at the base of the columella. This denticle is not present in all individuals identified as *Ph. mutabilis* by DNA-barcoding of the present material. One of the specimens genetically identified as *Ph. mutabilis* shows the typical shell shape of *Ph. richardi* (Fig. 13a and d, black arrow). Future research including nuclear genetic markers could elucidate possible hybridisation or incomplete lineage sorting in these species. Furthermore, *Ph. mutabilis* shows two distinct morphotypes: the individuals from Crete closely resemble *Ph. richardi* (Fig. 13c) in shell shape; two of the Crete specimens (e.g. Fig. 13c), however, have the columellar denticle and spiral lines typical for *Ph. mutabilis* but not for *Ph. richardi* (compare Philippi 1846). Specimens from Croatia rather resemble *Ph. turbinatus* (Figs. 13b and 14), a similarity already mentioned in the original description. They also show similarities to *Ph. articulatus* (Fig. 15).

In all *Phorcus mutabilis* specimens examined, the umbilicus is not as open as in *Ph. richardi*. It seems as if the umbilicus becomes smaller or is even completely closed as the shell grows (compare Philippi 1846).

Further research is needed to understand the mechanisms behind this high variability in shell morphology.

Phorcus turbinatus (Born, 1778)

Original combination: *Trochus turbinatus* Born, 1778: 340–341, n K.II.4

Type material: NHMW14002 (Naturhistorisches Museum Wien, Austria), two syntypes.

Phorcus turbinatus is originally described having an ovate shell with smooth whorls and a denticle at the columellar base. The specimens collected for this study match the original description and the type material (Fig. 14c) in their overall shape and the lack of an umbilicus, which is closed by a columellar fold. They differ, however, in lacking a denticle, and the presence of prominent spiral ridges (Fig. 14b). These differences may be due to the relatively small size of the collected shells. They seem to be immature and probably lose the shell sculpture with age.

Phorcus turbinatus shows a compact shell with a convex base. The slit like umbilicus is closed with age by a columellar fold. The aperture lip attaches on the round whorl edge, leaving a small shoulder and shallow suture.

Phorcus articulatus (Lamarck 1822)

Original combination: *Monodonta articulata* de Lamarck 1822: p 36, n 17

Type material: INVE 51532 (Muséum d'Histoire naturelle Genéve, Switzerland), four syntypes.

Phorcus articulatus is originally described as having a conical shell with very convex whorls. The umbilicus, round and small, is closed with age. The columella base is marked by a distinct, rounded denticle. The single specimen of *Ph. articulatus* in this study (Fig. 15b), as well as the syntypes (e.g. Fig. 15c), matches the original description.

The shell shape of *Phorcus articulatus* is characterised by a very high spiral height to diameter ratio. The columella shows a distinct denticle. The aperture lip attaches below the rounded whorl edge, leaving each whorl bulbous and without shoulder or suture. The aperture is comparatively small for *Phorcus* species, with attachment point set apart by just a quarter of a shell turn ($\alpha \approx 90°$).

Conclusion

Robust molecular phylogenetic results showing *Gibbula* as a paraphyletic group relative to *Phorcus* are calling for a taxonomic reassessment. Both COI barcoding and geometric morphometric analyses underscore the separation of the former subgenus of *Gibbula*, *Steromphala* Gray, 1847, from *Phorcus* Risso, 1826 and *Gibbula* s.s. Risso, 1826, and its elevation to full genus rank. Shell shape is an informative and reliable character to distinguish between *Steromphala* and *Phorcus* species significantly aiding identification even in the field.

More subtle differences in shell shape and the intraspecific variability make species identification within *Steromphala* and *Phorcus* difficult with the naked eye, even if most species are characterised by shell features. These species-specific shell shapes can only be resolved by combining lateral and ventral views with their information on the orientation of the aperture and umbilicus features. Thus, geometric morphometrics is a powerful tool to aid identification of most species in this study. It is also a non-destructive method for assessing type material or fossils. In combination with DNA-barcoding on recent material, it offers the possibility of objectively linking dry material to molecular markers. Although (morphologically trained) human vision is a powerful tool in separating morphotypes, gastropod shells with contour dissolving colourations can pose problems. Geometric morphometrics capture the true shape of the shell, regardless of colour and pattern. It can therefore show similarities among species otherwise perceived as very different due to colourations, intraspecific variability, or ontogenetic shapeshifts. Although it is not as easily applicable as classical morphometrics, e.g. length measurements, it serves significantly better in separating taxa in the present study.

However, the spatial resolution of sliding landmarks may be too low to detect some of the smaller shell features like the aperture denticle in *Ph. mutabilis*. Alternative methods such as outline analysis or a more narrow set of fixed landmarks could potentially overcome this problem. The problem of defining homologous landmarks on gastropod shells is a limiting factor.

Further analyses including nuclear genes and/or microsatellites on a broader taxon sample are required for resolving the internal relationships in the *Gibbula*-*Steromphala*-*Phorcus* group.

Some species, like *Steromphala nebulosa*, need further morphological investigations. Ongoing research on the remaining syntypes will result in the designation of additional lectotypes and, in the cases of missing type material, neotypes for the remaining *Gibbula*, *Steromphala*, and *Phorcus* species. The clarification of ambiguous type material, like in *St. divaricata*, will continue with further geometric morphometric analyses and appropriate sampling of live specimens in combination with molecular barcodes for taxon assignment and the designation of epitypes (Schrödl and Haszprunar 2016).

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