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Integrating traditional taxonomy and DNA barcoding for the identification of some bivalves in the Northern Red Sea, Egypt

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ABSTRACT

In attempting to reach integration between the morphological characteristics and DNA barcoding analysis, the present study was designed to confirm the identification of some bivalve molluscs collected by a fishing vessel working in the Suez Gulf, North of the Egyptian Red Sea, with special reference to the ambiguous species. Some external and internal morphology of the studied shells were characterized (e.g., the shape of the shell and umbo; colour patterns of the external and internal of the shell; the shape of the pallial line; cardinal and lateral teeth; the external and/or internal ligament, which hinged the two valves together). The mitochondrial cytochrome oxidase subunit I (*COI*) gene, which is highly varied among the studied bivalve species, was also used to evaluate the molecular identification of bivalve samples. The morphological and molecular analyses resulted in the six species namely: *Dosinia amphidesmoides* (Reeve, 1850); *Gafrarium pectinatum* (Linnaeus, 1758); *Circenita varia* (Forsskål in Niebuhr, 1775); *Circenita callipyga* (Born, 1778); *Mactra glauca* (Born, 1778), and *Gari depressa* (Pennant, 1777). Morphological characterization and molecular identification were effective for distinguishing the bivalve species collected from the Gulf of Suez, Red Sea and will provide a base for future phylogenetic analyses for additional bivalve research.

INTRODUCTION

Molluscs comprise the marine domain's second-largest animal phylum **(Sharabati, 1984; Rusmore-Villaume, 2008; Carpenter and De Angelis, 2016)**. Within any aquatic ecosystem, molluscs play significant parts both economically and ecologically **(Coen and Bishop, 2015)**. Economically, some industries of beneficial aquaculture and wild harvest depend on molluscs. Ecologically, their populations participate in the recycling of nutrients, determine the structure of benthic communities and maintain water quality by decreasing the levels of some heavy metals in environments. Moreover, higher trophic levels organisms, such as fishes, depend on it as a resource of food. Bivalvia is the second-largest molluscan class, with around 7500 species inhabiting a variety of marine

habitats. All the previous taxonomical studies on marine bivalves of the Red Sea have depended only on morphological characterization (e.g., **Hassan, 1983; El-Mekawy, 2016; El-Mekawy, El-Sayed *et al.*, 2019)**, while the most of the phylum Mollusca, especially bivalves, have a wealth of shell colour variations either inside or outside the valves, which may lead to the misidentification of some molluscan taxa and consider it as an ambiguous species in the environment. It is well-known that the process of colouration is achieved in both a matrix of crystalline calcium carbonate and its organic horny cover layer **(Taylor *et al.*, 1969)**. DNA barcoding is a molecular taxonomic approach that identifies species by using short genetic markers in an organism's genome. DNA barcoding is an interesting technique for rapidly and unambiguously identifying species. It also assists in determining genetic distances between different taxa. Therefore, DNA barcoding can be a resolving for some of the problems associated with traditional taxonomical identification because it can reveal cryptic species, the different life stages or dimorphisms, identify the individual samples, and offer a cost-effective means for species identification **(Hebert *et al.*, 2003)**. Due to the phenotypic variations, as well as the prevalence of closely related taxa, certain bivalve species are extremely difficult to recognize by traditional morphological identification **(Feng *et al.*, 2011)**. However, several investigations have demonstrated the use of DNA barcoding in resolving morphologically ambiguous species complexes in a variety of molluscan families **(Zou *et al.*, 2012; Sun *et al.*, 2016; Johnson *et al.*, 2017)**. Based on the available publications, only two genetic analyses have been carried out on the marine molluscs in the Egyptian Red Sea. **Radwan *et al.* (2014)** performed molecular phylogenetic analyses on four bivalve species found in the Egyptian Mediterranean and the Red Sea (*Paphia* sp. and *Polititapes* sp. from Lake Timsah, *Modiolus* sp. from Marsa-Alam and *Brachidontes* sp. from Lake Bardawil). The other study was conducted by **Sarhan *et al.* (2021)** on seven different Gastropoda species (cone snails) found along the Egyptian coast of the Red Sea (*Conus arenatus*, *C. miliaris*, *C. pennaceus*, *C. sanguinolentus*, *C. vexillum*, *C. textile* and *C. striatus*). As a result, the DNA-based species distinction assumes that genetic information can be used to determine species' bounds more objectively and successfully than morphological information alone **(Liu *et al.*, 2017)**. Alternatively, no substantial barcode variation was found in two Donax clams, and they were all classified as being of the same species **(Carstensen *et al.*, 2009)**. Simultaneously, barcodes have shown that certain molluscan species lack genetic differentiation **(April *et al.*, 2011)**. Additionally, genetic diversity and its relationship to the population's genetic structure can be used to manage and protect a commercially important species **(Ward, 2000; Ortega-Villaizan Romo *et al.*, 2006)**. Thus, this work was designed to study both morphological and genetic characterization of some bivalves inhabiting the Suez Gulf with special reference to the ambiguous species that have external variability. It is also worthy annotation that the current study has provided new information to the GenBank database about the Egyptian marine invertebrates in the Red Sea represented in the molluscan group.

MATERIALS AND METHODS

1. Samples collection

Samples were collected by a fishing vessel working in the Abu Zenima area of the Gulf of Suez, which is located north of Egypt's Red Sea. Specimens were collected for

each species and kept frozen in the icebox until they were transferred to the laboratories for examination. The soft parts of the samples were removed from the shells, dissected, and immediately stored in 99% ethanol to preserve these parts until DNA analysis could be performed on the samples. The species were identified and classified based on their internal and exterior morphology, which was studied in detail.

2. Morphological and morphometric characteristics

It is well-known that bivalves are almost always completely enclosed within their shells. The following parameters were used to characterize the external morphology of bivalves: the shape of the shell and umbo; colour patterns of the shell (externally and internally); shell's sculpture including growth lines and/or its perpendicular vertical rays of the shell when found. Alternatively, internally other parameters were examined, such as the shape of insertions of both anterior and posterior adductor muscles, the shape of the pallial line; cardinal and lateral teeth; the external and/or internal ligament, which hinged the two valves together. Fig. (1) demonstrates the main features of the interior bivalve shell. To confirm the identification, the obtained parameters' results were compared to those of the available literature (e.g. **Sharabati, 1984; Oliver, 1992; Rusmore-Villaume, 2008; Carpenter and De Angelis, 2016**).

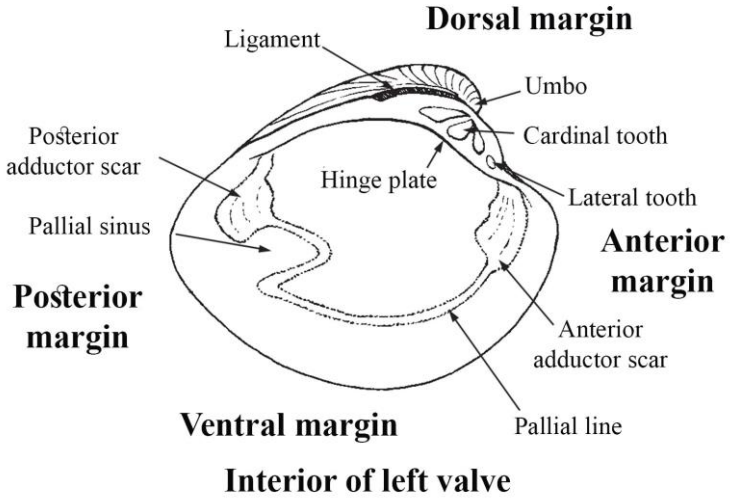

Figure 1. Main features of the interior bivalve shell, modified after Carpenter and De Angelis (2016).

3. Molecular identification and data analysis

In a TES buffer (10 mM Tris-HCl; 140 mM NaCl; 25 mM EDTA; pH 7.8) containing 1% SDS and 0.5 mg mL^{-1} proteinase K, tissue samples (10-20 mg) from the muscles were homogenized. Incubation at 56 °C for two hours resulted in the completion of the reactions. The standard phenol-chloroform method was used to extract genomic DNA from each of the studied species. The DNA was dissolved in TE buffer after it was recovered (100 mM Tris-HCl, 10 mM EDTA, pH 8). To determine the concentration of DNA samples, spectrophotometry was performed using a Nanodrop (Biodrop, Cambridge, England). DNA was stored at 4°C. The partial coding region of the cytochrome oxidase subunit 1 (*COI*) gene was amplified by PCR using a set of primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, Hoeh, Black, & Vrijenhoek,

1994). The amplification reaction was performed in a 25 µl reaction volume containing 12.5 µl of 1X MyTaq^{TM} HS Red Mix (Bioline, London, UK), 20 ng of template DNA from each sample and 0.4 µM of the primer. The conditions for PCR were as follows: 5 minutes of initial denaturation at 95°C, 35 cycles of denaturation for 30 seconds at 95°C, 1 minute of annealing at 45-48°C, 1.30 minutes of extension at 72°C, and 7 minutes of final elongation at 72°C. The amplified product was electrophoresed at 130V using 1.5% agarose gel (100 mg/ml) stained with ethidium bromide. PCRs generated with targeted bands were purified using Isolate II PCR and Gel Kit (Bioline). The BigDye Terminator cycle sequencer version 3.1 (adapted by **Abbas *et al.*, 2011**) and the ABI 3730 sequencing kit were used to sequence purified DNA fragments (both the kit and machine are Applied Biosystems). After two minutes of 96°C sequencing PCR, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and four minutes of 60°C PCR followed. Chromas Lite v2.1 software (Technelysium Pty Ltd., accessible at http://technelysium.com.au/) was used to edit the *COI* gene sequencing data for the bivalves' species and read with the BioEdit 7.2.6.1 test tool. Clustal-W software was also used to align the sequences **(Thompson *et al.*, 1994)**. The sequences of the coding region of the *COI* gene portion of the molluscan species were compared with a reference sequence in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for identity confirmation using BLAST search. Partial coding regions of the *COI* gene for molluscan samples were submitted to the GenBank/EMBL/DDBJ database with the accession numbers (OM524513, OM523103, OM509651, OM509652, OM509653, OM509654, OM509655, OM509656, OM509657, OM509658, OM420554, OM420555, OM420556, OM420557, OL741733 and OL711929). Phylogenetic tree constructed by MEGA X software using the Neighbour-Joining method **(Saitou and Nei, 1987)**. The best-fitting model was applied for the *COI* datasets of nucleotide composition depending on the Kimura 2-parameter model **(Kimura, 1980)** with a tool from MEGA-X software **(Kumar *et al.*, 2018)** with 1000 bootstrap replications.

RESULTS

1. Morphological and morphometric description

Six species of bivalves were identified in this study as follows:

1.1. *Dosinia amphidesmoides* Reeve, 1850 (Fig. 2)

Systematics description:

Class: Bivalvia
Subclass: Autobranchia
Infraclass: Heteroconchia
Subterclass: Euheterodonta
Superorder: Imparidentia
Order: Venerida
Superfamily: Veneroidea
Family: Veneridae
Subfamily: Dosiniinae
Genus: *Dosinia*
Species: *Dosinia amphidesmoides* Reeve, 1850

Synonyms:

Artemis amphidesmoides **Reeve, 1850**: 95, pl. 8, fig. 48 a & b.
Artemis radiata **Reeve, 1850**: 12, pl. 7, fig. 37.
Pardosinia amphidesmoides **Reeve, 1850**
Dosinia dilatata **Deshayes, 1853**
Pardosinia colorata **Iredale, 1929**
Dosinia miticula **Viader, 1951**: 142, pl. 3, fig. 1

Diagnosis: The exterior of the shell is a pale-yellow and dirty-white, the interior is porcelaneous. Solid shell; circular to slightly sub-circular; equivalve; inequilaterally; umbo slightly anterior to the midline, directed forwards and strongly inwards; with a dark-brown ligament that deeply grooved, extending about two-thirds of the way to the posterior margin; anterodorsal margin shows prominent upward elevated curvature; shell commonly sculptured with a large number of alternating raised and flattened concentric ridges; the strong hinge plate with three cardinal teeth; left valve with a dwarf, strong ridge in front of the anterior cardinal tooth, fitting in a suitable cavity lying in the right valve; anterior adductor muscle scar slightly elongated; pallial line posteriorly indented with a deeply triangular sinus margin smooth.

Figure 2. *Dosinia amphidesmoides* **(Reeve, 1850)** with accession number.

1.2. *Gafrarium pectinatum* Linnaeus, 1758 (Figs. 3 & 4)

Systematics description:

Subfamily: Gouldiinae
Genus: *Gafrarium*
Species: *Gafrarium pectinatum* Linnaeus, 1758

Synonyms:
Circe pectinata **Linnaeus, 1758**: 698.
Crista pectinata **Linnaeus, 1758**
Gafrarium pectinatum pectinatum **Linnaeus, 1758**
Gafrarium tumidum **Röding, 1798**
Venus pectinata **Linnaeus, 1758**: 689.
Gafrarium angulatum **Röding, 1798**
Gafrarium cardiodeum **Röding, 1798**
Gafrarium costatum **Röding, 1798**
Gafrarium depressum **Röding, 1798**
Cytherea gibbia **Lamarck, 1818**: 577-578.
Cytherea pectinata **Lamarck, 1818**: 577.
Cytherea ranella **Lamarck, 1818**: 578.
Cytherea pectinata var. *immaculata* **Sowerby I, 1835**: 47.
Circe pythinoides **Tenison Woods, 1878**: 60-61.

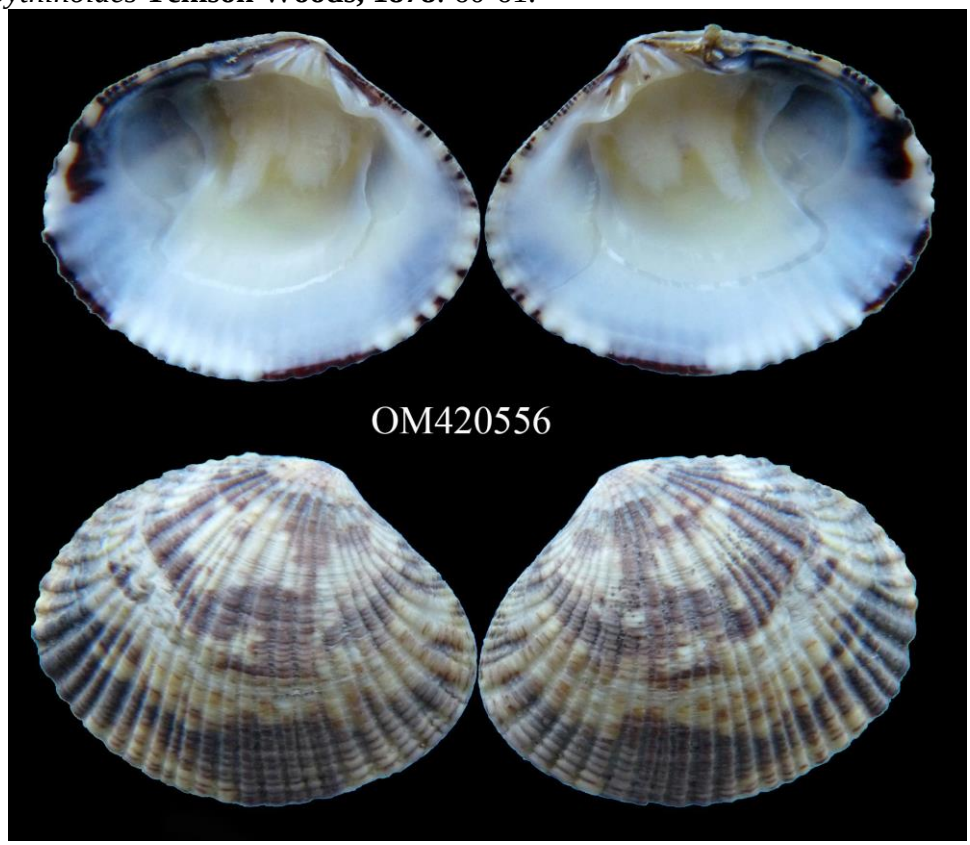

Figure 3. *Gafrarium pectinatum* **(Linnaeus, 1758).**

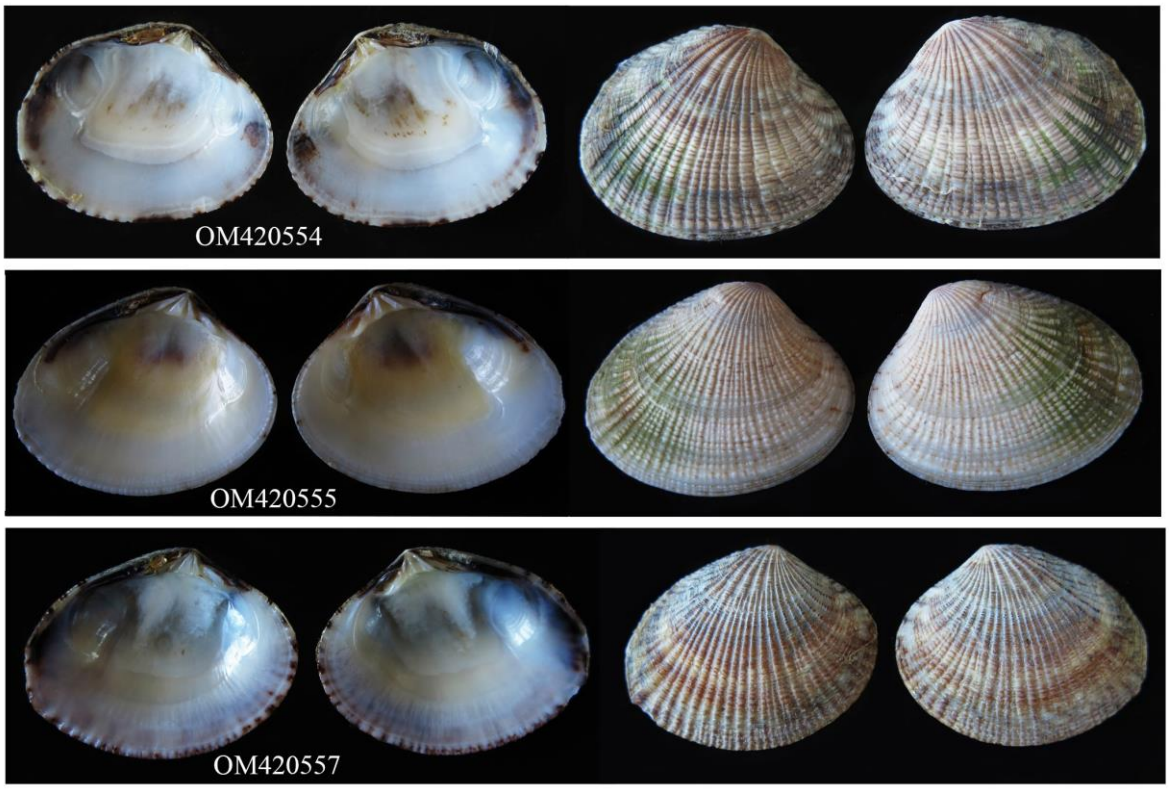

Figure 4. Colour variations of the shell for *G. pectinatum*.

Diagnosis: Exterior beiges-white, with variable light to dark brown mottling, and tent marks; interior white and may have dim purple colouration on the sprite. Strong nodulose radial ribs. Solid shell; ovate; equivalve; with a more rounded posterior margin and less inflated valves. Dorsally, nearly the anterior margin is straight, but the posterior one is slightly convex. Shell's sculpture consists of growth lines, and radial lines of nodules; with more prominent nodules at the central region of the flank, without co-marginal ribs**.** The lines of nodules may split as the animal grows, resulting in more lines of slightly finer nodules at the ventral margin. The umbo points slightly to the anterior. A quarter of the posterior dorsal edge is covered by the external ligament. It is supported by three cardinal teeth and one anterior lateral tooth; the anterior and posterior cardinal teeth are not bifid but the central one is slightly bifid. Neither of the three cardinal teeth nor the two anterior lateral teeth that prop up the right valve are bifid. Without the pallial sinus, the anterior adductor muscle injury is smaller than the posterior.

1.3. *Circenita varia* Forsskål in Niebuhr, 1775 (Figs. 5- 7)

Systematics description:

Genus: *Circenita*
Species: *Circenita varia* **Forsskål in Niebuhr, 1775**
Synonyms:
Venus varia **Forsskål in Niebuhr, 1775**: pl. XXXI.

Venus caliste **Gmelin, 1791**
Cytheraea lentiginosa **Chemnitz, 1795**
Cytherea arabica **Chemnitz, 1795**
Meretrix arabica **Chemnitz, 1795**
Venus arabica **Chemnitz, 1795**: 224. pl. 201 figs. 1968-1970.
Venus bicolorata **Chemnitz, 1795**: 223, pl. 201, figs. 1965-1967.
Venus arabica **Dillwyn, 1817**
Venus lentiginosa **Dillwyn, 1817**: 148.
Gafrarium arabicum **Lamarck, 1818**
Circe litturata **Gray, 1838**: 307.
Cytheraea subelliptica **Sowerby II, 1851**: 644, pl. 135, fig. 169.
Cytherea (Lioconcha) deshayesiana **Issel, 1869**: 67.
Lioconcha savignyi **Pallary, 1926**: 107, pl. 13, fig. 5.

Figure 5. *Circenita varia* **(Forsskål in Niebuhr, 1775)**.

Diagnosis: Exterior white beiges, radial stripes or tent marks, with purple or dark brown co-marginal stripes near the ventral margin. Interior white, having a pink or purple blush through the interior, may also be with dark brown-purple colouration at either anterior or posterior margins, or on both. Strong ovate shell; equivalve; both anterior and posterior dorsal margins nearly straight, with truncated posterior margin. The growth lines and co-marginal ribs form the shell's sculpture. The ribs may merge at the anterior and posterior ends of the flank. A narrow shallow groove is gently impressed and marked the lunule that extends of the anterior dorsal margin. The subcentral umbo points slightly to the anterior. Escutcheon with faded ribs, extending about two-thirds of the posterior

dorsal margin. This ligament extends over half of the posterior dorsal margin, which is supported by the nymphal ridges, and over half of the lateral margin. There are three cardinal teeth and one anterior lateral tooth that support the left valve; none of the cardinal teeth is bifid, although the central tooth may have a somewhat rugose texture, and the posterior tooth is united to the nymphal ridge. In the right valve, there are three

Figure 6. Colour variations of the shell for *C. varia*.

cardinal teeth and one anterior lateral tooth that support the left valve; none of the cardinal teeth is bifid, although the central tooth may have a somewhat rugose texture, and the posterior tooth is united to the nymphal ridge. In the right valve, there are three cardinal teeth and two anterior lateral teeth that support it; the front cardinal tooth is not bifid, but the centre and posterior teeth are mildly bifid. A very fine ridge also runs parallel to the posterior dorsal margin of the right valve. The scar on the anterior adductor muscle is slightly smaller than the scar on the posterior adductor muscle, with an extremely shallow pallial sinus. It is worth mentioning that *Circenita varia* is similar to

C. callipyga, but the posterior margin of *C. varia* is more truncated than that of *C. callipyga*.

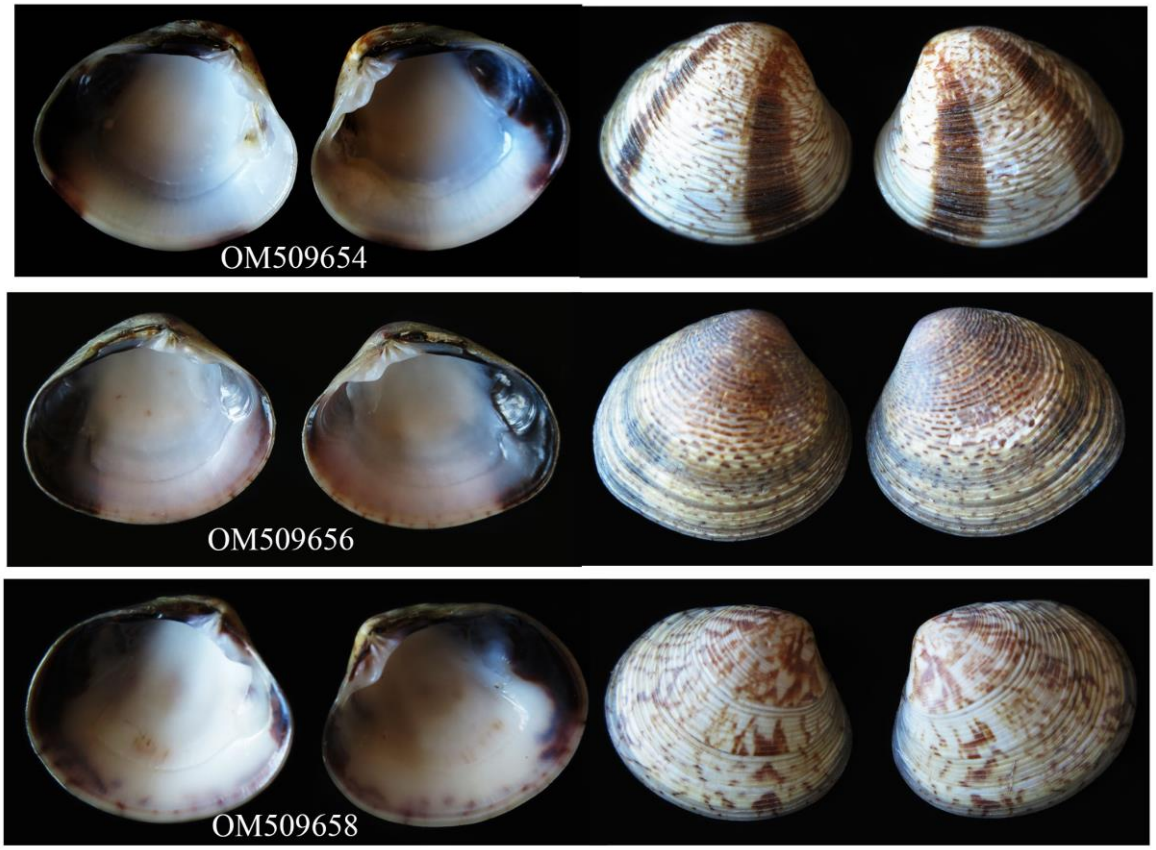

Figure 7. Other patterns of colour variations of the shell for *C. varia*.

1.4. *Circenita callipyga* Born, 1778 (Fig. 8)

Systematics description:
Genus: *Circenita*
Species: *Circenita callipyga* **Born, 1778**

Synonyms:

Venus callipyga **Born, 1778**: 55
Gafrarium callipygum **Born, 1778**
Meretrix lentiginosa **Chemnitz, 1795**
Venus lentiginosa **Chemnitz, 1795**: 223, pl. 201, figs. 1963-1964.
Cytherea arabica **Lamarck, 1818**: 571.
Circe crachrodii **Gray, 1838**: 307.
Cytheraea elliptica **Sowerby II, 1851**: 645, pl. CXXXV, figs. 173 &174.
Circe pulchra **Deshayes, 1854**: 6.
Circe fumata **Reeve, 1863**: 194, pl. VIII, fig. 35.
Tapes amphidesmoides **Reeve, 1864**: 214, pl. X, fig. 50.

Figure 8. *Circenita callipyga* **(Born, 1778)**.

Diagnosis: Exterior white or light tan, with purple and/or brown tent marks, radial stripes, co-marginal stripes or blotches. Interior white, with weak brown in the central area and/or concentrated brown or purple at the posterior margin. Strong oval shell; equivalve; with rounded anterior and ventral margins, although the posterior margin may be slightly truncated; with rounded anterior and ventral margins, but with rounded posterior margins. The front margin is sub-concave, whereas the posterior margin is sub-convex on the dorsal side. Sculptured growth lines and co-marginal ribs are the basis of Shell's work. In the anterior dorsal margin, the lunule is gently impressed and marked by a narrow shallow groove that runs the length of the anterior dorsal margin. The umbo is tilted slightly to the anterior in this position. The external ligament is extended over a portion of the posterior dorsal edge that is equal to one-third to half of the posterior dorsal margin and is supported by the nymphal ridges. Three cardinal teeth and one anterior lateral tooth are found inside the left valve; the central cardinal tooth is somewhat bifid, while the anterior and posterior ones are not bifid. The posterior cardinal tooth is united with the nymph, with the nymph protruding more than the posterior cardinal tooth. A total of three cardinal teeth and two anterior lateral teeth support the right valve; none of the cardinal teeth is bifid. A fine ridge extends along to the posterior dorsal margin of the right valve and runs parallel to the anterior dorsal margin of the left valve. The anterior adductor muscle scar is slightly smaller and less circular than the posterior adductor muscle scar, and it has a pallial sinus that is extremely shallow. *Circenita callipyga* is similar to *C. varia*, but the first is more elongated anteroposterior and has a rounder posterior margin than the latter.

1.5. *Mactra glauca* Born, 1778 (Fig. 9)

Systematics description:
Superfamily: Mactroidea
Family: Mactridae
Subfamily: Mactrinae
Genus: *Mactra*
Species: *Mactra glauca* **Born, 1778**

Synonyms:
Mactra glauca **Born, 1778***:* 51, pl. 3, figs. 11& 12.
Mactra neapolitana **Poli, 1791**: lxxi, pl. XVIII, figs. 1-14.
Mactra helvacea **Lamarck, 1818**: 473.
Mactra epidermia **Deshayes in Reeve, 1854**: 55, pl. III, fig. 11.
Mactra glauca var. *luteola* **Jeffreys, 1864**: 425.
Mactra sericea **Brusina, 1865**: 33.

Figure 9. *Mactra glauca* **(Born, 1778)**.

Diagnosis: Exterior pale grey with concentric grey-blue stripes and yellowish. Interior variable from bluish-grey to violet. The shell is nearly solid, widely oval to triangular in shape with rounded anterior and posterior borders; slightly inequilaterally; umbo just ahead in front of midline; left valve with three cardinal teeth, anterior and central teeth forming an inverted L-shape. The scar on the anterior adductor muscle is substantially smaller than the scar on the posterior adductor muscle, with a short, nearly rounded pallial sinus.

1.6. *Gari depressa* **Pennant, 1777** (Fig. 10)

Systematics description:
Order: Cardiida
Superfamily: Tellinoidea
Family: Psammobiidae
Genus: *Gari*

Species: *Gari depressa* **Pennant, 1777**

Synonyms:
Psammobia depressa **Pennant, 1777**
Tellina depressa **Pennant, 1777**: 73, pl. 47, fig. 27.

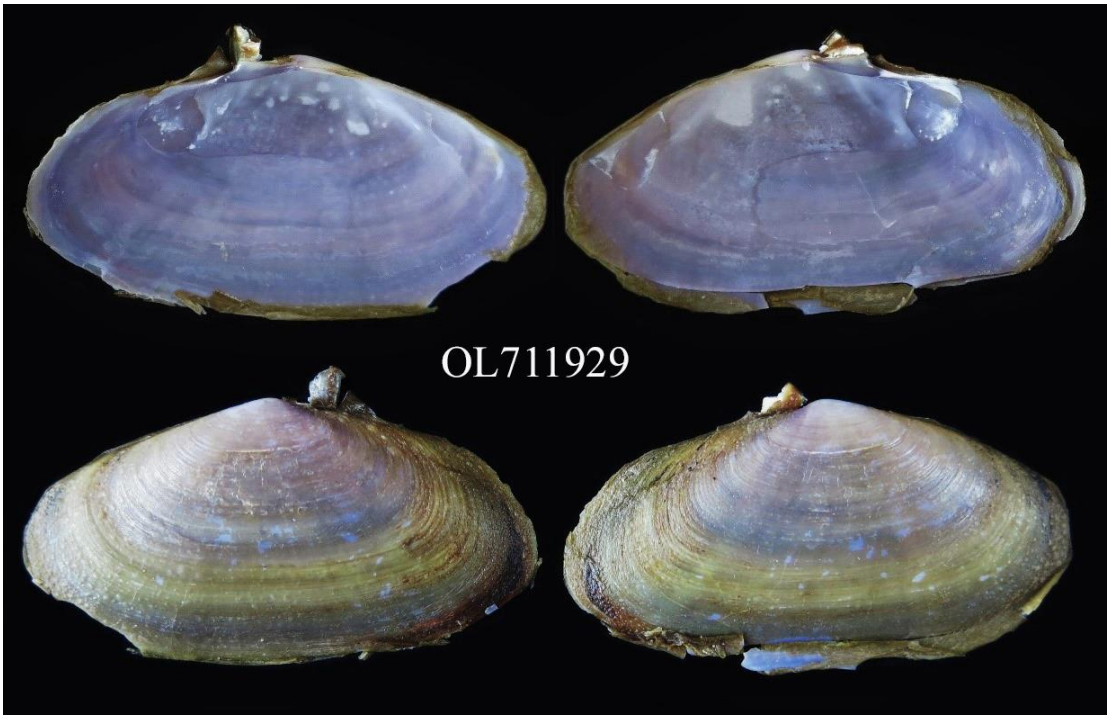

Figure 10. *Gari depressa* **(Pennant, 1777)**.

Diagnosis: Purple, rosy, white, variegated with brown, white and yellow shades. Solid shell; glossy; equivalve; sub-equilateral; with prominent posterior truncation; umbo anterior to the midline, directed inwards; with numerous fine concentric ridges and radiating striae; hinge with two small cardinal teeth, those of the right valve and the anterior of the left valve bifid; without lateral teeth; pallial sinus deep extending beyond the umbo and its lower limb united with the pallial line; inner margin smooth.

2. Molecular identification

The resultant *COI* datasets for each species were 570 bp long. The molluscan sequences were originally deposited in the GenBank database. The *COI* data sequences of

nucleotide composition were analyzed using the Kimura 2-parameter method and a software tool from MEGA X to determine evolutionary distances. The NJ tree constructed using the Neighbor-Joining method (NJ) for the molluscan species under investigation is displayed in (Fig. 11). There were three major clades in the molluscan phylogeny, according to the NJ tree. The first clade was divided into two sub-clades. The first subclade included three species namely *Circenita varia*, *Circenita callipyga*, and *Gafrarium pectinatum* that are affiliated to the subfamily Gouldiinae. Meanwhile, *Dosinia amphidesmoides* that descend from subfamily Dosiniinae was separated in the second subclade. However, these four species do belong to the family Veneridae. The other two species, *Mactra glauca* (family Mactridae) and *Gari depressa* (family Psammobiidae) were branched into the two main clades.

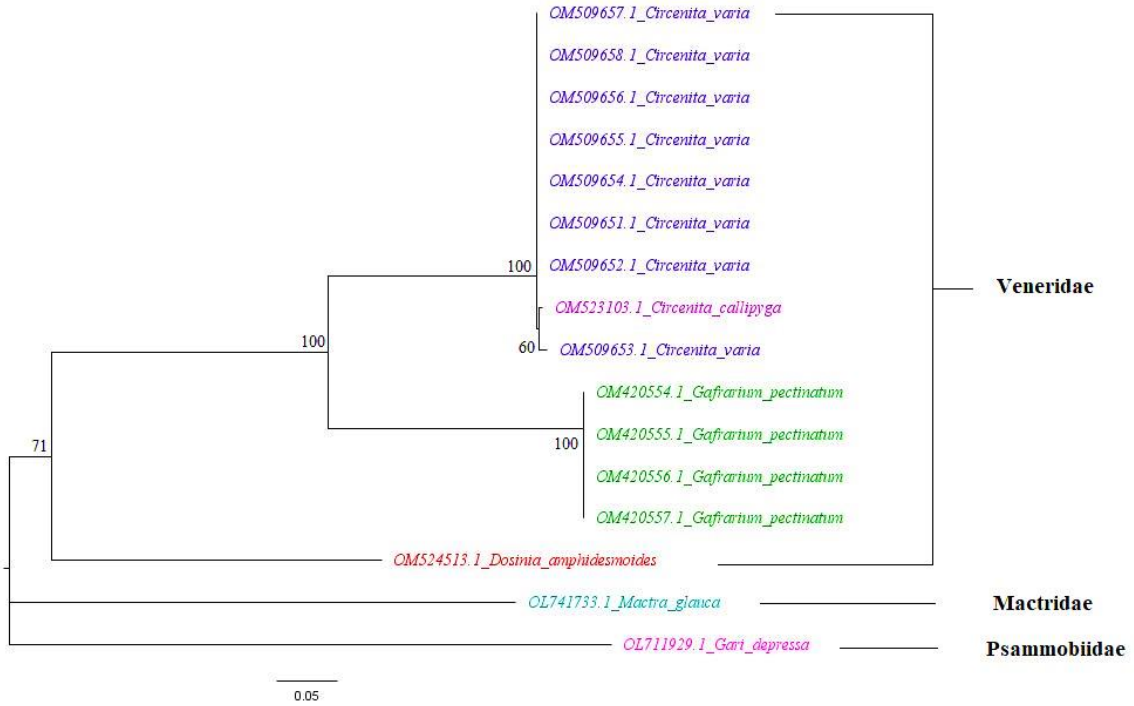

Figure 11. Neighbour Joining (NJ) phylogenetic tree for the six species of Egyptian bivalves based on partial Cytochrome oxidase subunit I (*COI*) gene using K2P method. The numbers above the branches are bootstrap values.

DISCUSSION

Classification of several marine bivalve groupings based on shell characteristics frequently presents difficulties for taxonomists and phylogeneticists due to the absence of identifiable morphological characteristics. Shells of bivalves can exhibit great morphological plasticity as a result of the variety of settings and ecological situations in which they might be found during their lifetime **(Baker *et al.*, 2003)**. In the present study, six species of bivalves belonging to the family (Veneridae, Mactridae, and Psammobiidae) were classified by traditional taxonomy and molecular identification that were collected from the Gulf of Suez, Northern Red Sea. Results of morphological examination of shell structure indicated that species of *Gafrarium pectinatum* and

Circenita varia were varying in the shell colours representing ambiguous species (Figs. 3-7). Observations of external of these shells revealed different colours and patterns. The different colours of external bivalves' shells are assumed to be as attributed to the pigments that are more easily integrated into the organic material than into calcareous shells **(Grant and Williams, 2018)**. The epithelial cells of the mantle tissue, which lies underneath the developing edge, are responsible for the formation of the shell. Primary determinants of shell colour are found in the presence of pigments produced by the mantle, while other factors such as colour and microstructure of shell-composing calcium carbonate crystals, as well as the presence of food, and may also play a role in this process **(Williams, 2017)**. A similar difference can be seen, in the inner shells of the two species such as, in *Circenita varia*, the dark brown-purple colour is mainly concentrated at the posterior margin of the shell, sometimes appears as a spotted patch in the anterior margin, and rarely a connecting linkage between them is displayed. Colours found in the interior of bivalve and gastropod shells, such as pearly iridescence, are caused by nacre, a composite substance made of ordered aragonite plate configurations and organic material **(Saenko and Schilthuizen, 2021)**. On the other hand, the morphological features are regarded as reliable and distinct individual identifying traits for the other four bivalve species. Figs. (2, 8-10) show the images of the investigated species, as well as their most significant external morphological distinctions. Simultaneously, the identification of these species was supported by molecular technique with *COI* gene and the sequence data for these six bivalve species that did not exist before this study and was used in the GenBank database as the sequences for bivalves that were collected from the Gulf of Suez, Egyptian Red Sea. *Circenita varia* and *Gafrarium pectinatum* were characterized by a variety of shell colouration and could be distinguished from different shell patterns. On the otherwise, *COI* sequences obtained for these species with the different colours and patterns confirmed that all individuals from both groups belong to each species and that the sequences were clustered together in two sub-clades of the first main clade, one for *Circenita varia* and the other for *Gafrarium pectinatum*. The individuals of bivalves belonging to the same genetic clade may have shells of different colours **(Reunov *et al.*, 2021)**. *Circenita callipyga* was branched with one individual of *Circenita varia* in the same clade indicating that both species are the grouping of such a genus Circenita and share the same synonyms. *Dosinia amphidesmoides* was branched in the second sub-clade with the species of the family Veneridae. *COI* sequence data were used to reconstruct a phylogenetic relationship of bivalve taxa along the coast of mainland China for the family Veneridae to evaluate the classification of the family by the traditional and molecular methods **(Chen *et al.*, 2011)**. The current phylogenetic study demonstrated that the use of DNA barcoding was effective and proven to be a good strategy. The findings were clear enough to identify and taxonomize the several bivalve species gathered in Egypt. Furthermore, in the present study, the CO1 sequences proved their ability to distinguish across bivalve groups (Veneridae, Mactridae and Psammobiidae). Also, molecular identification confirmed the classical taxonomy to release the ambiguous discrimination within the same species with different colours and patterns.

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