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Non-indigenous giant mud crab, *Scylla serrata* (Forskål, 1775) (Crustacea: Brachyura: Portunidae) in Malaysian coastal waters: a call for caution

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Abstract

Introduction of non-indigenous species into a well-established ecosystem can be detrimental, resulting in both ecological and economical damage. Two specimens of giant mud crab, *Scylla serrata* were found for the first time in two geographically distinct mud crab landing sites (Matang Mangrove Forest Reserve, Perak and Kota Marudu Mangrove Forest, Sabah) in Malaysia. Their identities were confirmed using a combination of morphological, morphometric and molecular (partial COI gene) analyses. *Scylla serrata* is regarded as non-indigenous species within Malaysian coastal waters as this is the first confirmed report of their occurrence in Malaysia and no established population was found in both landing sites. Accidental release or escape was considered as the possible vector for the introduction of the two specimens found in this study as frequent import of *S. serrata* from other countries were reported in both landing sites. Urgent intervention is needed to prevent further introduction and possible establishment of *S. serrata* population in Malaysian coastal waters.

Keywords: Giant mud crab, Introduction, Malaysia, Non-indigenous species, *Scylla serrata*, Species identification, Partial COI gene

Background

A new species introduced to an ecosystem outside its native range is termed as “non-indigenous species” and can occur deliberately or accidentally by the means of humans (Holmes and Simons 1996; Manchester and Bullock 2000). If the new environment is suitable, the non-indigenous species may establish a viable population, and if it spreads fast and causes harm to either the environment, human health, economy or any other valuable resources, it will be termed as an “invasive species” (Manchester and Bullock, 2000). Aquatic ecosystems are even more susceptible to the spread of non-indigenous species due to the high reproduction rate of most aquatic organisms and rapid dispersal of propagules

that is possible in water (Strayer and Dudgeon 2010; Wood et al. 2016).

The mud crab genus *Scylla* De Haan, 1833 (Decapoda: Portunidae) is widely distributed in the brackish and coastal mangrove area of Indo-West-Pacific region and supports important commercial, recreational and indigenous fisheries (Keenan et al. 1998; Ikhwanuddin et al. 2011). Known for their succulent meat and delicate flavour and texture, mud crabs are highly sought after in seafood restaurants, both locally and internationally. The global capture fishery production of mud crab is approximately 38,000 t in the year 2014 (FAO, 2016). Mud crabs are highly adaptable to various environments due to their ability to tolerate broad ranges of temperature (16–35 °C) and salinity (1–56 ppt) (Alberts-Hubatsch et al. 2015). They also show movement up to an average of 3.7 km for foraging purposes (Hyland et al. 1984). Mud crabs are opportunistic omnivores and feed mainly on crustaceans, molluscs and fish (Viswanathan and Raffi 2015). Thus, if introduced, either deliberate or accidental,

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mud crabs could potentially disrupt the balance of the local ecosystem.

There are four species of mud crab within this genus, *Scylla serrata* (Forskål, 1775), *Scylla tranquebarica* (Fabricius, 1798), *Scylla olivacea* (Herbst, 1796) and *Scylla paramamosain* (Estampador, 1949). *Scylla serrata* is the largest and most widespread species of genus *Scylla* (Keenan et al. 1998; Alberts-Hubatsch et al. 2015). *Scylla serrata* has been reported in most tropical and subtropical coastal regions of Indo-West-Pacific such as Bangladesh (Begum et al. 2009), India (Ali et al. 2011), Sri Lanka (Tharminie et al. 2014; Amarasekara et al. 2016), Indonesia (Roza and Hatai 1999; Nordhaus et al. 2009), Philippines (Baylon et al. 2004; Quintio et al. 2007), Australia (Keenan et al. 1998) and around the oceanic islands of Indo-Pacific (Alberts-Hubatsch et al. 2015), except around South China Sea (Albert-Hubatsch et al. 2015; Fazhan et al. 2017). Prior to the revision of genus *Scylla* by Keenan et al. (1998), the species identification of mud crabs within the genus *Scylla* has been controversial and only one species, *S. serrata*, was being recognized (Stephenson and Campbell 1960). The division of genus *Scylla* into four distinctive species has led to the re-validation of species identification in most locations. However, since the revision of the genus *Scylla* (Keenan et al. 1998), *S. olivacea* has been the most common species in Malaysian waters, although *S. paramamosain* and *S. tranquebarica* also occur (Ikhwanuddin et al. 2011; Waiho et al. 2015; Waiho et al. 2016).

A recent nationwide survey of mud crab species composition revealed two specimens of the giant mud crab, *S. serrata* in Malaysian waters. This study describes the species identification of *S. serrata* found using (i) morphological (ii) morphometrics and (iii) molecular identification methods. The confirmed identity of *S. serrata* in this study serves as the first report of its

occurrence in Malaysian waters. The possible vector and impact of its introduction are discussed.

Methods

Sample collection

A bimonthly nationwide survey of mud crab species composition was conducted in Malaysian waters from April 2012 to July 2013 (Fig. 1). A total of 1325 mud crabs caught using standard crab pots were identified to species level based on Keenan et al. (1998). Interestingly, two specimens of *S. serrata* was found for the first time from Malaysian coastal waters. One specimen (immature female) was collected from Matang Mangrove Forest Reserve, Perak (4°45'N100°37'E) (Straits of Malacca) on 27 March, 2013 by H. Fazhan and another (mature male) was found in Kota Marudu Mangrove Forest, Sabah (6°44'N117°1'E) (Sulu Sea) (Fig. 1) on 12 May, 2013 by K. Waiho. Fishing and crabbing are commonly conducted in both estuaries by local communities. Matang Mangrove Forest Reserve was gazetted as a permanent mangrove forest reserve more than a century ago whereas Kota Marudu is protected under the jurisdiction of local forestry department.

Morphological and morphometric identification

The collected specimens were identified morphologically based on the key characters (i.e. frontal lobe spines shape and height, cheliped carpus and propodus spine, cheliped color, and polygonal patterning on pereopods) provided by Keenan et al. (1998).

Only mature male (>95 mm carapace width, CW) was subjected to morphometric identification. The measurements of 24 body parts were taken to the nearest 0.01 mm using Vernier calipers based on Keenan et al. (1998). Five morphometric ratios used by Keenan et al.



Fig. 1 Two specimens of *Scylla serrata* was found in Malaysia (in white), one in Matang Mangrove Forest Reserve, Perak (labelled A) and another in Kota Marudu Mangrove Forest, Sabah (labelled B)

(1998) for species comparison and identification was applied in this study as well.

Molecular identification

Additionally, validation using molecular method was conducted. Both specimens were stored in $-80\text{ }^{\circ}\text{C}$ prior DNA extraction. DNA was extracted from the muscle tissue of walking leg using GF-1 Nucleic Acid Extraction Kits (Vivantis Tech., MY). Partial cytochrome oxidase subunit I (COI) gene from the mitochondrial genome was amplified using polymerase chain reaction (PCR). The temperature profile used was: pre-denaturation at $95\text{ }^{\circ}\text{C}$ for 10 mins, denaturation at $94\text{ }^{\circ}\text{C}$ for 30s, annealing at $50\text{ }^{\circ}\text{C}$ for 30s, extension at $72\text{ }^{\circ}\text{C}$ for 45 s (30 cycles) and final extension at $70\text{ }^{\circ}\text{C}$ for 10 mins. Standard universal primers LCO-1490 (5'-GGTCAACAAATCATAAAGA TATTGG-3') and HCO-2198 (5'-TAAACTTCAGG GTGACCAAAAAATCA-3') were used (Folmer et al. 1994; Fazhan et al. 2016). PCR products (Fig. 2) were sent for sequencing (First BASE Lab, MY), checked for quality and subjected to BLAST (Basic Local Alignment Search Tool) analysis for comparison and identification based on deposited sequences in GenBank, NCBI (National Center for Biotechnology Information) (Altschul et al. 1997).

Results

Systematic account

Order: Decapoda Latreille, 1806.

Family: Portunidae Rafinesque, 1815.

Genus: *Scylla* De Haan, 1833.

Scylla serrata (Forskål, 1775) (Fig. 3).

Description

The carapace is oval and is 1.56 times (mature ♂) / 1.57 times (immature ♀) broader than long. There are nine (9) anterolateral spines on both sides of the carapace with straight or slightly concave outer margin, and six (6) high frontal lobe spines with concave and rounded interspaces. Cervical grooves are present on the anterior of the slightly raised carapace. Overall, the carapace, walking legs and chelipeds are smooth. Two obvious spines are located at the carpus of chelipeds on distal half of the outer margin. Another two sharp spines can be found at the dorsal margin of chela (propodus), near the insertion of the dactyl. Teeth-like structures are found in between the cavity of the dactyl and the distal margin of the chela. Obvious polygonal patterning is present on chelipeds and all walking legs. The chela is greenish to brownish with bright blue coloration. The overall coloration is a mixture of light brown and green.

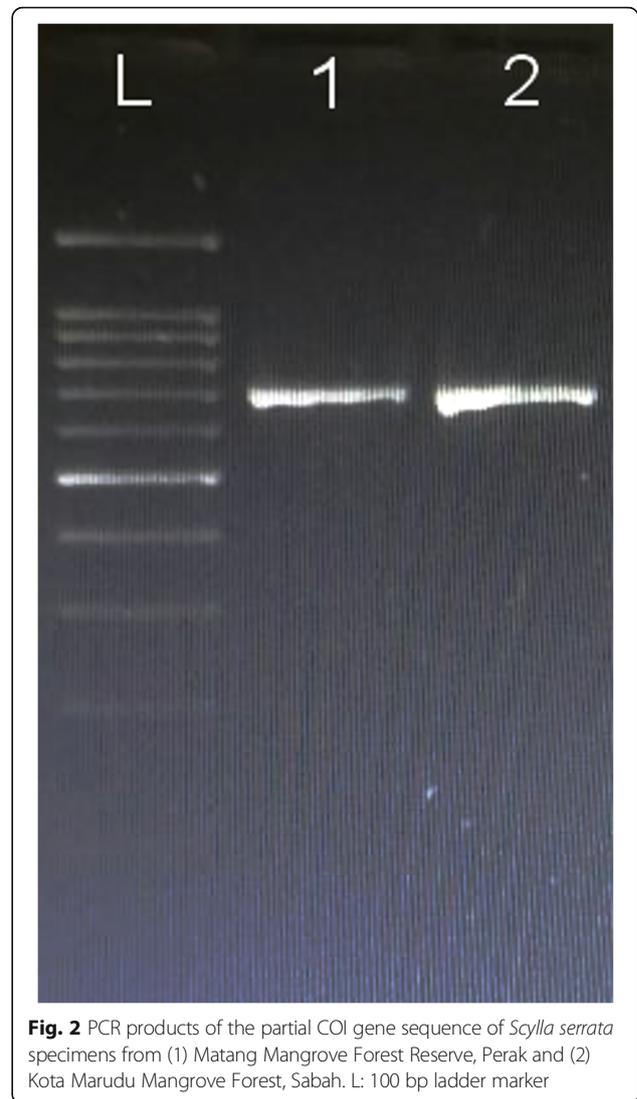


Fig. 2 PCR products of the partial COI gene sequence of *Scylla serrata* specimens from (1) Matang Mangrove Forest Reserve, Perak and (2) Kota Marudu Mangrove Forest, Sabah. L: 100 bp ladder marker

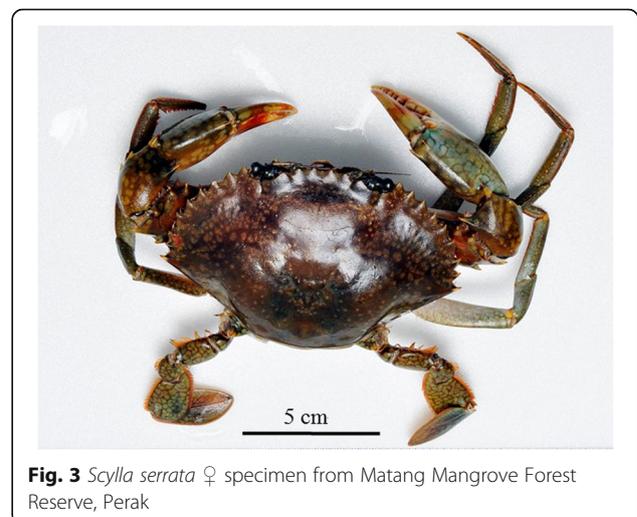


Fig. 3 *Scylla serrata* ♀ specimen from Matang Mangrove Forest Reserve, Perak

The measurements of *S. serrata* collected in this study is presented in Table 1.

Habitat

Resides in the coastal estuaries, coastlines and brackish mangrove forests area with tidal influence and occasional salinity reflux. Prefer muddy bottoms and seagrass beds.

Distribution

Perak and Sabah, Malaysia (current study); Philippines; Indonesia; Japan; Taiwan; Bangladesh; Sri Lanka; Australia; oceanic islands of the Indo-Pacific; Maldives; Mauritius; South Africa; Africa; the Red Sea; Gulf of Aden; the Persian Gulf.

Table 1 Measurements (mm) of *Scylla serrata*

Measurement parts	Immature ♀	Mature ♂
CW	75.11	142.72
ICW	69.83	139.81
8CW	74.15	140.71
PCW	24.84	46.53
CL	47.81	91.31
FW	28.8	52.67
FMSH	1.62	3.21
DFLS	3.35	7.13
DFMS	3.5	7.61
SW	37.4	71.86
AW	42.61	32.68
3PML	31.2	54.83
3PCL	28.7	33.01
5PDL	22.2	44.64
5PDW	22.86	21.76
CDL	32.10	53.61
CPD	16.74	43.68
CPL	69.81	110.9
CML	37.00	63.74
CPW	17.23	25.81
IPS	2.17	3.13
OPS	2.11	2.21
ICS	1.83	2.42
OCS	2.24	2.31

CW carapace width, ICW internal carapace width, 8CW carapace width at spine 8, PCW posterior width of carapace, CL carapace length, FW carapace frontal width, FMSH frontal median spine height, DFLS distance between frontal lateral spines, DFMS distance between frontal median spines, SW sternum width, AW abdomen width, 3PML third periopod merus length, 3PCL third periopod carpus length, 5PDL fifth periopod dactyl length, 5PDW fifth periopod dactyl width, CDL cheliped dactyl length, CPD cheliped propodus depth, CPL cheliped propodus length, CML cheliped merus length, CPW cheliped propodus width, IPS inner propodus spine, OPS outer propodus spine, ICS inner carpus spine, OCS outer carpus spine

Morphometrics measurements

The two morphometric characters and five morphometric ratios of mature male *S. serrata* specimen found in this study fall within the range of *S. serrata* when compared with the data of four *Scylla* species provided by Keenan et al. (1998) (Table 2). However, they were distinct from the other three species, i.e. *S. tranquebarica* (frontal median spine height (FMSH)/ carapace frontal width (FW) and FMSH/ distance between frontal median spines (DFMS)), *S. paramamosain* (inner carpus spine (ICS)/ outer carpus spine (OCS)) and *S. olivacea* (ICS/OCS, FMSH/FW and FMSH/DFMS) (Table 2). This further confirms the identity of our specimen as *S. serrata*.

Molecular validation

BLAST analysis of the partial COI gene sequence from both specimens confirmed their identity as *S. serrata*, in which the sequences of both specimens were identical to that of other *S. serrata* specimens found in GenBank (query cover = 100%; identity = 100%; E value = 0.0). The partial COI gene sequences of *S. serrata* ♂ from Sabah and ♀ from Perak were deposited into GenBank with the accession number KX249605 and KX249606, respectively [see Additional file 1].

Discussion

The identity of *S. serrata* specimens found in the current study was successfully identified and validated using a combination of morphological, morphometric and molecular analyses. All three analyses were previously used by Keenan et al. (1998) to separate the long recognised single species (i.e. *S. serrata*) within the genus *Scylla* into four distinct species, with the addition of *S. tranquebarica*, *S. olivacea* and *S. paramamosain*. Similar methods were also employed by Imai and Takeda (2005) to validate the identity of a natural hybrid mud crab genus *Scylla* in Japan.

The discovery of *S. serrata* in Malaysian coastal waters is unprecedented. It was reviewed by Alberts-Hubatsch et al. (2015) that despite their wide geographical distribution, *S. serrata* has never been reported in Malaysian waters after the division of genus *Scylla* into four species. The species composition of mud crab population in Malaysian waters is predominantly made up of *S. olivacea*, with *S. tranquebarica* and *S. paramamosain* being frequently found in smaller numbers (Ikhwanuddin et al. 2011; Waiho et al. 2016).

Both *S. serrata* specimens found in this study were captured in intertidal mangrove forests. The presence of only one specimen in each location despite our extensive survey involving large number of mud crabs suggests that very few individuals were introduced and there is possibly still no viable *S. serrata* population in the wild.

Table 2 Comparison of the ranges and means (in parentheses) of two morphometric characters and four morphometric ratios among collected specimen (mature ♂) and the four *Soylla* species provided by Keenan et al. (1998)

Ratio (mm)	Mature ♂	<i>S. serrata</i>	<i>S. tranquebarica</i>	<i>S. paramamosain</i>	<i>S. olivacea</i>
	n	n	n	n	n
CW	142.72	102.4–204.4 (147.3)	102.5–143.8 (120.8)	111.1–145.7 (122.5)	98.8–138.1 (112.2)
ICW	1.39.81	95.5–191.7 (138.4)	97.1–137.8 (113.7)	104.8–134.1 (114.7)	95.0–133.9 (107.5)
ICS/OCS	1.048	0.500–1.583 (0.940)	0.467–1.429 (0.980)	0.188–0.941 (0.352)	0.000–0.250 (0.006)
FMSH/FW	0.061	0.041–0.095 (0.061)	0.031–0.053 (0.043)	0.040–0.081 (0.058)	0.018–0.037 (0.029)
FW/ICW	0.377	0.335–0.406 (0.371)	0.383–0.443 (0.412)	0.364–0.386 (0.377)	0.371–0.451 (0.415)
LSH (CW-ICW)/ICW	0.021	0.018–0.046 (0.031)	0.022–0.044 (0.031)	0.022–0.049 (0.034)	0.015–0.037 (0.022)
FMSH/DFMS	0.422	0.224–0.544 (0.418)	0.216–0.371 (0.310)	0.283–0.483 (0.369)	0.143–0.316 (0.221)

CW carapace width, ICW internal carapace width, ICS inner carapace spine, OCS outer carapace spine, FMSH frontal median spine height, FW carapace frontal width, DFMS distance between frontal median spines

Personal communication with local fishermen revealed that some mud crab traders import mud crabs, specifically *S. serrata* from overseas due to their large sizes and high market price. Several traders from Perak sourced *S. serrata* from India and Indonesia whereas traders from Sabah imported *S. serrata* from the neighbouring Philippines. In addition, it was observed that local traders and fishermen frequently discard unhealthy mud crabs directly onto the ground nearby during screening before selling them off to restaurants. With most of these traders and fishermen living just nearby their mud crab fishing ground (the same sampling sites in this study), it is very likely that both *S. serrata* specimens found in our study were imported from overseas and accidentally released or escaped into the wild.

Other alternative vectors that have been reported to facilitate the introduction of non-indigenous marine and estuarine invertebrate species are the unintentional release of invertebrate larvae via ballast water of large cargo ships and escape from aquaculture activities (Fuller et al. 2014). Invertebrate larvae are taken on-board of large cargo ships, travel far from their natural geographical habitat and release into pristine non-native environments with the loading and discharge of ballast water. This unintentional introduction method was postulated to be one of the vectors responsible for the introduction of Asian shore crab, *Hemigrapsus sanguineus* (De Haan, 1853), to Northwest Atlantic (Epifanio et al. 1998) and Asian tiger shrimp, *Penaeus monodon* Fabricius, 1798 (Fuller et al. 2014). Since their introduction, both *P. monodon* and *H. sanguineus* have successfully colonised their new introduced environment, displaced other local invertebrate species and altered biodiversity (Ruiz et al. 1997; Fuller et al. 2014). Both locations where *S. serrata* specimens were found (Perak and Sabah) are main shipping channels (i.e. Straits of Malacca connects Indian Ocean and the Pacific Ocean whereas Sulu Sea is situated between South China Sea and the Pacific Ocean). However, introduction of *S. serrata* via ballast water in the form of larvae is less likely as the number of discovered *S. serrata* specimens was too low. If this postulate is true, the high volume of ballast water would have introduced a high number of *S. serrata* larvae, and subsequently resulted in the formation of a sizeable population instead of just one specimen being found in each location. However, further study is needed to determine the possibility of *S. serrata* larvae being transferred via ballast water. Another vector, i.e. escape from aquaculture activities is unlikely as no mud crab aquaculture activity is reported and observed in both locations. Only several small-scale fattening and

soft-shell crab farming are sighted in Perak, and all sourced juvenile crabs from local fishermen.

The first record of *S. serrata* in Malaysian waters reported in this study may have adverse impact towards the environment and could pose as threats to the local mud crab populations. Well known for their resilience – highly tolerance to water salinity changes, ability to survive out of water and without food for a long period (Quinitio et al. 2011), released or escaped *S. serrata* imported from other countries could easily survive and invade the local environment. Comparatively larger in terms of average body size than the other three *Scylla* species (Keenan et al. 1998), *S. serrata* may have greater nutritional requirements and a better advantage in its competition for food compared to local species i.e. *S. olivacea* that are of smaller size. Once a satellite population is established, crabs such as *S. serrata* may spread and colonise nearby habitat effortlessly via their natural larval dispersal (Behrens Yamada and Gillespie 2008), and may pose as competitors to the local mud crab populations. In addition, the report of natural hybrid mud crab crossed between *S. serrata* and *S. olivacea* (Imai and Takeda 2005) in Japan also highlights the possible inter-species crossbreeding of the escaped *S. serrata* with the dominant local *S. olivacea* population in Malaysian waters. Ultimately, it may result in the extinction of the local inhabiting mud crab species following with the emergence of a new hybrid species (Rhymer 1996). In addition, potential invasion of *S. serrata* may also negatively affect other intertidal communities, especially the preys (e.g. gastropods, crabs, shrimps and mollusks) along the mud crab food chain. Thus, frequent monitoring of wild mud crab populations, strict regulations on the import of non-indigenous *S. serrata* and the appropriate handling procedures should be introduced and imposed in near future to prevent future possible establishment of *S. serrata* satellite population in Malaysian waters.

Additional file

Additional file 1: The partial COI gene sequences of male and female *Scylla serrata* from Sabah and Perak, respectively. (DOCX 13 kb)

Abbreviations

COI: Cytochrome oxidase subunit 1; DFMS: Distance between frontal median spines; FMSH: Frontal median spine height; FW: Frontal width; ICS: Inner carpus spine; NCBI: National Center for Biotechnology Information; OCS: Outer carpus spine; PCR: Polymerase chain reaction

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Availability of data and materials

The genetic data supporting the results of this study are available in GenBank under the accession numbers KX249605 (male *S. serrata*) and KX249606 (female *S. serrata*).

Authors' contributions

HF and KW discovered the specimens during sampling. HF, KW and MI identified the specimens and prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All sampling sites are commercial fishing grounds and no special approval is needed. The work was approved by the Ethics Committee of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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