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The first record of an eastern Pacific invasive crab in Taiwanese waters: *Amphithrax armatus* (Saussure, 1853) (Brachyura: Majoidea: Mithracidae), with notes on the taxonomy of the genus

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ABSTRACT

The tropical Eastern Pacific mithracid spider crab *Amphithrax armatus* (Saussure, 1853), known thus far only from Mexico, Nicaragua, and Panama, is here reported for the first time from southern Taiwan in the Indo-West Pacific region. The invasive species probably entered via ballast water or as fouling fauna on large ships that ply the Pacific shipping routes. This is only the second record of an American majoid entering Asian seas, the first being the inachoidid *Pyromaia tuberculata* (Lockington, 1877) from the American Pacific to Japan. The genetic data, using three mitochondrial genes (12S rRNA, 16S rRNA and COI) and two nuclear genes (18S rRNA and H3), indicates that *A. armatus* does not belong to the genus *Amphithrax* Windsor & Felder, 2017, as currently defined, with the species genetically closer instead to taxa in *Nonala* Windsor & Felder, 2014 and *Ala* Lockington, 1877. This is also supported by a number of morphological characters. *Amphithrax* thus needs to be revised.

Key Words: Indo-West Pacific region, invasive species, spider crabs, tropical Eastern Pacific region, Taiwan, taxonomy

INTRODUCTION

Global trade has opened up many new ways for marine animals to be transported outside their natural distribution, and the challenge of invasive species has become a serious issue, creating innumerable problems for conservation biologists, ecologists, and managers (Bax *et al.*, 2003; Brockerhoff & McLay, 2011; Carlton, 2011; McLay, 2015). Among the most invasive and problematic species among brachyuran crabs have been portunoids like *Carcinus maenas* (Linnaeus, 1758), *Charybdis hellerii* (A. Milne-Edwards, 1867), *Charybdis japonica* (A. Milne-Edwards, 1861), and *Callinectes sapidus* Rathbun, 1896; xanthoids like *Rhithropanopeus harrisi* (Gould, 1841), and grapsoids like *Eriocheir sinensis* H. Milne Edwards, 1853, *Hemigrapsus sanguineus* (De Haan, 1835), and *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (see review in Brockerhoff & McLay, 2011; McLay, 2015).

With regards to spider crabs of the superfamily Majoidea (*sensu* Ng *et al.*, 2008), 10 species have been listed as invasive. Three

species have entered the Mediterranean via the Suez Canal, and the numbers will surely grow as the canal is expanded and more shipping flows through it: *Menaethius monoceros* (Latreille, 1825) (Epialtidae MacLeay, 1838) (Falciai, 2003); *Hyastenus hilgendorfi* De Man, 1887 (Epialtidae) (Galil, 2006); and *Micippa thalia* (Herbst, 1803) (Majidae Samouelle, 1819) (Enzenross & Enzenross, 1995). One species, *Herbstia nitida* Manning & Holthuis, 1981 (Epialtidae) (Galil, 2007) is believed to have entered the Mediterranean from the eastern Atlantic. *Chionoecetes opilio* (O. Fabricius, 1788) (Oregoniidae Garth, 1958) (Alvsvag *et al.*, 2009) is now believed to have entered the Barents Sea from the subarctic North Pacific. Another cold-water species, *Hyas araneus* (Linnaeus, 1758) (Oregoniidae) (Tavares & de Melo, 2004) has been transported from the northern Atlantic to the Southern Ocean. *Pyromaia tuberculata* (Lockington, 1877) (Inachoididae Dana, 1851) has spread widely from northeastern Pacific shores of America to the Atlantic, Japan, Australia, and New Zealand (Furota, 1996). A Western

Atlantic species, *Libinia dubia* H. Milne Edwards, 1834 (Epiplatidae) (Enzenross *et al.*, 1997; Zenetos *et al.*, 2005) has been transported to the Mediterranean. With regards to two western Pacific species; *Hyastenus spinosus* A. Milne-Edwards, 1872 (Epiplatidae) (Coles *et al.*, 1997 [incorrectly spelled “*Hyastenus spinosus*”]; Carlton & Eldredge, 2009) has entered the Hawaiian Islands; while *Schizophrys aspera* (H. Milne Edwards, 1834) (Majidae) (Edmondson, 1951; Coles *et al.*, 1997; Coutts & Taylor, 2004; Coutts & Dodgshun, 2007) has been found in the Hawaiian Islands and New Zealand (see also Castro, 2011; Lee *et al.*, in press).

In mid-2016 and early 2017, inshore fishermen in southern Taiwan around the major port of Kaohsiung began collecting in tangle nets substantial numbers of a large spider crab which they were not familiar with. The crab also started to appear in intertidal areas around the port. Examination of the material confirmed that they belonged to a tropical Eastern Pacific majoid species of Mithracidae, now identified as *Amphithrax armatus* (Saussure, 1853). No species of mithracid is known to be an invasive and there have not been any records of brachyuran species from the tropical Eastern Pacific region (western coasts of the Americas from the southern tip of Baja California Peninsula to northern Ecuador) other than *Pyromaia tuberculata* being transported to East Asian waters, in the Indo-West Pacific region.

The taxonomy of Mithracidae was revised by Windsor & Felder (2014), who separated *Mithrax Latreille, 1816*, into six genera (*Mithrax* s. str., *Mithraculus* White, 1847, *Nemausa* A. Milne-Edwards, 1875, *Nonala* Windsor & Felder, 2014, *Omalacantha* Streets, 1871, and *Petramithrax* Windsor & Felder, 2014), recognizing 38 species, all from the Pacific and Atlantic coasts of the Americas (see also Carmona-Suárez & Poupin, 2016). Klompmaker *et al.* (2015) later added a new genus, *Maguimithrax* Klompmaker, Portell, Klier, Prueter & Tucker, 2015. Windsor & Felder (2017) subsequently changed the name of one genus for nomenclatural reasons, with members of *Damithrax* Windsor & Felder, 2014 synonymized under *Mithrax Latreille, 1816* s. str., their original concept of *Mithrax* requiring a new name, *Amphithrax* Windsor & Felder, 2017.

Garth (1958) had placed the taxonomy of the eastern Pacific Mithracidae on a very firm foundation with his important revision of the western American spider crabs. The present material from Taiwan, which includes an excellent series of small and large individuals, including ovigerous females, agree very well with Garth's detailed descriptions and figures of *Mithrax armatus* Saussure, 1853, which was transferred to *Amphithrax* by Windsor & Felder (2017). Garth (1958) argued that *Mithrax (Mithrax) orcutti* Rathbun, 1925, described from three males and four females collected from Puerto Ángel, Oaxaca, Mexico as well as Panama (Rathbun, 1925), was synonymous with *Mithrax armatus* obtained from Mazatlán, Mexico (Saussure, 1853), and we agree with his prognosis. *Amphithrax armatus* is known thus far only from Mexico, Nicaragua, and Panama (Saussure, 1853; A. Milne-Edwards, 1875; Miers, 1886; Rathbun, 1925; Crane, 1947; Garth, 1958). *Amphithrax* currently has 10 recognised species from both Atlantic and Pacific coasts of the Americas (Windsor & Felder, 2014, 2017).

MATERIAL AND METHODS

Specimens examined are deposited in the National Taiwan Ocean University (NTOU), Keelung, Taiwan; National Museum of Marine Biology and Aquarium (NMMBA), Pingtung, Taiwan, and the Zoological Reference Collection (ZRC) of the Lee Kong Chian Natural History Museum, National University of Singapore. The following material of *Amphithrax armatus* from Taiwan was preserved: 1 male (50.7 × 50.5 mm) (ZRC 2017.988), from tangle nets, local fishermen, 31 October 2014; 1 male (75.9 × 82.7 mm) (NMMBCD 4082), Kezailiao fishing port, north of Kaohsiung, from tangle nets, 10 December 2014; 4 males (62.1 × 70.2 mm, 59.8 × 65.4 mm, 41.7 × 47.9 mm, 16.9 × 18.9 mm) (NMMBCD

4083), Kezailiao fishing port, north of Kaohsiung harbor, from tangle nets, collected by C.-W. Lin from local fishermen, 14 April 2016; 2 males (74.7 × 73.7 mm, 75.8 × 75.1 mm), 1 female (49.2 × 49.8 mm) (NTOU), 1 male (72.0 × 71.8 mm), 1 female (52.6 × 55.2 mm) (ZRC 2017.214), Kezailiao fishing port, north of Kaohsiung Harbor, Taiwan, from tangle nets, collected by Y.-P. Li, 10 January 2017; 6 males (60.5 × 59.3 mm – 82.1 × 79.8 mm) (NTOU), Kezailiao fishing port, north of Kaohsiung Harbor, Taiwan, collected by Y.-P. Li & P.-T. Tsai from tangle nets, 23 June 2017; 6 males (36.7 × 39.3 mm to 81.6 × 79.6 mm), 3 females (50.9 × 52.5 mm to 58.4 × 60.2 mm) (NTOU), Sizihwan, north of Kaohsiung Harbor, from tangle nets, collected by J.-J. Li, 10 June 2017. All specimens are from southwestern Taiwan.

The abbreviations G1 and G2 are used for the male first and second gonopods, respectively. Measurements provided, in millimetres, are of the maximum carapace width and length (including spines), respectively.

The smallest male specimen (16.9 × 18.9 mm, NMMBCD 4083) was used for DNA analysis. Crude genomic DNA was extracted from the muscles of the right cheliped using QIAGEN® DNeasy Blood and Tissue Kit (cat. no. 69504, Valencia, CA, USA) following the protocol of the manufacturer. Three mitochondrial genes (12S rRNA, 16S rRNA and COI) and two nuclear genes (18S rRNA and H3) were selected, the same genes used by Windsor & Felder (2017). Five primer sets were used to amplify partial sequences of the targeted genes by polymerase chain reaction (PCR): 12S rRNA (L13337-12S/H13845-12S, ~570 bp; Yamauchi *et al.*, 2002), 16S rRNA (16Sar/16S1472, ~550 bp; Simon *et al.*, 1994; Crandall & Fitzpatrick, 1996), COI (LCO1490/HCO2198, 657 bp; Folmer *et al.*, 1994), 18S rRNA

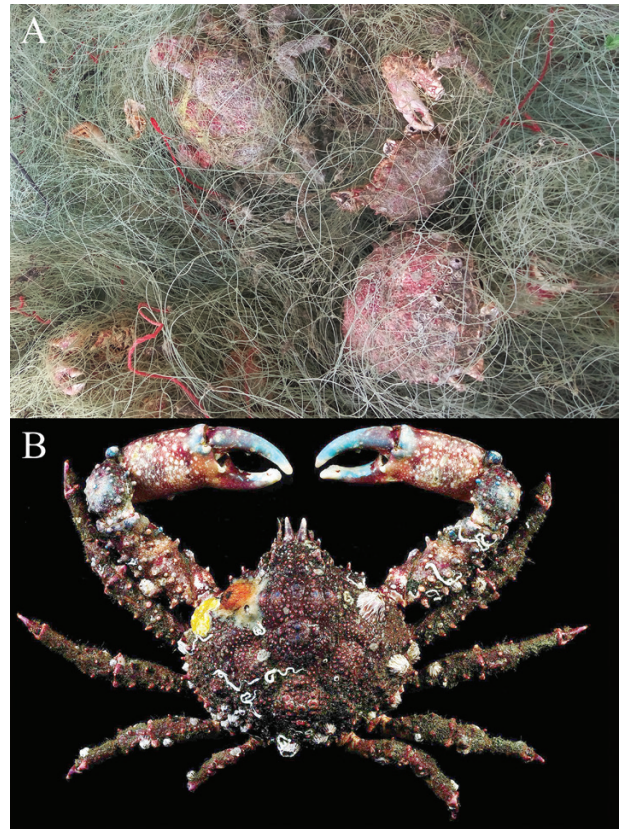


Figure 1. *Amphithrax armatus*; fishing net in Kezailiao, Kaohsiung, Taiwan, that is full of crabs (2017) (A). Colour in life, male (62.1 × 70.2 mm) (NMMBCD 4083), Kezailiao, Kaohsiung, Taiwan (B). This figure is available in colour at *Journal of Crustacean Biology* online.

(18SA/18SL, 18SC/18SY, 18SB/18SO, ~1800 bp; Medlin *et al.*, 1988; Apakupakul *et al.*, 1999), and H3 (H3AF/H3AR, ~350 bp; Colgan *et al.*, 1998). All amplifications were performed in 25 μ l reactions with 50–250 ng of the DNA templates using TaKaRa Taq™ kit (Takara Bio, Kasatsu, Japan), included 2.5 μ l of 10X polymerase buffer (Mg²⁺ plus), 0.5 μ l of 2.5 mM of deoxyribonucleotide mixture (dNTPs) and 0.5 U of Taq polymerase (5U/ μ l) and additional 10–25 mM magnesium chloride (MgCl₂) (depending on gene). Finally, 0.5 μ l of 10 μ M for each primer (Mission Biotech, Taipei, Taiwan) were added and supplemented with sterile, double-distilled water (ddH₂O) to a total of 25 μ l volume. PCR cycling conditions were as follows: 5 min at 95°C for first denaturation, 40 cycles of 30 seconds at 94°C, 40 seconds at 46–52°C (depending on genes), and 40 seconds at 72°C, with final extension for 10 min at 72°C. PCR products of correct size and quality checked by 1% agarose gel were sent to a commercial company (Mission Biotech) for sequencing. SeqMan Pro™ (LASERGENE®; Dnastar, Madison, WI, USA) was used to clean and edit two strands of sequences for contig assembly.

The sequences of COI, 16S rRNA, 18S (18SA/18SL+18SC/18SY segments) and H3 were successfully obtained from *A. armatus* (MG281842–MG281845). To test the taxonomic position of *A. armatus*, this species was added to the datasets of Windsor & Felder (2014, 2017) that were downloaded from the GenBank. Sequences were aligned by MAFFT v.7 (Katoh & Standley, 2013) and the missing data were treated as the fifth nucleotide. GBlocks v.0.91b website (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) (Castresana, 2000) was used to remove poorly aligned positions on 12S rRNA and 16S rRNA genes datasets. The amino acid sequence of the COI dataset was analysed to ensure there were no stop codons. The best model of DNA substitution and parameters for individual alignment was determined by jModelTest v.2.1.3 (Darriba *et al.*, 2012) based on Akaike's Criterion (AIC). Individual datasets were concatenated into 3 genes (12S+16S+COI, for Mithracidae) and 5 genes (12S+16S+COI+18S+H3, for Majoidea) and partitioned by genes for the subsequent analyses. The 3- and 5-gene separations followed that used by Windsor & Felder (2014). Maximum likelihood (ML) and Bayesian inference (BI) methods

were used to construct the phylogenetic trees by RAxML v.7.2.6 (Randomized Accelerated Maximum Likelihood; Stamatakis, 2006) and MrBayes v. 3.2.1 (Ronquist & Huelsenbeck, 2003), respectively. Maximum likelihood (ML) analysis settings followed the model of general time reversible with a gamma distribution and proportion invariant (GTRGAMMAI) for the both of partitioned datasets. Branch confidence of the tree topology was assessed using 1,000 bootstrap replicates (MLb; Felsenstein, 1985). Two independent BI runs were performed with 20 million generations and sampled one tree every 1,000 generations. Tracer v.1.6 (Rambaut *et al.*, 2013) was used to evaluate the convergence of Bayesian runs, and the majority rule trees from the 3- and 5-gene datasets were constructed to estimate the posterior probabilities (Pp). Only values of MLb > 50 and Pp > 0.5 are presented on the phylograms (see Figs. 5, 6).

The approximately unbiased (AU) test (Shimodaira, 2002) was implemented in Consel v.0.1i (Shimodaira & Hasegawa, 2001) to test the hypothesis that *A. armatus* does not belong to *Amphithrax*. The identical two concatenated datasets were examined for the ML analysis based on GTRGAMMAI model in RAxML v.7.2.6. The alternative tree topologies were also constructed and optimized by RAxML. The algorithm 'f g' was used to compute per site log-likelihood scores for those hypothetical trees and evaluate the significance ($P < 0.05$) with the present molecular trees in Consel.

RESULTS

Amphithrax armatus is easily recognized by its relatively large adult size (carapace width exceeding 50 mm) (Figs. 1B, 2); a relatively broad carapace, with the dorsal surfaces densely covered with tubercles and spinules, without deep grooves on the branchial regions (Figs. 1B, 2); the rostral spines are relatively long and acute (proportionately shorter in large individuals), with the tips sometimes gently curving inwards (Fig. 3A); a proximal tubercle on the outer surface of each chela (Fig. 4D, E); and the G1 has a distinct structure with the tip distinctly acute, the presence of a prominent swelling on the dorso-subterminal part, and an area with three uneven folds between the tip and the swelling (Fig. 4F–H) (Garth, 1958). Rathbun (1925: 398) described the fresh colours of this species (as *Mithrax orcutti*) as "Crimson predominating, mixed with white; outer surface of chelipeds crimson with small white dots; abdomen about equally crimson and white, mottled." This agrees well with the fresh colours of the present Taiwanese material (Fig. 1B).

The final dataset for each of the five genes tested was 387 bp of 12S rRNA, 425 bp of 16S rRNA, 657 bp of COI, 1978 bp for 18S rRNA and 327 bp for H3 after trimming. The 3- and 5-gene datasets include 41 species (38 species of Mithracidae and three species of Epialtidae as outgroups) each with 1469 bp and 82 species (78 species of Majoidea and four species of *Ethusa* (Ethusidae) as outgroups) each with 3774 bp, respectively. The optimal model for 12S rRNA, 16S rRNA, and COI genes datasets assessed are all with gamma-distributed (G) and invariant sites (I). The tree topologies by ML and BI analyses are generally similar and the ML phylogram is presented to show the phylogenetic relationship of *Amphithrax armatus* on both the 3- and 5-gene results (see Figs. 5, 6).

The 3-gene tree shows that *Amphithrax armatus* is sister to *Nonala holderi* but not with a very strong nodal support (MLb = 74, Pp = 0.97) (see Fig. 5), and some distance from the clade with the three species of *Amphithrax*. The same relationship was revealed on the 5-gene tree but the support is stronger (MLb = 91, Pp = 1.0) between *Amphithrax armatus* and *Nonala holderi* on the 3-gene tree. The result of the AU test, however, could not reject the hypothesis that *Amphithrax armatus* does not belong to the clade of *Amphithrax* ($P = 0.394$ and 0.442 on the 3- and 5-gene trees, respectively).

The molecular data therefore challenges the current taxonomic placement of *Mithrax armatus* Saussure, 1853 in *Amphithrax* Windsor & Felder, 2017 by these authors. Windsor & Felder (2014,

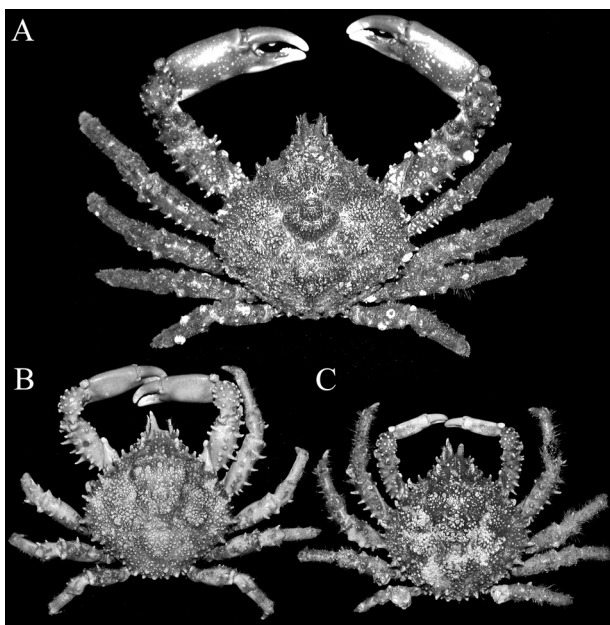


Figure 2. *Amphithrax armatus*, overall view, specimens from Kezailiao, Kaohsiung, Taiwan. Male (72.0 × 71.8 mm) (ZRC 2017.214) (A). Female (52.6 × 55.2 mm) (ZRC 2017.214) (B). Male (50.7 × 50.5 mm) (ZRC 2017.988) (C).

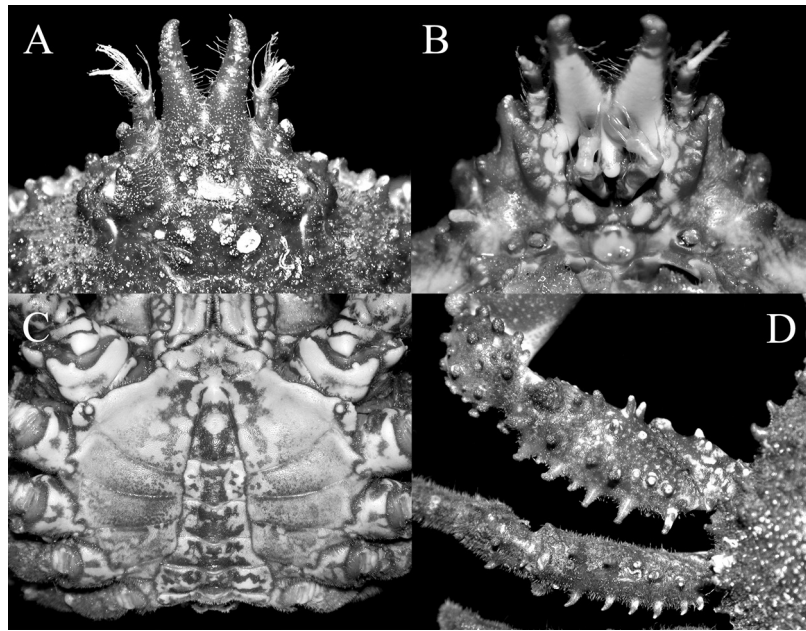


Figure 3. *Amphithrax armatus*, male (72.0 × 71.8 mm) (ZRC 2017.214), Kezailiao, Kaohsiung, Taiwan. Dorsal view of rostrum (A). Ventral view of rostrum showing antennae, antennules and epistome (B). Anterior thoracic sternum and pleon (C). Left meri of cheliped and first ambulatory leg (D).

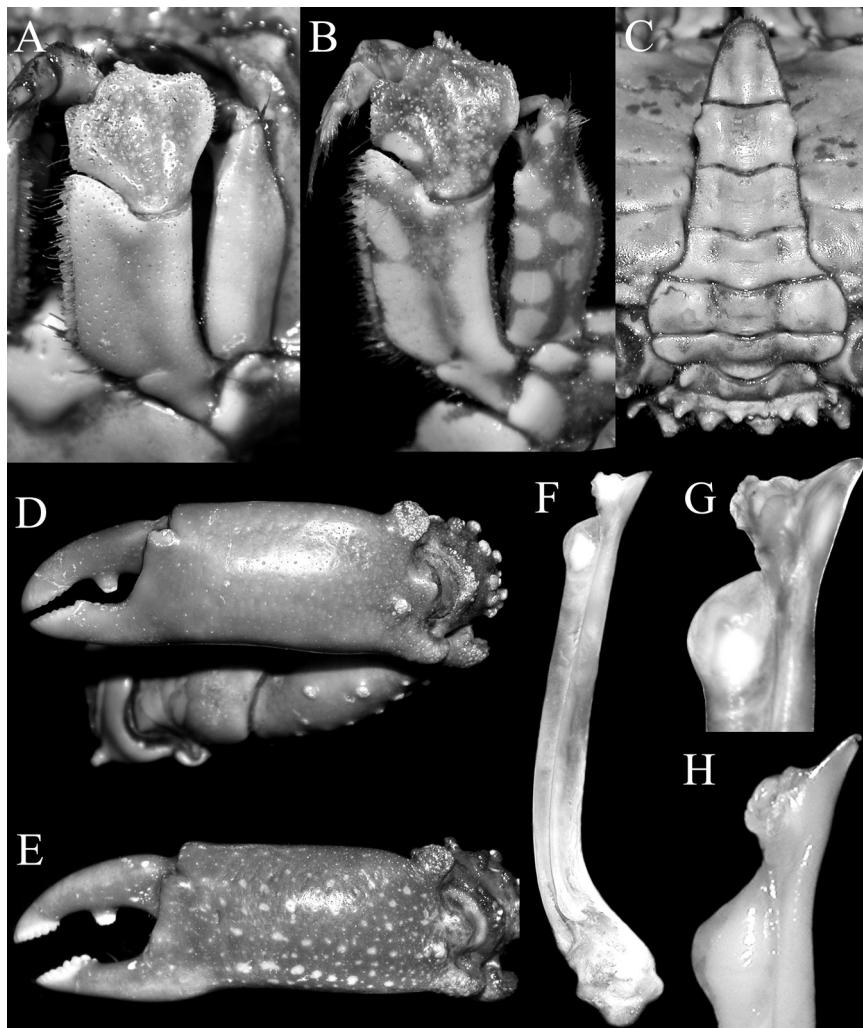


Figure 4. *Amphithrax armatus*; male (50.7 × 50.5 mm) (ZRC 2017.988) (A, C, D, F, G); male (72.0 × 71.8 mm) (ZRC 2017.214) (B, E, H); specimens from Kezailiao, Kaohsiung, Taiwan. Left third maxilliped (denuded) (A, B). Male pleon (C). Outer view of left chela (D, E). Left G1 (ventral view) (F). Distal part of left G1 showing folds (ventral view) (G, H).

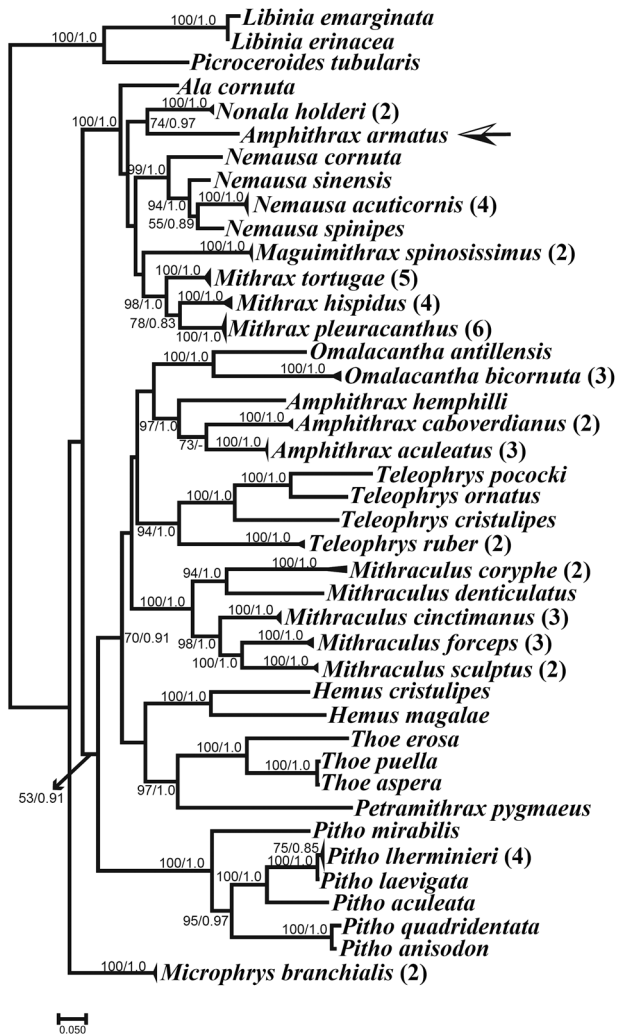


Figure 5. Maximum-likelihood phylogram for *Amphithrax armatus* and selected Mithracidae. Three species of Epialtidae (*Picroceroides tubularis* Miers, 1886, *Libinia emarginata* Leach, 1815, and *L. erinacea* (A. Milne-Edwards, 1879)) were chosen as outgroups, and tree based on the concatenated dataset of 12S rRNA, 16S rRNA and COI genes. Maximum likelihood bootstrap values (MLb) and posterior probabilities (Pp) are represented as percentages above or below the branches. Values < 50% are not shown.

2017) had DNA for only *Amphithrax aculeatus*, *A. caboverdianus*, and *A. hemphilli* in their analysis, although they did examine material of *A. armatus* in the Smithsonian Institution, Washington, DC. There is no doubt that *A. armatus* should be included in *Amphithrax* when applying the morphological characters Windsor & Felder (2014) used to define the genus. *Amphithrax armatus*, however, possesses some morphological features that suggest that such a placement in *Amphithrax* is questionable. Windsor & Felder (2014: 163) had already suggested as much when they wrote “As here treated, *Mithrax* s.s. [now *Amphithrax*] remains a diverse genus that exhibits wide variation in body morphology. Several of the above-listed species, unavailable for inclusion in our molecular phylogenetic analyses, default to continued treatment in this genus for now, while those included in our analyses group together in this genus with low support. Even with some questionable remainders in this genus, not all species that superficially resemble *M. aculeatus* can remain within *Mithrax* s.s. on the basis of our molecular phylogenetic analyses.” The G1 structure, which was not used substantially in the revision by Windsor & Felder (2014, 2017) may be indicative in showing relationships in the future. The G1 of

Amphithrax armatus has a prominent swelling on its inner subdistal margin just before it curves (Fig. 4F–H). This is a character it shares most closely with *Nemausa sinensis* and *Amphithrax clarionensis* (Garth, 1958: pl. 5, figs. 5, 6). It is also similar to that of *Amphithrax tuberculatus* (Garth, 1958: pl. 5, fig. 3) except that its carapace is completely different (Garth, 1958: pl. 40, fig. 1). In *Mithrax hispidus*, and to some degree, *Amphithrax pilosus*, the swelling on the G1 is proportionately lower and less prominent (Wagner, 1990: figs. 19, 20, 30). This structure is also shared by members of *Teleophrys* Stimpson, 1860 and *Microphrys* H. Milne Edwards, 1851 (Garth, 1958: pl. W, figs. 1–5). Species of *Mithraculus* White, 1847 also have a relatively prominent swelling on the G1 but the subterminal segment is quite different in shape (Wagner, 1990: fig. 55). The G1 structures of the other species of *Amphithrax* are without any swelling. The inner angle of the ischium of the third maxilliped of *Amphithrax armatus* is also relatively more acute and the inner angle of the merus at the articulation point is proportionately less wide compared to congeners (Fig. 4A, B).

The genetic data indicates that *A. armatus* is closest to *Ala* Lockington, 1877 and *Nonala* Windsor & Felder, 2014, even though their carapaces are very different. The anterolateral angle of the basal antennal article of *Nonala* and *Ala* are conspicuously elongated, but the rostrum is proportionately shorter compared with *Amphithrax*, and in *Nonala* the G1 distal part is strongly recurved. The morphological evidence at least suggests that *Amphithrax armatus* is not a member of *Amphithrax* and must eventually be referred to its own genus. This should, however, only be done in the context of a more complete revision of *Amphithrax* as suggested by Windsor & Felder (2014). For the moment, we keep this species in *Amphithrax*.

DISCUSSION

The oldest specimen in our collections dates back to 31 October 2014 indicating that *A. armatus* has been in Taiwan for several years. Some fishermen in the Kezailiao fishing port who were interviewed said that they first saw this crab in their nets between 2012 and 2013. At that time, the a few crabs were only occasionally caught in the by-catch. In the last one or two years, and in the present, more than 1,000 specimens can be caught daily (Fig. 1A), with the crab occurring even in shallow waters, including intertidal areas.

Amphithrax armatus almost certainly arrived in Taiwan accidentally through ballast water or as part of fouling communities on vessels. Kaohsiung is the twelfth busiest port in the world by tonnage, processing some 10 million TEU (Twenty-foot Equivalent Units) annually. The shipping routes include major routes to California, Mexico, and Panama. For example, the major Mexican ports of Ensenada and Manzanillo are within the natural distributional range of *A. armatus*. Container ships take on average a month to travel from the eastern Pacific to Taiwan, and this is longer than the known larval development of mithracids. The complete larval development of five species of *Mithrax*, *Mithraculus*, *Amphithrax*, and *Maguimithrax* are known, and it takes 8–18 days for newly hatched zoeae to the first crab stage depending on temperature and salinity (Goy et al., 1981; Renggel et al., 1993; Wilson et al., 1979; Lárez et al., 2000; Magalhães et al., 2017), although Provenzano & Brownell (1977) recorded that it only took five to six days in *Maguimithrax spinosissimus* (Lamarck, 1818). As such, it is possible that if newly hatch larvae from the eastern Pacific survived and metamorphosed inside the ballast water tanks and discharged as juvenile crabs in Kaohsiung on arrival. It is also possible that the crabs were part of the fouling community on large ships that made their way across the Pacific. Since Kaohsiung is also a major industrial city with significant petrochemical facilities and supporting industries, it is also possible that the crab may have entered via various kinds of oil platforms which may have spent time in the eastern Pacific (see Yeo et al., 2009).

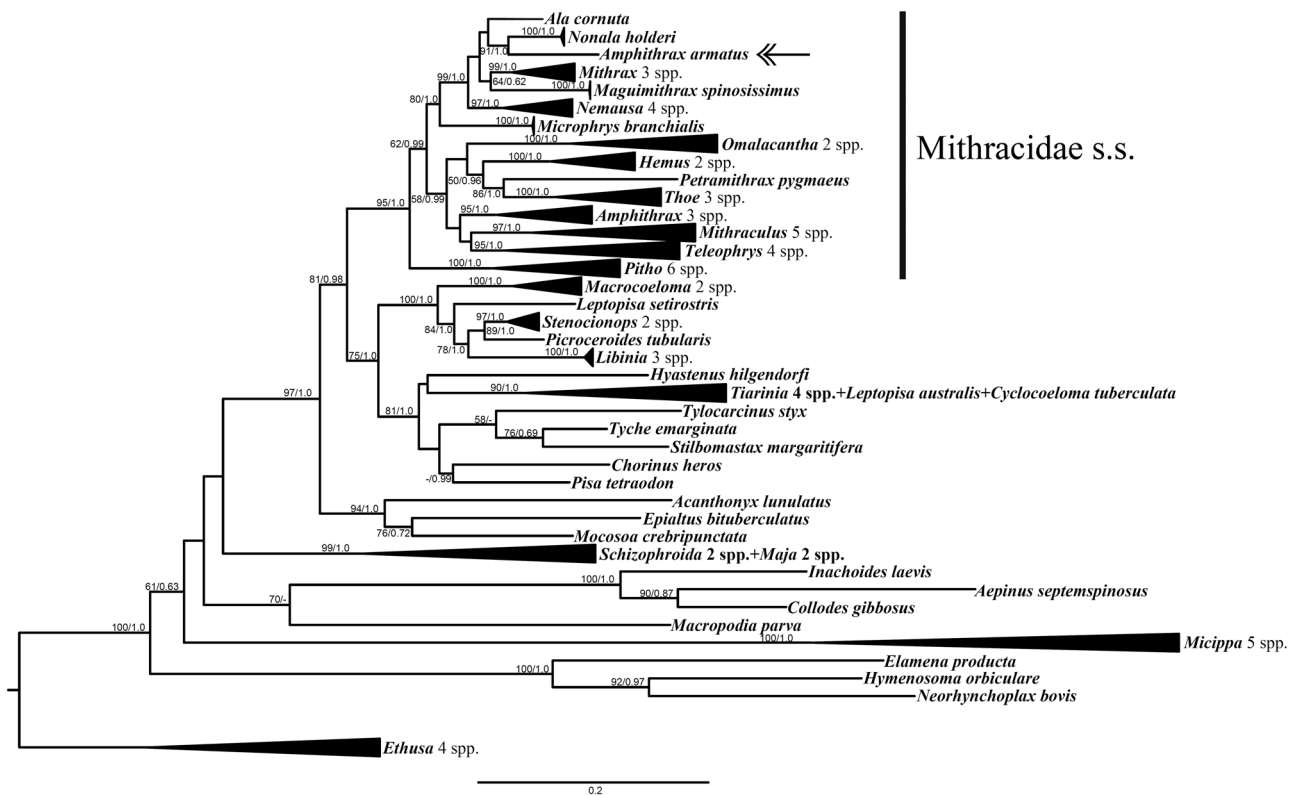


Figure 6. Simplified maximum-likelihood phylogram for *Amphithrax armatus* and selected majoid species with Mithracidae. Four species of *Ethusa* Roux, 1830 (Ethudisae Guinot, 1977) were chosen as outgroups, and tree based on the concatenated dataset of five genes (12S rRNA, 16S rRNA, COI, 18S rRNA and H3). Maximum likelihood bootstrap values (MLb) and posterior probabilities (Pp) are represented as percentages above or below the branches. Values < 50% are not shown.

Although *A. armatus* grows to a relatively large size, it has no commercial value and has not been harvested for export as with many kinds of high-value seafood. It is thus not likely to have entered the island this way (see Brockerhoff & McLay, 2011; Yeo *et al.*, 2011; McLay, 2015). Neither has it been imported via the aquarium trade, its large size being generally unsuitable for home marine aquaria, although the authors have seen the much smaller *Mithraculus sculptus* (Lamarck, 1818) for sale in the aquarium trade in Taiwan.

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