Size-associated morphological changes in *Epigrapsus notatus* (Heller, 1865) and the taxonomic status of *E. villosus* Ng, 2003 (Crustacea: Decapoda: Brachyura: Gecarcinidae)

Tohru Naruse¹, Hsi-Te Shih², Yoshihisa Fujita³, Jose Christopher E. Mendoza⁴, and Peter K. L. Ng⁴

**Abstract.** The identities of what have been referred to as *Epigrapsus notatus* (Heller, 1865) and *E. villosus* Ng, 2003, are evaluated using morphological and molecular methods. Morphological comparisons showed that the main characters used to diagnose *E. villosus* (viz. the setation of the carapace, the condition of the epigastric cristae, the granulation on the branchial region, and proportions of the ambulatory legs) can be attributed to the small size of the type specimen; and the extreme condition of these structures can be connected to adult forms of *E. notatus* through transitional phases. One morphological feature (the laterally expanded first epibranchial tooth) is seen only in the holotype of *E. villosus*, but it can also be regarded as a character state specific to small individuals of *E. notatus*, and signifying the extreme end of possible variation in that species. The analysis of mitochondrial cytochrome c oxidase subunit I (COI) reveals three haplotypes in the *E. notatus*/*E. villosus* complex, but with only as much as 0.61% of nucleotide divergence between them. In conclusion, it is clear that *E. notatus* undergoes dramatic morphological change during its growth, and that *E. villosus* should be considered a junior synonym of *E. notatus*.

**Key words.** Decapoda, Grapsoidae, land crab, *Epigrapsus*, intraspecific variation, mitochondrial COI

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**INTRODUCTION**

The gecarcinid land crab genus, *Epigrapsus* Heller, 1862, currently contains three species: *E. notatus* (Heller, 1865), *E. politus* Heller, 1862, and *E. villosus* Ng, 2003 (viz. Ng et al., 2008). *Epigrapsus notatus* is one of the most studied species in terms of taxonomy and morphology and has been reported to vary significantly in morphology as it grows. Türkay (1973: 105) synonymised Mystacocarcinus crendens Hilgendorf, 1888, *Epigrapsus (Grapsodes) notatus punctatus* Sendler, 1923, and *Epigrapsus (Grapsodes) wolffii* Sendler, 1923, with *E. notatus* after comparing their types. He discussed the differences in five supposed distinguishing characters: granulation on the anterolateral margin, granulation on the anterolateral region, relative length and width of the male pleonomal somites, cheliped armature, and shape of the distal end of the male first gonopod (Türkay, 1973: 106); and argued that these differences are connected by transitional individuals. In addition, Sendler (1923) collected both *E. notatus* and *E. punctatus* from the same place in Bertrand and Liebliche islands (presently Tarawai Island and Arawe Islands, respectively, in Papua New Guinea). Tesch (1918: fig. 3) had earlier also observed that a female specimen (carapace length 18.0 mm × carapace width 29.0 mm) had relatively large granules on the branchial region when compared with a larger male (28.0 × 32.5 mm) that only possessed some faint granules. Ng et al. (1998) subsequently examined a large number of specimens from Taiwan and documented that the striae and granules of the branchial region is distinct in females, with a tendency for these to be more prominent in smaller individuals. They also mentioned that this is a sex-related character as males (the smallest they had measured 20.1 × 23.4 mm) have a relatively smoother carapace, with the striae and granules on the branchial and supraorbital regions very weak or undiscernible (Ng et al., 1998: 76).

*Epigrapsus villosus* Ng, 2003, was described based on a small male (8.9 × 9.4 mm), from a cave in Guam. It was distinguished from the two congers, *E. notatus* and *E. politus*, by the shape of the carapace, presence of scattered tufts of short black setae on its dorsal surface, presence of sharp epigastric cristae, and by the structure of the external orbital and first anterolateral teeth, proportions of the ambulatory legs, and armature of the ambulatory meri. Recently, additional specimens that have been identified as *E. villosus* were collected from the Ryukyu Islands (= Ryukyus), Dongsha Island (Shih, 2012: fig. 154 [right]), the Philippines, and Vanuatu (Ng et al., 2008: fig. 160). The present study was initiated after the discovery of specimens from the Ryukyu Islands which were intermediate in morphology between *E. villosus* and *E. notatus*. Fujita (2017b) had
reported on the intermediate features of *E. villosus* and *E. notatus*, and commented on the possible conspecificity of the two species. To resolve this question, we examined a good series of specimens of various sizes from many localities and used a suite of morphological and genetic characters to determine their synonymy.

**MATERIALS & METHODS**

Material examined and used for molecular study are deposited in the Museum Zoologicum Bogoriense, Cibinong, Indonesia (MZB); Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOOL); National Museum of Natural Science, Taichung, Taiwan (NMNS); Ryukyu University Museum, Fukuoka, Okinawa, Japan (RUMF); Florida Museum of Natural History, University of Florida, USA (UF); and Zoological Reference Collection, Lee Kong Chian Natural History Museum (previously Raffles Museum of Biodiversity Research), National University of Singapore (ZRC).

Measurements provided are carapace length (CL) × carapace width (CW) in millimetres (mm). Discriminative characters of *E. villosus* (setation of the carapace and ambulatory legs, condition of the epigastric cristae, and the relative positions of the external orbital angle and epibranchial teeth) as well as the degree of granulation on the branchial region were examined.

Specimens used for molecular study were collected from the Ryukyus, Taiwan (including Dongsha Island), West Java in Indonesia, and Guam (Table 1), and included the holotype of *E. villosus*. Specimens of *E. politus* used were from Taiwan and the Philippines. Sequences of mitochondrial cytochrome c oxidase subunit I (COI) from the above specimens were obtained following the method described by Shih et al. (2016) and analysed. Sequences were obtained by automated sequencing and were aligned after the verification with the complementary strand, and have been deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers in Table 1). Basepair (bp) difference, as well as the pairwise estimates of Kimura 2-parameter (K2P) distance (Kimura, 1980) and the uncorrected p-distance (recommended in Srivathasan & Meier, 2011) for genetic divergence between haplotypes were also calculated by MEGA (version 5.2.2, Tamura et al., 2011).

**Material examined.** Ryukyu Islands: 1 male, 17.1 × 19.8 mm, RUMF-ZC-1132, Nakama-gumui, Kume Island, KUMEJIMA 2009, st. intertidal 10, 26°22′57.3″N
Table 1. The haplotypes of COI gene of specimens of *Epigrapsus notatus*, *E. politus* and the holotype of *E. villosus*.

<table>
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<tr>
<th>Locality</th>
<th>Catalogue no.</th>
<th>Sample size</th>
<th>Haplotype of COI</th>
<th>Figure</th>
<th>DDBJ Accession no.</th>
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<td>Iriomote: Sonai</td>
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<td></td>
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Fig. 2. Various-sized males of Epigrapsus notatus (Heller, 1865) (not to scale). a, ZRC2002.0011 (8.9 × 9.4 mm) (holotype of E. villosus); b, RUMF-ZC-1129 (11.2 × 12.7 mm); c, RUMF-ZC-2140 (13.4 × 15.6 mm); d, RUMF-ZC-1130 (17.0 × 20.0 mm); e, RUMF-ZC-2141 (20.9 × 24.3 mm); f, ZRC 1998.0826 (29.3 × 36.1 mm).

**Comparative material.** *Epigrapsus politus* Heller, 1862: 1 female, 11.8 × 13.9 mm, NCHUZOOL 13606, Siouliouciou I., Pingtung County, Taiwan, coll., coll. J.-H. Lee, 13 November 2011; 1 male, 11.1 × 13.4 mm, NCHUZOOL 13607, Dongsha I., Kaohsiung City, Taiwan, coll. H.-T. Shih et al., 3 September 2011.

**RESULTS**

**Morphological comparisons.** *Setation of the dorsal surfaces of the carapace and ambulatory legs.* In males, tufts of short black setae on the dorsal surface of the carapace were observed in relatively small individuals up to CW 20.0 mm (Figs. 1a, b, 2a–c). Larger individuals more than CW 24.3 mm (RUMF-ZC-2141, Figs. 1c, d, 2e, f) have completely lost such setae, and the dorsal carapace surface is, consequently, glabrous. The change in the setation of the pereopods also occurs at about the same size range: specimens up to CW 20.0 mm (Figs. 1b, 2a–c) have tufts of short black setae with a few long setae along the flexor margins of the ambulatory propodi. A photograph of the holotype of *E. notatus* clearly shows such setation (CW 19.0 mm, Türkay, 1974: fig. 19).

Larger specimens (Figs. 1d, 2e, f) have glabrous chelipeds and their ambulatory legs are lined mainly with long setae instead.

In females, the change in the setation of the carapace occurs at similar sizes as in males: specimens up to CW 18.9 mm (e.g., Fig. 3a, b) have tufts of short black setae on the dorsal surface of carapace, while the specimens larger than CW 21.1 mm (Fig. 3c) lack these setae. The change in pereopod setation in females also occurs in the same size range as with carapace setation. There is one exception – one female with a smooth dorsal carapace surface (CW 24.7 mm, Fig. 3d) still had tufts of short black setae on the pereopods.

**Epigastric cristae.** The epigastric cristae are more prominent and sharp in small specimens (Fig. 1a). There was a male specimen with CW 15.6 mm (Fig. 2c), however, with relatively lower cristae, while much larger specimens had weak and rounded cristae (e.g., Figs. 1c, 2d).

Female specimens measuring CW 15.2 mm (RUMF-ZC-2139) and CW 18.1 mm (ZRC 1998.0828, Fig. 3b) already have rounded epigastric cristae, and these become almost indiscernible in a female with CW 21.1 mm (Fig. 3c).

**External orbital tooth and anterolateral margin.** The anterolateral margin of the carapace has three teeth (one external orbital tooth and two epibranchial teeth). The anterolateral margins of the smallest male specimen (CW 9.4 mm, holotype of *E. villosus*, ZRC 2002.0011, Fig. 2a) are slightly divergent posteriorly, but still almost straight and not produced laterally. The first epibranchial tooth is the largest tooth. The condition is markedly different in a larger male with CW 12.7 mm (RUMF-ZC-1129, Figs. 1b, 2b) in which the anterolateral margin is expanded laterally and becomes more arcuate up to the point of the second epibranchial tooth, after which the anterolateral margins are again only slightly divergent. Furthermore, the external orbital tooth is now as large as the first epibranchial tooth. This condition is retained in the male measuring CW 20.0 mm (Fig. 2d), where the part of the anterolateral margin encompassing the external orbital tooth to the second epibranchial tooth remains distinctly arcuate, but posterior to that the anterolateral margin is more subparallel, and the posterolateral margin is distinctly convergent posteriorly. In much larger and fully grown individuals, i.e. the largest examined male with CW 36.1 mm (ZRC 1998.0826, Figs. 1d, 2f) and the holotype of *E. wolfi* (CW 27.5 mm, Türkay, 1973: fig. 59), the first and second epibranchial teeth are almost completely fused.

The smallest females with CW 15.2 mm (RUMF-ZC-2139) and CW 15.4 mm (ZRC 2017.0498, Fig. 3a) already show a laterally expanded and arcuate anterolateral margin. This condition is commonly seen in the other females.

**Granulation on epibranchial region.** The granulation on the branchial region is often more pronounced in small females. In some, the granules even extend over to the epigastric region, as observed in the female with CW 15.4 mm (ZRC 2017.0498, Fig. 3a). This character, however, seems to be variable. Females with CW 24.7 mm (ZRC 2001.0726, Fig. 3d) and CW 28.7 mm (RUMF-ZC-2142, Fig. 3e) have clearly observable granulation on the postorbital to epibranchial regions, although they lack tufts of black setae on the entire dorsal carapace surface. On the other hand, similar-sized females, with CW 21.1 mm and CW 28.6 mm (both ZRC 1998.0830, Fig. 3e), are almost completely devoid of granules on the dorsal surface of the carapace.

The granulation in males is indistinct in most specimens except for two individuals, CW 9.4 (ZRC 2002.0011, Fig. 2a) and CW 20.0 (RUMF-ZC-1130, Fig. 2c), which still have some minimal granulation.

**Ambulatory legs.** The second and third ambulatory propodi and dactyls are relatively longer in the holotype of *E. villosus*, but this is also a character associated with size; with gradually larger specimens having relatively slightly shorter appendages (Fig. 2b–f; Ng, 2003: fig. 3A–C). The extensor margins of the ambulatory meri of *E. villosus* are distinctly serrate (Ng, 2003: fig. 3A–C) but in larger specimens where the carapace features are intermediate in condition, the margins are relatively more entire (Fig. 2c, d), and when they reach CW of 20 mm, the margins are smooth (Figs. 1d, 2e, f).

**DNA results.** A 658-bp segment of COI from 21 specimens of the *E. notatus*/*E. villosus* complex and five specimens of *E. politus* was amplified, resulting in eight different haplotypes.
Fig. 3. Various-sized females *Epigrapsus notatus* (Heller, 1865) (not to scale). a, ZRC 2017.0498 (13.3 × 15.4 mm); b, ZRC 1998.0828 (15.2 × 18.1 mm); c, ZRC 1998.0830 (18.9 × 21.1 mm); d, ZRC 2001.0726 (21.9 × 24.7 mm); e, RUMF-ZC-2142 (24.2 × 28.7 mm); f, ZRC 1998.0828 (23.7 × 29.1 mm).

(Table 1). The studied segment of the COI sequences was AT rich (62.9%) (T, 35.0%; A, 27.9%; G, 16.9%; C, 20.2%). In this fragment, 66 positions were variable and 58 were parsimoniously informative. The nucleotide divergence with the K2P distance between those haplotypes of COI are shown in Table 2. Because the distances of sequences estimated between K2P and the p-distance are similar (intraspecific divergences are the same; average interspecific divergences are 9.95 and 9.19, respectively), the K2P distance is chosen to allow a consistent comparison with most barcoding studies.

The holotype of *E. villosus* (CW 9.4 mm, ZRC 2002.0011, Figs. 1a, 2a) shares the same haplotype with an intermediate female form of *E. notatus* and *E. villosus* from Guam (CW 24.7 mm, ZRC 2001.0726, Fig. 2d). These two specimens from Guam showed only 0.61% of nucleotide divergence (or 4 bp difference) from the common haplotype of the remaining *E. notatus*/*E. villosus*, including an intermediate form (CW 12.7 mm, RUMF-ZC-1129, Fig. 2b) as well as the fully grown adult form (CW 36.5 mm, NCHUZOOL 13601). This degree of difference can be considered as intraspecific. That is, the average intraspecific nucleotide divergence (and bp difference) is 0.16% (and 1.05 bp) in *E. notatus*/*E. villosus*. There are five haplotypes in *E. politus*, with an average intraspecific nucleotide divergence (and bp difference) of 0.43% (and 2.80 bp). On the other hand, the interspecific divergence between *E. notatus*/*E. villosus* and *E. politus* is at least 12 times more than the intraspecific values (Table 2).

**DISCUSSION**

The character states of the examined specimens are summarised in Fig. 4. It shows clearly that male individuals with carapace widths between 12.7 and 20.0 mm, including the holotype of *E. notatus*, are in a “transitional state” (Fig. 2a–c), with features of both larger adults and what has been defined as “*E. villosus*” sensu Ng (2003) for certain characters. Some female specimens start “transitioning” in character states when they reach carapace widths of 15.2–24.7 mm (Fig. 3b, d). Ng et al. (1998) hypothesised that the presence of the striae and granules on the branchial region of the carapace is characteristic of the female since the males they examined, including the smallest one (20.1 × 23.4 mm), had
a relatively smooth carapace. The present study, however, found that much smaller males (CW 8.9 × 9.4 mm, ZRC 2002.0011, Fig. 2a; CW 20.0 mm, RUMF-ZC-1130, Fig. 2c) also possess such striae and granules. The presence of a suite of transitional morphological forms indicate that *E. notatus* and "*E. villosus*" are conspecific, with the various forms connected by a remarkable series of morphological changes as they grow in size. As to the structure of the male first gonopods, we find no discernible differences between intermediate size and adult *E. notatus*, and that of *E. villosus* is confirmed here to be of a juvenile form (see Türkay, 1974: figs. 6, 9; Ng, 2003: fig. 3F).

Comparison of the COI sequences of *E. notatus*/*E. villosus* show that although the Guam haplotypes (including the holotype of *E. villosus*) are not shared with the common haplotype of *E. notatus*, the maximum intraspecific nucleotide divergence (0.61%) is similar to the intraspecific COI
dissolve. For example, 0.61% for Mictyris thailandensis Davie, Wisespongand & Shih, 2013 (Mictyridae, Davie et al., 2013), ≤ 0.61% for Scopimera spp. (Dotilliidae, Wong et al., 2011), ≤ 0.61% for Helice latimera complex (Varunidae, Shih & Suzuki, 2008) and ≤ 1.86% for Paraleptus splendida (Stimpson, 1858) (Ocyopodidae, Shih et al., 2012, 2016). It shows that all Guam haplotypes (including holotype of E. villosus) as well as other E. notatus/E. villosus should be regarded as the same species.

In summary, considering the entire suite of morphological and molecular evidence, E. villosus Ng, 2003, is here treated as a junior subjective synonym of E. notatus (Heller, 1865).

ECOLOGICAL NOTES

Adult Epigrapsus notatus are known to inhabit the supralittoral zone, from vegetated sandy coasts to coastal forests, sometimes foraging during the day. They are usually not encountered in open habitats and/or in and around their burrows under forest cover (Ng et al., 1998; Liu & Jeng, 2005; Naruse, 2005; Shih, 2012, 2013; Fujita, 2017a). Liu & Jeng (2005) noted that E. notatus is rarely observed outside the forest even on rainy nights.

Small individuals of E. notatus had been discovered in rather unusual environments. Ng (2003) noted that the small male holotype of E. villosus (ZRC 2002.0011, 8.9 × 9.4 mm) was collected from about 50 metres from the entrance of a cave, which itself is situated about 100–200 metres from the shoreline in Guam. A small female (ZRC 2017.0498, 13.3 × 15.4 mm) from Palau Island, northern Philippines, was collected from under a rock on the mud bank of a small stream that runs through a coastal forest with limestone substratum, ca. 50 m from the coast (JCEM & TN, pers. obs.). Shih (2012) noted that young individuals could be found under driftwood in the supralittoral zone of Dongsha Island, northern South China Sea, while Shih (2013) mentioned that adults were collected under rocks in the tropical coastal forest of southern Taiwan. Fujita (2017b), however, collected young and adult forms of E. notatus from the lower supralittoral zone, under boulder/rubble substratum in the southern Ryukyus, which was occasionally splashed with seawater by wave action.

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LITERATURE CITED


Fujita Y (2017b) Distributional records of four rare land crabs (Gecarcinidae) in Sakishima Island Group, southern Ryukyus, Japan. Fauna Ryukyuana, 36: 19–26. [In Japanese with English abstract and figure captions]


Ocypodidae (Crustacea: Brachyura), based on molecular evidence, E. villosus Ng, 2003, is here treated as a junior subjective synonym of E. notatus (Heller, 1865).

Phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of the endemic mudflat crab Helice/Chasmagnathus complex.
Naruse et al.: Morphological change and taxonomy of *Epigrapsus notatus*


