[new species]

Diversity of Taiwanese Brackish Crabs Genus *Ptychognathus* Stimpson, 1858 (Crustacea: Brachyura: Varunidae) through DNA Barcodes, with Descriptions of Two New Species

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Species of the brackish crab genus *Ptychognathus* are common in the seashore and estuary habitats with freshwater input. Due to the similar morphology and dull coloration, it is always difficult to identify the correct species of this genus. In this study, the DNA barcode gene, *COI* (cytochrome *c* oxidase subunit I), was used to help the identification of *Ptychognathus* from Taiwan. The results showed the 10 species can be identified successfully by *COI*, with intraspecific distances below 1.54% and interspecific distances ranged 12.2%–19.57%. In addition, two new species of *Ptychognathus* are described from Taiwan. *Ptychognathus makii* sp. nov. from southern Taiwan is similar with *P. altimanus* (Rathbun, 1914); and *P. stimpsoni* sp. nov. from southern Taiwan and southern Philippines resembles *P.* aff. *barbatus* (A. Milne-Edwards, 1873) and *P. pusillus* Heller, 1865. Both species can be distinguished from other congeners by a suite of characters, including the carapace, orbital region, frontal region, telson of male pleon, male first gonopod, and setae on ambulatory legs.

Key words: *Ptychognathus*, *P. makii*, *P. stimpsoni*, New species, Morphology, Mitochondrial cytochrome *c* oxidase subunit I (*COI*), Barcodes.

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BACKGROUND

The brackish crab genus *Ptychognathus* Stimpson, 1858, usually inhabiting under stones or interspaces of pebbles along estuaries or seashores influences by flows of fresh water, is a diverse genus of the family Varunidae, with 25 species reported (Osawa and N.K. Ng 2006; Ng et al. 2008; N.K. Ng 2010; Sasaki 2019). The first report of this genus in Taiwan was *P. barbatus* (A. Milne-Edwards, 1873) (Maki and Tsuchiya 1923). Sakai (1939) established two new species from Taiwan, *P. ishii* Sakai 1939 (type locality: Lanyu, Taitung) and *P. takahasii* Sakai 1939 (type locality: Danshuei, New Taipei City). Later, *P. hachijoensis* Sakai, 1955, *P. affinis* De Man, 1895, *P. altimanus* (Rathbun, 1914), *P. insolitus* Osawa & N.K. Ng, 2007 and *P. pilosus* De Man, 1892 were reported successively (Fukui et al. 1989; Lee 2001; Naruse et al. 2005; Li 2015; Li et al. 2019). In total, based on morphological identification, there are 8 species of this genus from Taiwan (Ng et al. 2001 2017; Li et al. 2019).

Because most species of *Ptychognathus* have minor interspecific morphological difference and the dull coloration, it is always difficult to identify the correct species (*e.g.*, Chen 2001; Lee 2001; Nakasone and Irei 2003). Several taxonomic studies of crabs have used the evidence of *COI* (cytochrome *c* oxidase subunit I) and other markers to support the recognition of new or reinstated species (cf. Chu et al. 2015), including the taxa within the family Varunidae (*e.g.*, Chu et al. 2003;

Shih and Suzuki 2008; Naser et al. 2012; N.K. Ng et al. 2018; Shih et al. 2019a 2020) and Sesarmidae (Li et al. 2019a b; Ng et al. 2020). To help identify species of *Ptychognathus* from Taiwan, the *COI* barcodes approach (Hebert et al. 2003a b) was undertaken in our study.

Two species of *Ptychognathus* collected from southern Taiwan, with distinct morphology from similar species, supported genetically by *COI* gene, are described as new herewith.

MATERIALS AND METHODS

Specimens of *Ptychognathus* collected from Taiwan, Philippines and Indonesia were examined (Table 1), and these specimens were deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOOL). The following abbreviations are used: CW = carapace width, CL = carapace length; G1 = male first gonopod; P2–P5 = first to fourth ambulatory legs, respectively.

Genomic DNA was isolated from muscle tissue using kits (see Shih et al. 2016 for details). A portion of the *COI* gene was amplified with a polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR conditions for the above primers were 40 cycles of denaturation for 50 s at 94°C, annealing for 70 s at 45–47°C, and extension for 60 s at 72°C, followed by extension for 10 min at 72°C. Sequences were obtained by automated sequencing (Applied Biosystems 3730, USA), after verification with the complementary strand. Sequences of the different haplotypes were deposited in GenBank (with accession nos. given in Table 1).

A neighbor-joining (NJ) tree for *COI* sequences was established using Kimura (1980) 2-parameter (K2P) model with the complete deletion option using the program MEGA (vers. 10.0.5, Kumar et al. 2018). Basepair (bp) differences and pairwise estimates of K2P distances for genetic diversities between specimens were also calculated with MEGA.

RESULTS

Molecular analyses of *COI*

Molecular results of the *COI* gene comprise 35 specimens and 10 OTUs (operational taxonomic units) have been well supported (Fig. 1), which correspond to 10 species. Among them, the specimens identified as "*P. affinis*" previously are confirmed to be *P. altimanus*, as well as two new species (described as follows) and an unknown species (Table 1, Fig. 1).

The mean pairwise nucleotide divergences of K2P distances and bp differences of haplotypes of the 10 species are shown in table 2. The intraspecific nucleotide divergences (and bp differences) of the 10 species are all ≤ 1.54% (≤ 10 bp), with *P. makii* possessing the highest intraspecific divergence. The interspecific divergences among the 10 species range 12.2%–19.57%. (73–112 bp), with the lowest interspecific divergence between *P. hachijoensis* and *P. takahasii*, supporting the 10 OTUs being distinct species.

TANONOMY

Family Varunidae H. Milne Edwards, 1853

Subfamily Varuninae H. Milne Edwards, 1853

Genus *Ptychognathus* Stimpson, 1858

Ptychognathus makii sp. nov.

(Figs. 2A, B, 3)

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Material examined: Holotype: 1 ♂ (23.5 mm), NCHUZOOL 16062, Jhonggang River (= R.) estuary, Manjhou, Pingtung, coll. P.-Y. Hsu et al., 7 Nov. 2018. Paratype: 2 ♂♂ (13.2–13.6 mm), 1 ♀ (11.2 mm), NCHUZOOL 16049, Gangkou R. estuary, Hengchun, Pingtung, coll. P.-Y. Hsu and C.-Y. Chi, 4 Dec. 2016; 1 ♂ (20.3 mm), NCHUZOOL 16050, Gangkou R. estuary, Hengchun, Pingtung, coll. P.-Y. Hsu et al., 4 Sep. 2017; 4 ♂♂ (12.8–21.0 mm), 1 ♀ (16.3 mm), NCHUZOOL 16052, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-J. Li, 19 Mar. 2018; 5 ♂♂ (7.9–19.8 mm), 3 ♀ (6.6–10.6 mm), NCHUZOOL 16053, Gangkou R. estuary, Hengchun, Pingtung, coll. P.-Y. Hsu et al., 4 Sep. 2017.

Comparative material: *Ptychognathus pilosus*: 1 ♂ (20.2 mm), NCHUZOOL 15395, Gangkou R. estuary, Pingtung, coll. J.-J. Li, 10 Aug. 2015. *P. altimanus*: 2 ♂♂ (9.5–19.1 mm), NCHUZOOL 16055, Linbian R. estuary, Checheng, Pingtung, coll. J.-H. Lee, 6 Feb. 2000; 1 ♂ (17.4 mm), 1 ♀ (19.7 mm), NCHUZOOL 16051, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-J. Li, 10 Aug. 2015.

Description: Carapace (Figs. 2A, 3A) quadrate, slightly broader than long, 1.1 times as broad as long, flat; dorsal surface smooth, glabrous, regions weakly defined, with noticeable groove between epigastric regions. Front broad, flat, not sloping forward, part near orbital regions slightly convex; frontal margin straight, without lobes; postfrontal region indistinct, separated into two obscure lobes, without distinct shallow grooves. Anterolateral margins each with conspicuous three teeth including orbital tooth, first tooth largest and most distinct, third tooth smallest and most acute. Posterolateral margins divergent posteriorly, moderately sloping outwards; lateral and posterolateral margins regularly furnished with short, soft setae. Infraorbital ridge consisting of several small, rounded granules, decreasing in size laterally. Epistome broad, median part triangular, margin with tiny granules.

Third maxillipeds (Figs. 2B, 3B) broad, surface with short setae sparsely, exopod distinctly broader than ischium, exopod 1.7 times as broad as ischium; mesial part of merus with oblique groove, anteroexternal angle very broad, slightly sloping outwards; ischium with distinct vertical shallow groove.

Chelipeds (Fig. 3D) symmetrical in male. Merus without spines, margins lined with small, rounded granules, dorsal margins with long soft setae, ventral margins glabrous. Surface of carpus with several tiny granules, part near inner distal angle with single ridge consisting of large granules; inner surface sometimes with short setae sparsely; inner distal angle with single long blunt spine, slightly curving forward, and single smaller spine on margin in male (Fig. 3C); inner distal angle with single small spine in female. Outer surface of palm in male (Fig. 3D) smooth, without distinct granules; inner surface glabrous, middle part convex. Movable finger distinctly longer than palm; immovable finger with obscure horizontal ridge toward palm; cutting edges of both fingers with numerous blunt teeth, teeth near base of fingers larger; margins between movable finger and palm with short soft setae, without pulvinus at base of fingers. Female with outer surface of palm almost glabrous, inner surface glabrous and middle part convex; immovable finger with single prominent horizontal ridge toward palm; part near cutting edges with sparse short setae.

Ambulatory legs (Fig. 3F, G) slender, P3 and P4 longest, P4 and P5 obviously more flat than P2 and P3; anterior margins of each merus with long soft setae, posterior margins with short setae densely and long setae sparsely, with dense black short setae on margins near carpus; merus with single small spine on anterior margins near carpus in P2–P4, spine indistinct or absent in P5; propodus of P2–P4 narrower, propodus of P5 wider. Carpus and propodus of P2 covered with dense short setae on anterior margins, posterior margins of carpus without dense short setae, with dense black short setae on posterior margins of propodus, ventral surfaces of carpus and propodus with rows of short setae. P3 with anterior margins of propodus and carpus covered with dense short setae, ventral surface of propodus with rows of short setae, posterior margins of carpus without dense short setae, posterior margins of propodus with dense short setae and sparse long setae. P4 (Fig. 3F) shorter; carpus with anterior margins covered with dense short setae, posterior margins often glabrous; propodus two times as wide as long, anterior margins covered with dense short setae, posterior margins with dense long setae, more dense on distal part. P5 (Fig. 3G) relatively shortest, anterior margins of carpus covered with dense short setae (more dense on distal part); propodus oval, width as 1.5 times as length, anterior margins covered with dense short setae, posterior margins with dense long setae, more dense on distal part.

Male pleon (Fig. 3E) narrow, surface smooth, without any granules, margins covered with short setae, setae on margins of telson longer; telson tongue-shaped, approximately as 1.2 times long as sixth segment, distal margin of telson not concave, without tuft of setae.

Male G1 (Fig. 3H–K) slender, slightly curving dorsally; tip blunt, with short chitinous structure, semicircle-shaped lobes in lateral view (Fig. 3J, K), opened laterally and dorsally; G2 shorter than 1/4 length of G1.

Coloration: Preserved specimens with carapace and ambulatory legs gray, brown or dark brown; ventral surface of carapace and chelipedal palm light brown; setae dark brown or black.

Habitat: This species inhabits estuarine area, with sandy mud sediment. Individuals always hid under stones or objects and sometimes sympatric with *P. altimanus* and *Varuna litterata* in southern Taiwan.

Etymology: This species is named for the Japanese zoologist Moichiro Maki, who published the first monograph of Taiwanese decapods (Maki and Tsuchiya 1923; Ng et al. 2009) which was also the first record of *Ptychognathus* in Taiwan.

Distribution: Southern Taiwan.

Size: Large; largest male CW 23.5 mm, largest female CW 16.3 mm.

Remarks: Among the species of *Ptychognathus* from Taiwan, this species is similar to *P. altimanus* (Rathbun, 1914) and *P. pilosus* De Man, 1892 in morphology , but can be distinguished by the characters of frontal region, supraorbital margins, posterolateral margins of carapace, male chelipedal palms and G1s. *Ptychognathus makii* differs from *P. altimanus* by having the frontal region shorter with lateral margins not vertical (vs. longer with lateral margins vertical), supraorbital margins gently sinuous (vs. margins strongly sinuous) (Figs. 2A, 3A; Tesch 1918: pl. 4(5a), Naruse et al. 2005: fig. 1A); posterolateral margins of carapace distinctly divergent posteriorly (vs. almost parallel, not divergent posteriorly) (Figs. 2A, 3A; Tesch 1918: pl. 4(5a), Naruse et al. 2005: fig. 1A); male palms proximally almost smooth, without distinguishable granules (vs. with several tiny granules) (Fig. 3D; Naruse et al. 2005: fig. 1C), but the character is indistinct in smaller specimens; the margins of the chitinous structure of G1 without obvious notch (vs. with a notch) (Fig. 3H–K; Naruse et al. 2005: fig. 1E).

Ptychognathus makii and *P. pilosus* can be distinguished by the setae on chelipeds and G1. In *P. makii*, male and female with short soft setae on the margin between palm and movable finger (Fig. 3D), and cutting edge with sparse short setae in females. In *P. pilosus*, male and female have a small tuft of shorter setae on tip of fixed finger, and longer setae on both fingers only in males (Li et al. 2019: fig. 7B). The tips of male G1 is blunt, and the chitinous structure is large semicircle-shaped in lateral view in *P. makii* (vs. tapering, and the chitinous structure is small pyramid-shaped pyramid-liked in *P. pilosus*) (Fig. 3H–K; Li et al. 2019: fig. 7C, D).

***Ptychognathus stimpsoni* sp. nov.**

(Figs. 2C–F, 4)

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Material examined: Holotype: 1 ♂ (7.9 mm), NCHUZOOL 16501, Wanlitong, Hengchun, Pingtung, Taiwan, coll. J.-W. Hsu, 15 Aug. 2016. Paratype: 16 ♂♂ (7.3-10.9 mm), 4 ♀♀ (7.5-8.8 mm), NCHUZOOL 16502, Camiguin, Philippines, 31 Aug. 2003.

Comparative material: *Ptychognathus* aff. *barbatus* (see Remarks): 10 ♂♂ (7.7–12.4 mm), 5 ♀♀ (8.9–13.9 mm), NCHUZOOL 16063, Dasi, Toucheng, Yilan, coll. J.-W. Hsu, 15 Aug. 2016; 2 ♂♂ (10.4–14.7 mm), 1 ♀ (13.0 mm), 1 ♀ (ovig.) (13.2 mm), NCHUZOOL 16064, Yanliao, Shoufeng, Hualien, coll. J.-W. Hsu, 29 June 2016; 2 ♀♀ (6.7–7.4 mm), NCHUZOOL 16065, Dulanwan, Donghe, Taitung, coll. P.-Y. Hsu et al., 9 Aug. 2017; 1 ♂ (13.0 mm), NCHUZOOL 16066, Shanyuan, Taitung, 27 July 2014; 2 ♂♂ (10.9–11.0 mm), 1 ♀ (9.4 mm), NCHUZOOL

16067, Gihui, Chenggong, Taitung, coll. J.-W. Hsu, 28 Apr. 2017; 3 ♂♂ (12.1–14.9 mm), NCHUZOOL 16075, Fubao, Changhua, 16 Jan. 2017; 2 ♂♂ (18.9–20.2 mm), 1 ♀ (11.5 mm), NCHUZOOL 16074, Gaoping R. estuary, Linyuan, Kaohsiung City, 29 Apr. 2009; 4 ♂♂ (9.9–16.9 mm), 1 ♀ (8.6 mm), NCHUZOOL 16073, Houwan, Hengchun, Pingtung, 11 July 2017; 1 ♂ (13.0 mm), NCHUZOOL 16072, Baoli R. estuary, Checheng, Pingtung, 23 June 2014; 8 ♂♂ (9.3–12.3 mm), 3 ♀♀ (8.8–11.9 mm), 2 ♀♀ (ovig.) (8.2–9.7 mm), NCHUZOOL 16070, Wanlitong, Hengchun, Pingtung, coll. J.-W. Hsu et al., 11 Dec. 2018; 5 ♂♂ (7.8–11.4 mm), 1 ♀ (9.4 mm), NCHUZOOL 16071, Dingtanzih, Hengchun, Pingtung, coll. P.-Y. Hsu et al., 19 Mar. 2018; 3 ♂♂ (6.5–10.5 mm), 1 ♀ (8.9 mm), NCHUZOOL 16069, Gangkou R. estuary, Hengchun, Pingtung, 18 Aug. 2016; 1 ♂ (14.7 mm), NCHUZOOL 16068, Jhonggang R. estuary, Manjhou, Pingtung, 7 Nov. 2018; 2 ♂♂ (6.3–12.3 mm), 1 ♀ (10.8 mm), NCHUZOOL 16076, Watong, Baisha, Penghu, 2 Sep. 2014. *Ptychognathus ishii* Sakai, 1939: 3 ♂♂ (5.3–7.4 mm), 1 ♀ (5.8 mm), NCHUZOOL 16033, Dasi, Toucheng, Yilan, coll. J.-W. Hsu, 15 Aug. 2016; 2 ♂♂ (8.9–11.4 mm), 2 ♀♀ (8.9-9.1 mm), NCHUZOOL 16034, Dasi, Toucheng, Yilan, coll. J.-W. Hsu, 16 Aug. 2016; 1 ♂ (10.8 mm), NCHUZOOL 16035, Dulanwan, Donghe, Taitung, coll. P.-Y. Hsu, 29 June 2016; 1 ♂ (9.2 mm), 1 ♀ (8.4 mm), NCHUZOOL 16036, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-J. Li, 5 June 2015; 6 ♂♂ (11.0–13.2 mm), 7 ♀♀ (9.1-13.7 mm), NCHUZOOL 16037, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-J. Li, 10 Aug. 2015; 1 ♂ (8.2 mm), 1 ♀ (6.9 mm), NCHUZOOL 16038, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-W. Hsu et al., 19 Mar. 2018; 1 ♀ (ovig.) (10.27 mm), NCHUZOOL 16039, Gangkou R. estuary, Hengchun, Pingtung, coll. J.-W. Hsu et al., 18 Aug. 2016.

Description: Carapace (Figs. 2C–F, 4A) subquadrate, slightly broader than long, 1.2 times as broad as long, flat; dorsal surface smooth, glabrous, regions weakly defined, with noticeable groove between epigastric regions. Front broad, slightly sloping forward, part near orbital regions slightly convex; frontal margin slightly concave, weakly divided into indistinct two lobes; anterior margin lined with small, rounded granules, with row of small granules behind frontal margin, granulated ridge contiguous to anterior margin in lateral part, separated from anterior margin in middle part; postfrontal region indistinct, separated into obscure 2 lobes by shallow grooves. Anterolateral margins and supraorbital margins lined with small granules, anterolateral margin with three teeth including orbital tooth, first tooth most distinct and acute, slightly sloping forward; second and third teeth blunt, third tooth indistinct or notch. Posterolateral margins divergent posteriorly, moderately sloping outwards; lateral and posterolateral margins regularly furnished with short, soft setae. Infraorbital ridge (Fig. 4B) consisting of 12–15 small, rounded granules. Surface of pterygostome with sparse soft setae. Epistome broad, median part triangular, margin with tiny granules.

Third maxillipeds (Figs. 2D, F, 4C) broad, surface with short setae sparsely, exopod slightly broader than or equal to ischium; mesial part of merus with oblique groove, anteroexternal angle broad, slightly sloping outwards; ischium with obscure vertical shallow groove.

Chelipeds (Fig. 4E) symmetrical in male. Merus without spines, dorsal margins with long soft setae, ventral margins glabrous. Surface of carpus smooth, without distinct granules, inner surface sometimes with short setae sparsely, inner distal angle blunt or obtuse triangular in male (Fig. 4D); inner distal angle with single spines in female. Outer surface of palm in male (Fig. 4E) smooth, without distinct granules or horizontal ridge; inner surface glabrous, middle part convex. Movable finger approximately as long as palm, inner surface glabrous and middle part convex; cutting edges of both fingers with numerous small blunt teeth, immovable finger with 2–3 larger blunt teeth; proximal half of fingers with long dense soft setae in male (absent in female), margin between movable finger and palm with short soft setae, with single small pulvinus at base of fingers. Female with outer surface of palm glabrous and granulated, inner surface glabrous, middle part convex; immovable finger with ridge consisting of large granules toward palm, fingers with sparse short setae at tips; movable finger slightly longer than palm.

Ambulatory legs (Fig. 4G, H) slender, P3 and P4 longest; mesial half of anterior margins of each merus with long soft setae, without spines, posterior margins without setae; propodus as long as dactylus in P2–P4 (Fig. 4G), propodus about 2 times length of dactylus in P5 (Fig. 4H). Carpi of

P2 and P3 with dense short setae on anterior margins of distal half, anterior margins of propodus and dactylus all covered with dense short setae, posterior margins of propodus sparsely setose, ventral surfaces of carpus and propodus with rows of short setae. Carpi of P4 and P5 with dense short setae on anterior margins of distal 1/3–1/4, posterior margins of carpus glabrous. P4 (Fig. 4G) relatively long, anterior margins of propodus covered with dense short setae, posterior margins with sparse long setae; posterior margins of distal 1/2–1/3 propodus covered with dense short setae, ventral surface with rows of long setae sparsely. P5 (Fig. 4H) relatively short, anterior margins of propodus covered with dense short setae (more dense on distal part), posterior margins of distal 1/3 propodus covered with dense short setae.

Male pleon (Fig. 4F) narrow, surface smooth, without any granules, lateral margins covered with short setae; telson tongue-shaped, approximately as long as sixth segment, distal margin of telson not concave, without tuft of setae.

Male G1 (Fig. 4I–N) slender, almost straight, part near tip more slender and slightly curving outwards; tip with short chitinous structure, two semicircle-shaped lobes in lateral view (Fig. 4K, L), opened laterally and mesially, respectively; G2 shorter than 1/4 length of G1.

Coloration: Preserved specimens with carapace gray to dark brown, ventral body white; chelipeds and ambulatory legs light gray to light brown, with setae brown.

Habitat: This species inhabits the intertidal area, with sediment composed of coarse sand. Individuals always hid under gravel and sometimes sympatric with *P*. aff. *barbatus* in Wanlitong, Kenting, southern Taiwan.

Etymology: This species is named for the American zoologist William Stimpson, who established the genus *Ptychognathus* (Stimpson 1858 1907).

Distribution: Southern Taiwan; southern Philippines (Camiguin).

Size: Small, largest male CW 10.9 mm, largest female CW 8.8 mm, smallest ovigerous female 7.9 mm.

Remarks: This species can be distinguished from other congeners from Taiwan by a suite of characters of the frontal region, anterolateral margins of carapace, male cheliped, setae on ambulatory legs and male telson.

This new species is very similar to the East Asian "*Ptychognathus barbatus* (A. Milne-Edwards, 1873)" in morphology (see below), however the identification of *P. barbatus* is problematic. In East Asia, Maki and Tsuchiya (1923) was the first report to identify Taiwanese specimens as "*P. barbatus*", which was followed by subsequent studies for the East Asian species (*e.g.*, Sakai 1939 1976; Dai et al. 1986; Dai and Yang 1991; Ng et al. 2001 2017). The type locality of *P. barbatus* is New Caledonia and this species was only provided with a very brief description with the dorsal view illustration (A. Milne-Edwards 1873: 136, pl. 17(4)), without detailed description on the ambulatory legs and male telson. Even so, it is clear that the frontal region is divided into two lobes by a shallow groove, with the posterolateral margins of carapace distinctly divergent posteriorly, and the propodus and dactylus of forth ambulatory legs are relatively slender. However, for the specimens from East Asia, the frontal region is slightly divided into obscure four lobes, with the posterolateral margins of carapace only slightly divergent posteriorly, and the propodus and dactylus of forth ambulatory legs are relatively short (Sakai 1976: text-fig. 348, pl. 219(2); Dai and Yang 1991: pl. 65(8); Fukui et al. 1989: fig. 14). Further detailed morphological studies and molecular analyses are necessary to clarify this issue (N.K. Ng, personal communication). As a result, we temporally treat the species from East Asia as *P.* aff. *barbatus*.

Ptychognathus stimpsoni sp. nov. is very closely allied to the East Asian *P.* aff. *barbatus*, but they can be distinguished by the frontal region, setae on ambulatory legs and telson of male. *Ptychognathus stimpsoni* has a row of small granules behind the frontal margin (vs. without distinct granules behind the frontal margin in *P.* aff. *barbatus*) (Fig. 4A; Sakai 1976: pl. 219(2)); the anterior margins of ambulatory carpi and propodi covered with dense short setae (vs. anterior margins of ambulatory carpus glabrous, only distal part of anterior margins of propodus covered with dense short setae) (Fig. 4G, H; Sakai 1976: pl. 219(2)); and the distal margin of male telson not concave, without a tuft of soft setae (vs. distal margin of male telson concave, with a tuft of soft setae) (Fig. 4F; Dai et al. 1986: fig. 262(4), Dai and Yang 1991: fig. 262(4)). The females of two species can be separated by the setae on the ambulatory legs as males.

In addition, *P. stimpsoni* is also similar with *P. pusillus* in morphology, but can be separated by the characters of ambulatory legs. The carpus of ambulatory legs covered with dense short setae on anterior margins of distal part in *P. stimpsoni* (Fig. 4G, H) (vs. anterior margins of ambulatory carpus glabrous in *P. pusillus* (De Man 1895: 99, fig. 22, De Man 1905: 539, pl. 17(1–5)).

The new species is also similar with *P. ishii* because the surfaces of carpus and propodus of ambulatory legs are covered with dense short setae in both species. However, the two species can be clearly distinguished by the features of carapace and setae on male chelipedal palm. In *P. stimpsoni*, the dorsal surface of carapace almost glabrous, anterolateral margin with three teeth, only third tooth indistinct or notch (vs. carapace covered with short black setae, anterolateral margin with two or three teeth, third tooth indistinct or absent in *P. ishii* (Fig. 4A; Sakai 1976: 639, text-fig. 349a, pl. 219(3)); and the proximal half of both fingers with long dense soft setae in male (vs. fingers with a tuft of long setae on outer surface extending to base of palm, mostly expanding onto fixed finger in male *P. ishii* (Fig. 4E; Sakai 1976: 639, text-fig. 349b)).

DISCUSSION

Diversity and distribution of the East Asian species of *Ptychognathus*

In East Asia, the species diversity of *Ptychognathus* is highest in the Ryukyus region, with 11 species and 4 undescribed species (Nakasone and Irei 2003). The second highest is Taiwan, with 9 species and 1 unknown species (Ng et al. 2017; Li et al. 2019; see RESULTS). Only 1 and 6 species are reported from China and the main islands of Japan, respectively (Sakai 1976; Takeda 1984; Dai et al. 1986; Dai and Yang 1991; Yamamoto et al. 2007; Yokooka et al. 2015); and no record from

Korea (Ko and Lee 2012).

Among the East Asian species of *Ptychognathus*, two names published by Sakai need to be solved. Sakai (1939: 661) established a new species, *P. takahasii*, but Sakai (1976) used both *P. takahasii* (p. 638) and *P. takahashii* (p. 641). This is the case similar to *Metaplax takahasii* Sakai, 1939 (see Shih et al. 2019a: 13) and the correct spelling should be *Ptychognathus takahasii* Sakai, 1939 (ICZN 1999: Article 32.2). *Ptychognathus hachijoensis* was published in Sakai (1955), which is confused because the subtitle of this new species was *P. hachijoensis* (p. 199), but as "*P. hachijyoensis*" in the captions for the two text-figures (p. 198 and p. 199). Because this species was named after the island Hachijo and the locality name was used with 10 times (including in the title) in Sakai (1955), we here select *Ptychognathus hachijoensis* Sakai, 1955 as the correct spelling, according to the Article 24.2.3 of the Code (ICZN 1999) (P. K. L. Ng, personal communication).

The distributional pattern of this genus with higher diversity in oceanic islands and lower in continental coasts is suggested to be caused by the different sediment, salinity, and temperature of the two types of environment. With regard to Taiwan, it appears to be the southern limit of some species, *e.g.*, *P. hachijoensis* (Sakai 1976; Fukui et al. 1989; Nakasone and Irei 2003), *P. takahasii* (Sakai 1976; Nakasone and Irei 2003) and *P. insolitus* (Osawa and N.K. Ng 2006; Li 2015). On the other hand, Taiwan is also the northern limit of *P. pilosus* (Li et al. 2019).

Identification of *Ptychognathus* species via DNA barcodes

Because most species of *Ptychognathus* have similar morphology and without distinct coloration, it always causes the correct identification difficult. For example, the pictures of "*P. affinis*" in Lee (2001: 116) should be *P. altimanus* and *Varuna litterata*; "*P. barbatus*" in Chen (2001: 252) should be *P. hachijoensis*; and "*P.* cf. *hachijoensis*" in Kishino et al. (2001: pl. 1(5)) and "*P. johannae*" in Nakasone and Irei (2003: fig. 50) were confirmed as a new species, *P. insolitus* by Osawa and Ng (2006). DNA barcodes have been applied to help identify several groups of crabs (Chu et al. 2015). With regard to the genus *Ptychognathus*, the *COI* sequences deposited in GenBank are only available for a few species.

Based on the results of our study, the *COI* sequences are useful to distinguish the 10 species of *Ptychognathus* from Taiwan, and some from other countries. The interspecific distances are at least 12.2% which are larger than most species of crabs (see Chu et al. 2015), *e.g.*, the minimum interspecific distance 3.0% among species of *Eriocheir* sensu lato (Varunidae) (Naser et al. 2012); 3.8% between *Hemigrapsus penicillatus* (De Haan, 1835) and *H. takanoi* Asakura & Watanabe, 2005 (Varunidae) (Markert et al. 2014); 1.49% between *Leptarma liho* (Koller, Liu & Schubart, 2010) and *L. paucitorum* (Rahayu & Ng, 2009) (Sesarmidae) (Shih et al. 2019b); 3.2% between *Sesarmops imperator* Ng, Li & Shih, 2020 and *S. impressus* (H. Milne Edwards, 1837) (Sesarmidae) (Ng et al. 2020); 3.78% between *Tubuca urvillei* (H. Milne Edwards, 1852) and *T. alcocki* Shih, Chan & Ng, 2018 (Ocypodidae) (Shih et al. 2018); 4.59% between *Austruca citrus* Shih & Poupin, 2020 and *A. perplexa* (H. Milne Edwards, 1852) (Ocypodidae) (Shih and Poupin 2020); 5.63% between *Tortomon gejiu* Huang, Wang & Shih, 2020 and *T. puer* Huang, Wang & Shih, 2020 (Potamidae) (Huang et al. 2020); and 6.11% between *Tiwaripotamon pingguoense* Dai & Naiyanetr, 1994 and *T. xiurenense* Dai & Naiyanetr, 1994 (Potamidae) (Do et al. 2016).

CONCLUSIONS

In our study, the *COI* gene has been used to help identify the species of *Ptychognathus* from Taiwan. Ten species from Taiwan were identified successfully, with interspecific distances ranged 12.2%–19.57%. Two new species are described, supported by *COI*, with *Ptychognathus makii* sp. nov. from southern Taiwan and *P. stimpsoni* sp. nov. from southern Taiwan and southern Philippines.

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Authors' contributions: JWH performed the morphological description and part of the molecular analysis, and drafted the manuscript. HTS performed the molecular analysis, discussion and drafted the manuscript. Both authors read and approved the final manuscript.

Competing interests: JWH and HTS declare that they have no conflict of interests.

Availability of data and materials: Sequences generated in the study have been deposited in GenBank database (accession numbers in Table 1 in manuscript).

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Ethics approval consent to participate: Not applicable.

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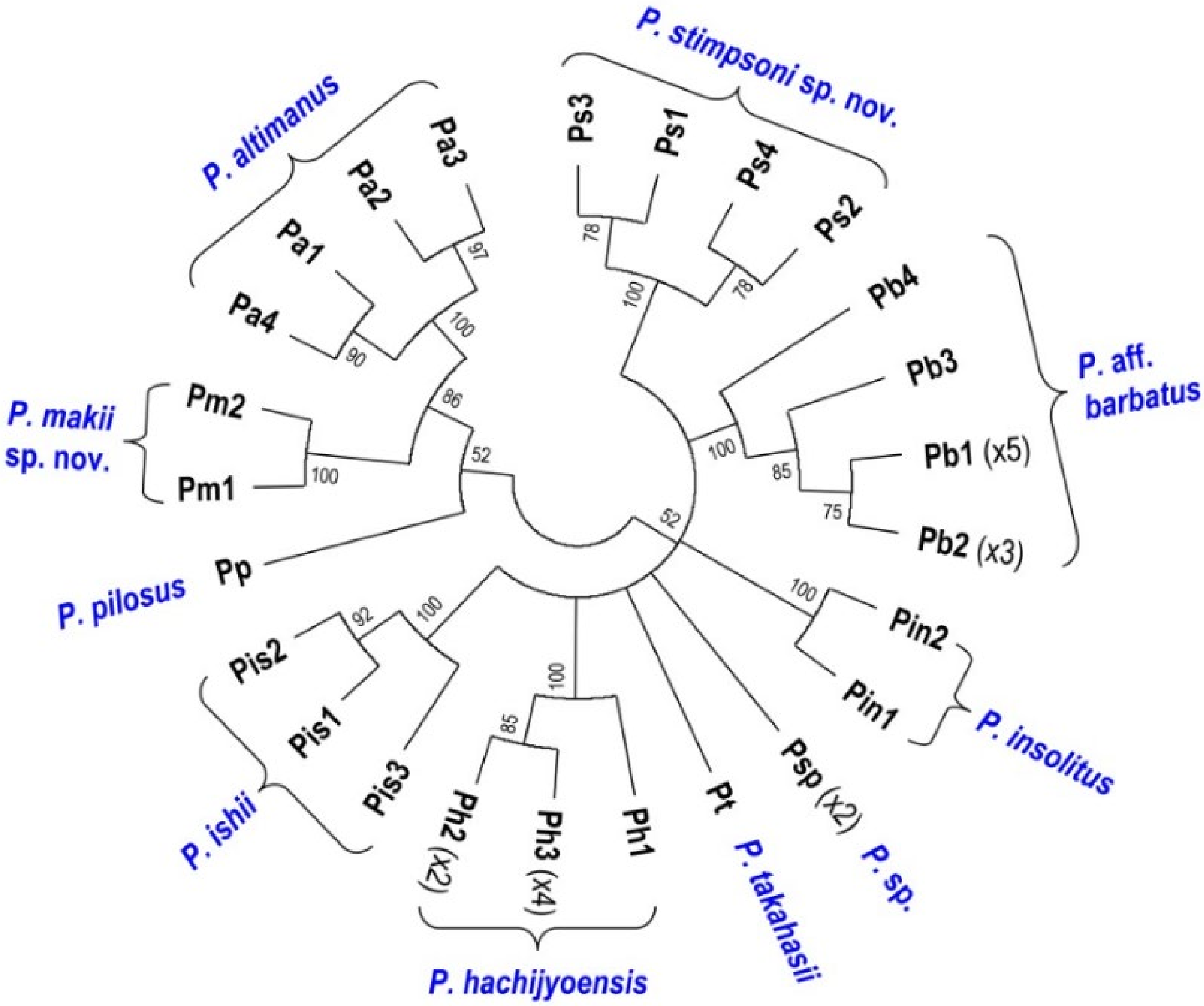

Fig. 1. A neighbor-joining tree for species of *Ptychognathus* from Taiwan, Philippines and Indonesia, based on the cytochrome *c* oxidase subunit I (*COI*) gene. Probability values at the nodes represent support values. Only values > 50% are shown. For haplotype names, see table 1.

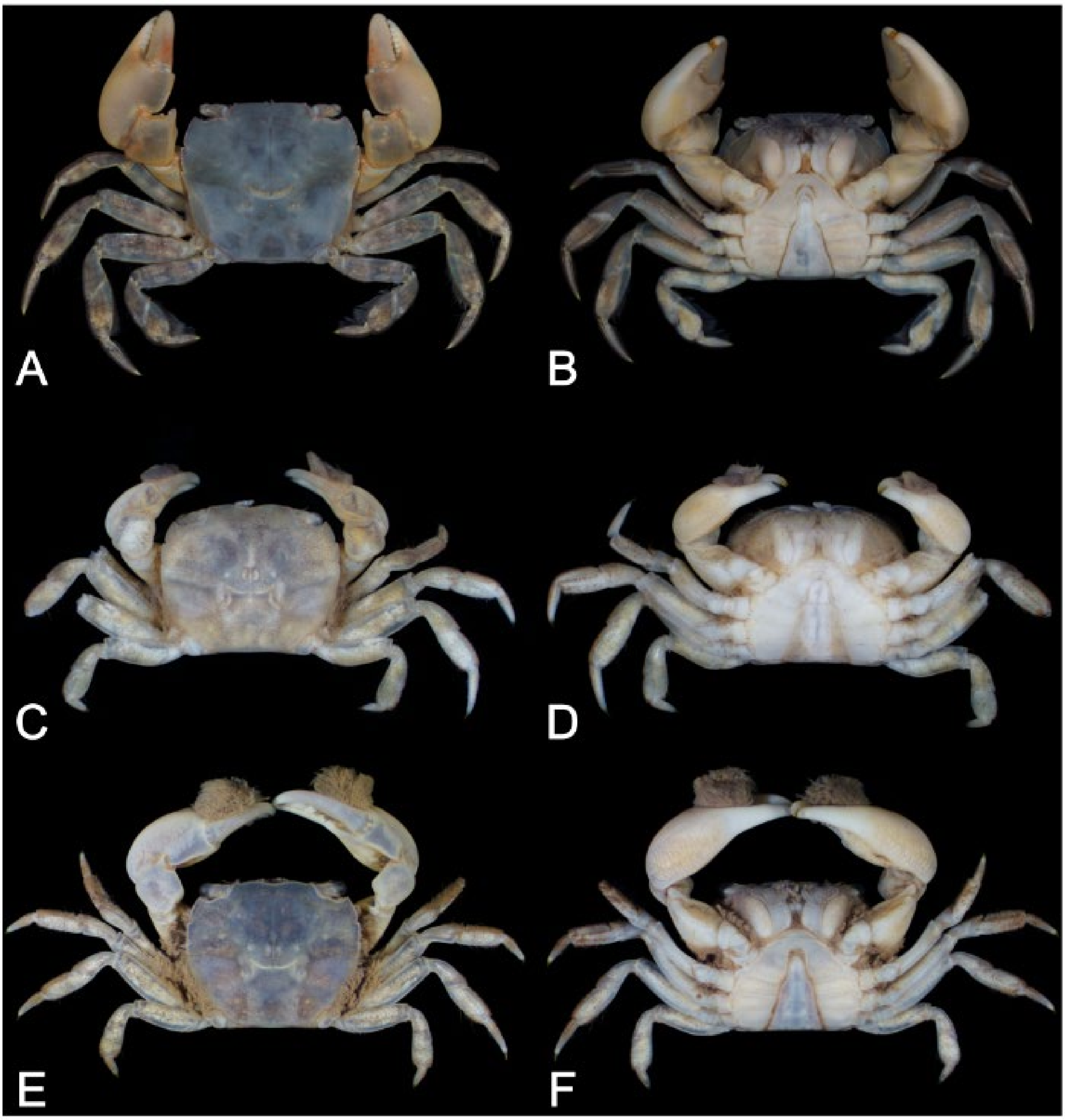

Fig. 2. A–B: *Ptychognathus makii* sp. nov., holotype (male, CW 23.5 mm; NCHUZOOL 16062). C–F: *Ptychognathus stimpsoni* sp. nov.; C, D: holotype (male, CW 7.9 mm; NCHUZOOL 16501). E, F: paratype (male, CW 10.9 mm; NCHUZOOL 16502).

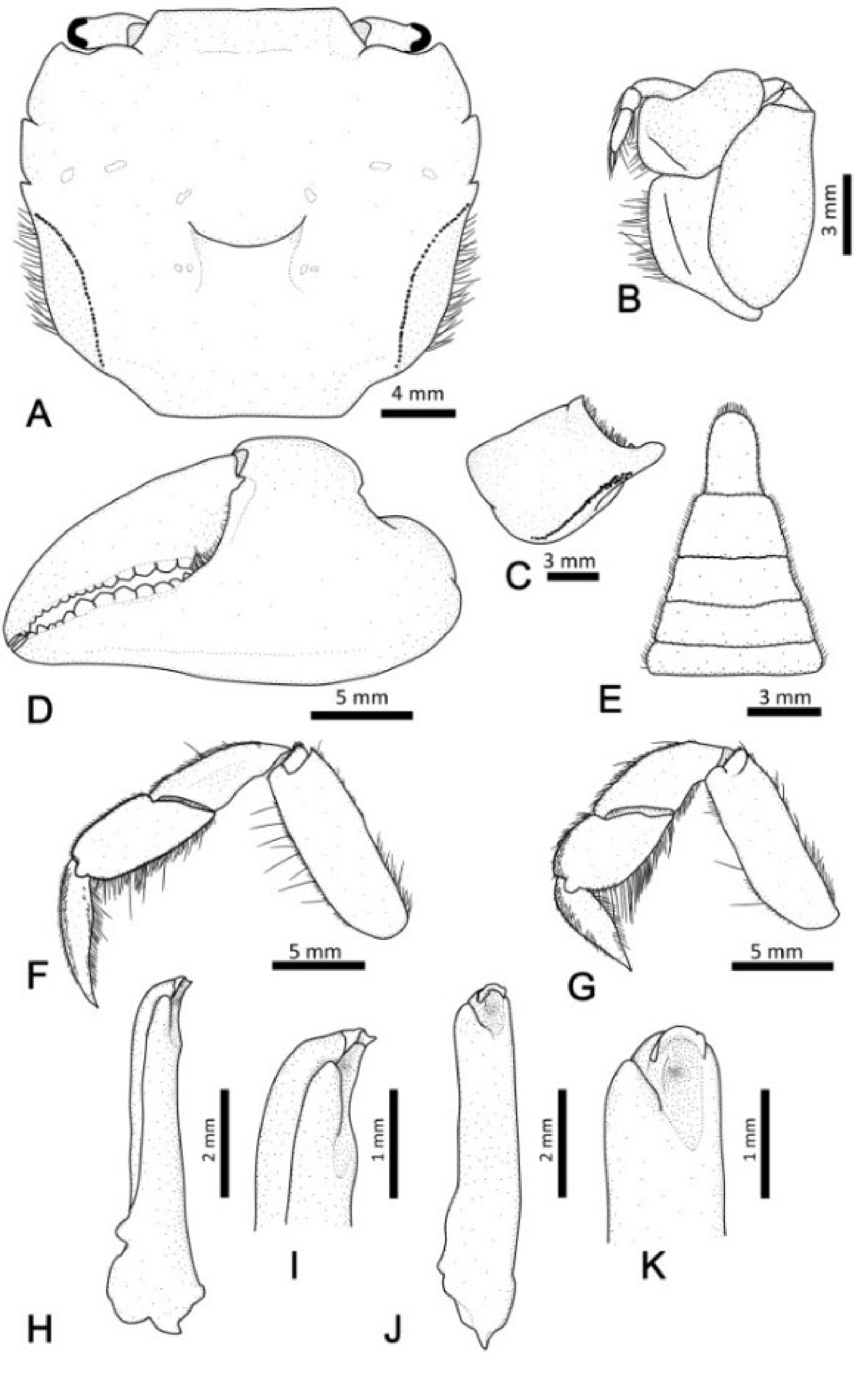

Fig. 3. *Ptychognathus makii* sp. nov., holotype (male, CW 23.5 mm; NCHUZOOL 16062). A, carapace; B, left third maxilliped; C, left carpus of cheliped (dorsal view); D, outer view of left cheliped; E, pleon; F, left third ambulatory leg; G, left forth ambulatory leg; H–I, right G1; H, I, dorsal view; J, K, lateral view.

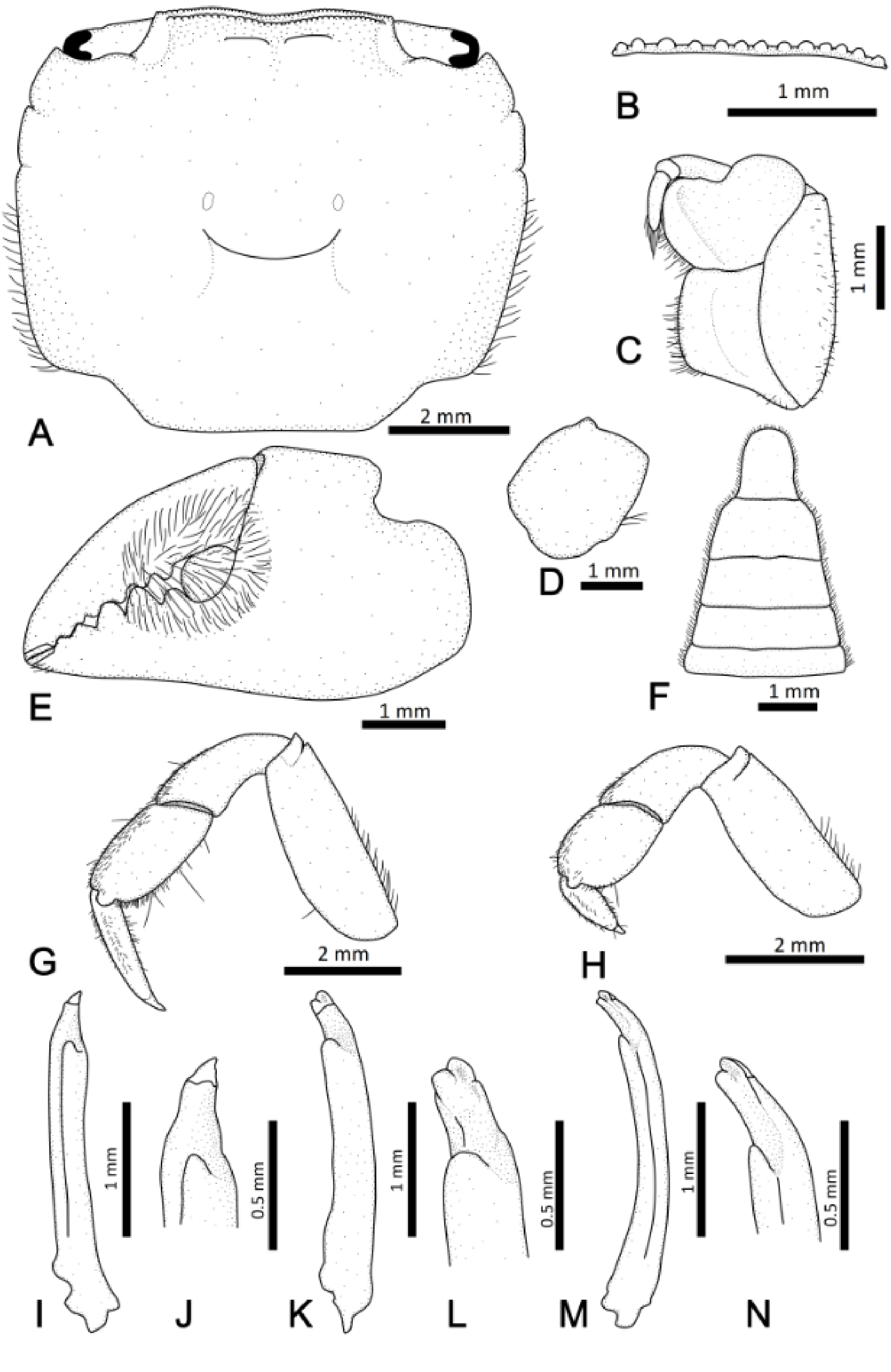

Fig. 4. *Ptychognathus stimpsoni* sp. nov., holotype (male, CW 7.9 mm; NCHUZOOL 16501). A, carapace; B, left infraorbital ridge; C, left third maxilliped; D, left carpus of cheliped (dorsal view); E, outer view of left cheliped; F, pleon; G, left third ambulatory leg; H, left forth ambulatory leg; I–N, right G1; I, J, dorsal view; K, L, lateral view; M, N, ventral view).

Table 1. The haplotypes of *COI* gene of the genus *Ptychognathus* used in this study. See MATERIALS AND METHODS for abbreviations of museums and universities. *, species identified in Lee (2001)

Species	Locality	Sample size	Catalogue no. of NCHUZOOL (unless indicated)	Haplotype	Access. no.
P. altimanus	Taiwan: Gangkou R. estuary, Pingtung	1	16052	Pa1	MW000763
	Taiwan: Gangkou R. estuary, Pingtung	1	16052	Pa2	MW000764
(id as "*P. affinis*")*	Taiwan: Linbian R. estuary, Pingtung	1	16055	Pa3	MW000765
	Taiwan: Linbian R. estuary, Pingtung	1	16055	Pa4	MW000766
P. aff. *barbatus*	Taiwan: Fubao, Changhua	1	16075	Pb1	MW000767
	Taiwan: Baoli R. estuary, Pingtung	1	16072	Pb2	MW000768
	Taiwan: Watong, Baisha, Penghu	1	16076	Pb2	MW000768
	Taiwan: Dasi R. estuary, Yilan	1	16063	Pb1	MW000767
	Taiwan: Yanliao, Hualien	1	16064	Pb3	MW000769
	Taiwan: Jihuei, Taitung	1	16067	Pb1	MW000767
	Taiwan: Dulanwan, Taitung	1	16065	Pb1	MW000767
	Taiwan: Shanyuan, Taitung	1	16066	Pb2	MW000768
	Taiwan: Shanyuan, Taitung	1	16505	Pb1	MW000767
	Indonesia: Bali	1	16504	Pb4	MW000770
P. hachijoensis	Taiwan: Chaishan, Kaohsiung	1	15807	Ph1	MW000771
	Taiwan: Houwan, Pingtung	1	15818	Ph2	MW000772
	Taiwan: Jioupeng, Pingtung	1	15821	Ph3	MW000773
	Taiwan: Dasi, Yilan	1	15820	Ph3	MW000773
	Taiwan: Yanliao, Hualien	2	15809, 15810	Ph3	MW000773
P. insolitus	Taiwan: Houwan, Pingtung	1	16044	Pin1	MW000774
	Taiwan: Houwan, Pingtung	1	16045	Pin2	MW000775
P. ishii	Taiwan: Gangkou R. estuary, Pingtung	1	16039	Pis1	MW000776
	Taiwan: Gangkou R. estuary, Pingtung	1	16506	Pis2	MW000777
	Taiwan: Dulanwan, Taitung	1	16035	Pis3	MW000778
P. makii	Taiwan: Gangkou R. estuary, Pingtung	1	16049	Pm1	MW000779
	Taiwan: Gangkou R. estuary, Pingtung	1	16050	Pm2	MW000780
P. pilosus	Taiwan: Gangkou R. estuary, Pingtung	1	15395	Pp	MW000781
P. stimpsoni	Taiwan: Wanlitong, Pingtung	1	16501	Ps1	MW000782
	Philippines: Camiguin	1	16502	Ps2	MW000783
	Philippines: Camiguin	1	16502	Ps3	MW000784
	Philippines: Camiguin	1	16502	Ps4	MW000785
P. takahasii	Taiwan: Jihuei, Taitung	1	16058	Pt	MW000786
Ptychognathus sp.	Taiwan: Dingtanzih	2	16503	Psp	MW000787
Total		35			

Table 2. Matrix of percentage pairwise nucleotide divergence with Kimura 2-parameter (K2P) distances (lower left) and mean numbers of differences (upper right) based on cytochrome *c* oxidase subunit I (*COI*) within and between 10 species of *Ptychognathus* from Taiwan, Philippines and Indonesia (see Table 1). Values of the range are shown in parentheses

	Intraspecific		Interspecific									
	Nucleotide divergence	Mean nucleotide difference	*P. altimanus*	*P.* aff. *barbatus*	*P. hachijoensis*	*P. insolitus*	*P. makii*	*P. ishii*	*P. pilosus*	*P. stimpsoni*	*P. takahasii*	*Ptychognathus* sp.
P. altimanus	0.31 (0–0.46)	2 (0–3)		101.9 (100–103)	104.67 (102–106)	104 (102–106)	99.33 (97–101)	84 (82–86)	95 (95)	101 (100–102)	100 (99–101)	99 (98–100)
P. aff. *barbatus*	0.15 (0–0.77)	1 (0–5)	17.49 (17.11–17.71)		86.43 (83–87)	91.3 (90–92)	96.63 (94–98)	101.4 (100–102)	104.9 (103–106)	77.85 (76–81)	83.8 (83–84)	76.9 (76–77)
P. hachijoensis	0.2 (0–0.61)	1.33 (0–4)	18.02 (17.5–18.29)	14.86 (14.16–14.98)		83.67 (81–87)	93.83 (91–96)	107.5 (105–110)	106.83 (105–108)	81.42 (79–83)	74.17 (73–76)	80.17 (79–82)
P. insolitus	0.46 (0.46–0.46)	3 (3)	17.95 (17.55–18.36)	15.67 (15.41–15.81)	14.26 (13.72–14.92)		99.83 (99–101)	111 (110–112)	105.5 (104–107)	90.25 (89–92)	86.5 (86–87)	79.5 (79–80)
P. makii	1.03 (0.15–1.54)	6.67 (1–10)	17.05 (16.59–17.37)	16.61 (16.09–16.89)	16.02 (15.48–16.44)	17.24 (17.06–17.48)		101.83 (99–106)	97.67 (97–98)	93.25 (92–95)	82.33 (82–83)	89 (88–91)
P. ishii	0.15 (0.15–0.15)	1 (1)	14.28 (13.89–14.67)	17.39 (17.11–17.51)	18.62 (18.13–19.14)	19.36 (19.15–19.57)	17.47 (16.91–18.3)		109.5 (109–110)	94.25 (93–96)	100.5 (100–101)	93.5 (93–94)
P. pilosus	—	—	16.17 (16.17)	18.05 (17.67–18.28)	18.47 (18.11–18.71)	18.27 (17.97–18.58)	16.73 (16.59–16.8)	18.98 (18.87–19.08)		91 (91)	105 (105–105)	91 (91)
P. stimpsoni	0.28 (0–0.46)	1.83 (0–3)	17.32 (17.12–17.52)	13.05 (12.7–13.65)	13.73 (13.28–14.03)	15.44 (15.19–15.79)	15.89 (15.65–16.23)	15.97 (15.73–16.31)	15.37 (15.37)		83.75 (83–85)	75.25 (74–77)
P. takahasii	—	—	17.12 (16.92–17.32)	14.19 (14.03–14.23)	12.43 (12.2–12.78)	14.77 (14.67–14.86)	13.8 (13.74–13.92)	17.24 (17.14–17.34)	18.11 (18.11)	14.14 (13.99–14.38)		80 (80)
Ptychognathus sp.	0	0	16.86 (16.67–17.06)	12.95 (12.78–12.97)	13.58 (13.34–13.93)	13.4 (13.31–13.5)	15.09 (14.89–15.47)	15.82 (15.73–15.92)	15.37 (15.37)	12.52 (12.29–12.85)	13.47 (13.47)	