

# The Synonymy of the Supratidal Crab Species *Parasesarma cognatum* Rahayu & Li, 2013 with *P. liho* Koller, Liu & Schubart, 2010 (Decapoda: Brachyura: Sesarmidae) Based on Morphological and Molecular Evidence, with a Note on *P. paucitorum* Rahayu & Ng, 2009

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*Parasesarma liho* Koller, Liu & Schubart, 2010 and *P. cognatum* Rahayu & Li, 2013 from East and Southeast Asia are similar species that have been confused in several studies. Here, we re-examined the type specimens of both species and found identical main characters, which is supported by the molecular identity of the mitochondrial cytochrome oxidase subunit I gene. As a result, we treat *P. cognatum* as a junior subjective synonym of *P. liho*. We also show that the male paratype of *P. paucitorum* Rahayu & Ng, 2009 is conspecific with *P. liho*, although *P. paucitorum* s. str. remains a distinct but allied species. The distribution of *P. liho* is updated to include Japan (Ryukyus), Taiwan, Philippines (Cebu) and Indonesia (Sulawesi).

**Key words:** *Parasesarma*, *P. liho*, *P. cognatum*, *P. paucitorum*, Morphology, Mitochondrial COI.

## BACKGROUND

Crabs of the family Sesarmidae Dana, 1851 form part of the dominant macrofauna of the Indo-West Pacific mangrove ecosystems, playing an especially important ecological role as “ecosystem engineers” (Lee 1998; Kristensen 2008). They inhabit landward regions of mangrove fringes and can tolerate high temperature and salinity fluctuations (Theurkauff et al. 2018). With 69 recognised species, *Parasesarma* De Man, 1895 is

the most speciose genus of this family, especially after most species of *Perisesarma* De Man, 1895 have been moved to *Parasesarma* and the recent descriptions of more new species (see Ng et al. 2008; Shahdadi et al. 2017 2018a 2019; Li et al. 2018; Shahdadi and Schubart 2017; Fratini et al. 2019). In Taiwan, 12 species of this genus have been reported, of which eight, viz. *P. cognatum* Rahayu & Li, 2013, *P. corallicum* Ng, Davie & Li, 2016, *P. kuekenthali* (De Man, 1902), *P. kui* Li, Rahayu & Ng, 2018, *P. lepidum* (Tweedie, 1950), *P. liho*

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Koller, Liu & Schubart, 2010, *P. macaco* Li, Rahayu & Ng, 2018, and *P. unguatum* (H. Milne Edwards, 1853), were added after the latest revision of Taiwanese crabs in 2001 (Ng et al. 2001 2016 2017; Koller et al. 2010; Rahayu and Li 2013; Li 2015; Hsu and Shih 2018; Li et al. 2018 2019).

Recently, several taxonomic studies of crabs have used molecular evidence to support the descriptions or recognition of new or reinstated species (cf. Chu et al. 2015); this is also the case within the family Sesarmidae (Schubart et al. 1998 2009; Gillikin and Schubart 2004; Koller et al. 2010; Naderloo and Schubart 2010; Ragionieri et al. 2012; Thiercelin and Schubart 2014; Cannicci et al. 2017; Shahdadi et al. 2017 2018a b). To help identify species of Taiwanese sesarmids, a DNA barcoding approach using the cytochrome oxidase subunit I (*COI*) marker (Hebert et al. 2003a b) was undertaken. A preliminary result of the *COI* analyses showed that only one clade was obtained for specimens labelled as *P. liho* and *P. cognatum* from Taiwan; and this required further investigation.

*Parasesarma liho* Koller, Liu & Schubart, 2010 (type locality: Hualien, Taiwan) is distributed in Hualien and Taitung, eastern Taiwan (Koller et al. 2010), as well as the Ryukyus, Japan (Okinawa, Miyako and Ishigaki; Maenosono and Naruse 2015). *Parasesarma cognatum* Rahayu & Li, 2013 (type locality: Pingtung, Taiwan) is distributed in Pingtung (southern Taiwan) and Hualien (eastern Taiwan), as well as in Cebu (the Philippines) according to Rahayu and Li (2013). Rahayu and Li (2013: 639) mentioned that *P. cognatum* is different from *P. liho* in the proportions of ambulatory propodi, structure of the male first gonopod, number and shape of dactylar tubercles of male chela, and coloration. However, the similarity between the two species has been remarked by Maenosono and Naruse (2015: 22), who questioned their identities.

In the present study, the types of these species, as well as more specimens of different sizes, were examined and their *COI* sequences compared, including specimens from various localities. The types of the allied *P. paucitorum* Rahayu & Ng, 2009 from Indonesia were also studied.

## MATERIALS AND METHODS

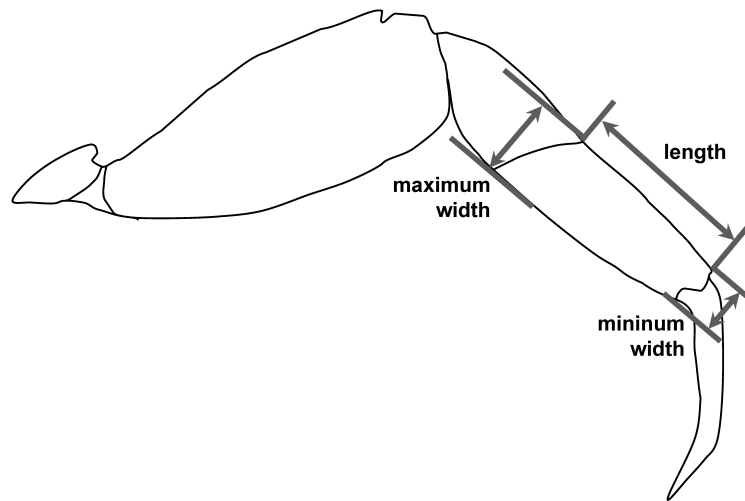
New specimens with the appearance of *Parasesarma liho* and *P. cognatum* were collected from their type localities and other areas in southern and eastern Taiwan, as well as from Cebu, Philippines. The holotypes and paratypes of *P. liho*, *P. cognatum* and *P. paucitorum* were also included. Individuals of other related species (see below) were also studied for

comparison (Table 1). Those specimens were deposited in the Biodiversity Research Museum, Academia Sinica, Taipei, Taiwan (ASIZ); the Muséum national d'Histoire naturelle, Paris (MNHN); the Museum Zoologi Bogor, Indonesian Institute of Sciences, Indonesia (MZB); the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOO); the National Museum of Marine Biology and Aquarium, Pingtung, Taiwan (NMMBA); National Museum of Natural Science, Taichung, Taiwan (NMNS); the Senckenberg Museum, Frankfurt am Main, Germany (SMF); Zoological Reference Collection of the Lee Kong Chian Natural History Museum, National University of Singapore (ZRC); and the Zoologische Staatssammlung, München (Munich), Germany (ZSM).

The abbreviations P4 is used for the fourth pereopods (third ambulatory legs), and G1 for male first gonopods. Measurements, all in millimeters (mm), are of the maximum carapace width (CW) and carapace length (CL). The G1s of different sized specimens were compared to determine whether there is a size effect on the morphology of G1. The ratios of length/width of the P4 propodus for different-sized specimens were calculated, as it was used to distinguish *P. liho* and *P. cognatum* by Rahayu and Li (2013). Because different authors could measure different positions from the photograph (Shih and Do 2014), both the proximal and distal widths of the propodi were measured to obtain the range of width in our study (Fig. 1). The morphology of the upper margin of the cheliped merus of the holotype was also examined to confirm the presence of a large subdistal spine, as mentioned in Rahayu and Li (2013: 637) and Maenosono and Naruse (2015: 22, fig. 7B).

Genomic DNA was isolated from the muscle tissue of the pleon or walking leg with different kits (Shih et al. 2016; Shahdadi and Schubart 2017). A portion of the *COI* gene was amplified with PCR using the primers LCO1490, HCO2198 (Folmer et al. 1994) or COL6 and COH6 (Schubart 2009). The PCR conditions for the above primers were: denaturation for 50 s at 94°C, annealing for 70 s at 45–47°C (45 s at 48–50°C for COL6-COH6), and extension for 60 s at 72°C (40 cycles), followed by extension for 10 min at 72°C. Sequences were obtained by automated sequencing (Applied Biosystems 3730) and verified with the complementary strand. Sequences of the different haplotypes were deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers in Table 1).

For comparative purposes, we included the species or species complexes used in the study of *P. liho*, *P. cognatum* and *P. paucitorum* in Koller et al. (2010), Rahayu and Li (2013) and Rahayu and Ng (2009), respectively, using the following as outgroups: *P. affine*



**Fig. 1.** Schematic drawing showing the measurement of the length, as well as the maximum and minimum widths of the propodi of the fourth pereopods (third ambulatory leg, P4) used in this study.

**Table 1.** Found haplotypes of the *COI* gene of *Parasesarma liho*, *P. cognatum* and *P. paucitorum*, as well as the outgroups. For abbreviations of museums and universities see MATERIALS AND METHODS

| Locality   | sample size | Catalogue no.  | Haplotype of <i>COI</i> | Access. no. of <i>COI</i> |
|--|-------------|--|-------------------------|---------------------------|
| <i>P. liho</i> or <i>P. cognatum</i>                       |             |  |                         |                           |
| Taiwan   |             |  |                         |                           |
| Hualien: Meilun R. estuary                                 | 1           | SMF 36266 (holotype of <i>P. liho</i> )                | — <sup>a</sup>          | LC490879                  |
|  | 1           | ZSM A20100040 (paratype of <i>P. liho</i> )            | — <sup>a</sup>          | LC490880                  |
|  | 1           | NCHUZOO 15027  | PRL-C1                  | LC490881                  |
| Taitung: Dulanwan  | 2           | NCHUZOO 15025  | PRL-C1                  | LC490881                  |
| Pingtung: Niou R. estuary                                  | 1           | NCHUZOO 15031  | PRL-C1                  | LC490881                  |
| Pingtung: Gangkou R. estuary                               | 1           | NMMBCD 3975 (holotype of <i>P. cognatum</i> )          | PRL-C1                  | LC490881                  |
|  | 2           | NMMBCD 3976 (paratypes of <i>P. cognatum</i> )         | PRL-C1                  | LC490881                  |
|  | 1           | NMMBCD 3976 (paratype of <i>P. cognatum</i> )          | PRL-C2                  | LC490882                  |
|  | 1           | NCHUZOO 15028  | PRL-C1                  | LC490881                  |
|  | 1           | ZRC 2013.1757  | — <sup>a</sup>          | LC490883                  |
|  | 1           | ZRC 2013.1757  | — <sup>a</sup>          | LC490883                  |
| Pingtung: Houwan   | 3           | NCHUZOO 15022  | PRL-C1                  | LC490881                  |
|  | 1           | NCHUZOO 15425  | PRL-C1                  | LC490881                  |
| Pingtung: Baoli R.   | 1           | NCHUZOO 15024  | PRL-C1                  | LC490881                  |
| Philippines  |             |  |                         |                           |
| Cebu: Kawasan  | 1           | ASIZCR   | PRL-C1                  | LC490881                  |
|  | 1           | ASIZCR   | PRL-C1                  | LC490881                  |
|  | 1           | NCHUZOO 15034  | PRL-C2                  | LC490882                  |
| Indonesia  |             |  |                         |                           |
| Sulawesi: Manado   | 1           | ZRC 2019.0578 (male paratype of <i>P. paucitorum</i> ) | — <sup>a</sup>          | LC490884                  |
| Others   |             |  |                         |                           |
| <i>P. paucitorum</i> : Sulawesi, Indonesia                 | 1           | MZB Cru 2243 (holotype)                                |                         | LC490885                  |
|  | 1           | ZRC 2008.0869 (female paratype)                        |                         | LC490886                  |
| <i>P. kui</i> : Gangkou R. estuary, Pingtung, Taiwan       | 1           | NMNS 7779-015 (holotype)                               |                         | LC490887                  |
| <i>P. macaco</i> : Baoli R. estuary, Pingtung, Taiwan      | 1           | NMNS-7779-005 (holotype)                               |                         | LC490888                  |
| <i>P. tripectinis</i> : Dajia R. estuary, Taichung, Taiwan | 1           | NCHUZOO 15428  |                         | LC490889                  |
| <i>P. pictum</i> : Nangan, Matsu, Taiwan                   | 1           | NCHUZOO 15427  |                         | LC490890                  |
| <i>P. affine</i> : Danshuei, New Taipei, Taiwan            | 1           | NCHUZOO 15426  |                         | LC490891                  |
| <i>P. dumacense</i> : Cebu, Philippines                    |             | ZRC 2008.0833  |                         | KX400929                  |

<sup>a</sup>sequences are shorter and not included for further analyses (see RESULTS).

(De Haan, 1837), *P. dumacense* (Rathbun, 1914), *P. leptosoma* (Hilgendorf, 1869) (now corresponding to *P. kui* Li, Rahayu & Ng, 2018, *P. macaco* Li, Rahayu & Ng, 2018 of this species complex; see Li et al. 2018), *P. pictum* (De Haan, 1835), and *P. tripectinis* (Shen, 1940) (Table 1). The *COI* sequences of *P. dumacense* was downloaded from GenBank (accession number: KX400929).

The best-fitting model for sequence evolution was determined by Partition Finder (vers. 2.1.1; Lanfear et al. 2017), selected by the Bayesian information criterion (BIC). The obtained best model (GTR + I) was subsequently used for a Bayesian inference (BI) analysis. The BI analysis was performed with MrBayes (vers. 3.2.3, Ronquist et al. 2012). The phylogenetic analyses were run with four chains for 10 million generations and four independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2019) and the first 3000 trees were discarded as burnin. The maximum likelihood (ML) analysis was conducted in RAxML (vers. 7.2.6, Stamatakis 2006). Because RAxML does not accept the GTR + I model, the second best model, GTR + G (*i.e.*, GTRGAMMA), was used with 100 runs, and the best ML tree was found by comparing the likelihood scores. The robustness of the ML tree was evaluated by 1000 bootstrap pseudoreplicates under the model GTRGAMMA. The relationships of the *COI* haplotypes among *P. liho* and other related species were examined by using the program PopART (vers. 1.7, Leigh and Bryant 2015). Basepair (bp) differences and the pairwise estimates of Kimura 2-parameter (K2P) distances (Kimura 1980) for genetic diversities between haplotypes were calculated with MEGA (vers. 10.0.5, Kumar et al. 2018).

*Material examined* (see Table 1): Holotype of *P. liho*: male (13.0 × 12.3 mm) (SMF 36266), Meilun R. (= River) estuary, Hualien, Taiwan, coll. H.-C. Liu, 31 October 2009; paratypes: 1 male (12.7 × 11.5 mm) (ZSM A20100040), same data as holotype; 1 male (14.2 × 13.1 mm) (SMF 36269), 1 male (14.5 × 13.1 mm) (MNHN B32312), same locality as holotype, coll. H.-C. Liu, 6 November 2000.

Holotype of *P. cognatum*: male (14.3 × 13.1 mm) (NMMBCD 3975), Gangkou R. estuary, Manzhou, Pingtung, Taiwan, coll. J.-J. Li, 1 September 2012; paratypes: 2 females (11.5 × 10.3 mm; 14.4 × 12.6 mm) (NMMBCD 3506), same locality as holotype, coll. J.-J. Li, 8 June 2012 (the data is different from that in Rahayu and Li 2013).

*Others*: Taiwan: 1 female (10.8 mm) (NCHUZOOOL 15024), Baoli R. estuary, Pingtung,

coll. P.-Y. Hsu et al., 11 July 2017; 1 male (14.2 mm) (NCHUZOOOL 15030), Houwan, Pingtung, coll. P.-Y. Hsu, 26 June 2012; 2 males (11.1–16.2 mm), 5 females (12.6–16.0 mm) (NCHUZOOOL 15022), Houwan, Pingtung, coll. R.-H. Lee, 19 September 2013; 3 males (9.7–14.9 mm), 1 female (9.3 mm) (NCHUZOOOL 15425), Houwan, Pingtung, coll. P.-Y. Hsu and C.-Y. Chi, 3 December 2016; 1 female (16.2 mm) (NCHUZOOOL 15029), Leidashih, Kenting, Pingtung, coll. J.-H. Lee, 18 August 2012; 1 male (16.7 mm) (NCHUZOOOL 15031), Niou R. estuary, Pingtung, coll. P.-Y. Hsu, 19 January 2016; 2 males (13.3–13.0 mm), 1 female (11.8 mm) (ZRC 2013.1757), Gangkou R. estuary, Pingtung, coll. J.-J. Li, 19 February 2013; 2 females (11.7–12.6 mm) (NCHUZOOOL 15023), Fushuei Bridge, Gangkou R. estuary, Pingtung, coll. P.-Y. Hsu et al., 12 July 2017; 2 females (8.6–13.9 mm) (NCHUZOOOL 15028), Gangkou R. estuary, Pingtung, coll. P.-Y. Hsu et al., 4 September 2017; 8 males (4.8–12.4 mm), 2 females (5.6–6.0 mm) (NCHUZOOOL 15025), Dulanwan, Taitung, coll. P.-Y. Hsu et al., 9 August 2017; 1 male (6.1 mm), 2 females (10.9–10.9 mm) (NCHUZOOOL 15032), Jihuei Fishing Port, Taitung, coll. P.-Y. Hsu et al., 10 August 2017; 1 male (11.6 mm) (NCHUZOOOL 15027), Meilun R. estuary, Hualien, coll. J.-H. Lee, 18 May 2012; 1 male (11.6 mm), 3 females (10.2–11.3 mm) (NCHUZOOOL 15026), Meilun R. estuary, Hualien, coll. P.-Y. Hsu et al., 10 August 2017. Philippines: 1 male (11.2 mm), 1 female (16.4 mm) (ASIZCR), Kawasan, Cebu, coll. H.-C. Liu, 4 December 2001; 2 males (13.0–13.3 mm) (NCHUZOOOL 15034), Kawasan, Cebu, coll. J.-J. Li, 6 September 2018. Indonesia: 1 male (15.5 mm) (ZRC 2019.0578, ex ZRC 2008.0869 partim) (paratype of *P. paucitorum* Rahayu and Ng, 2009), Manado, northern Sulawesi, Indonesia, coll. P. K. L. Ng, 17 July 2003.

*Comparative material: Parasarma paucitorum*: 1 male (19.7 mm) (MZB Cru 2243, holotype), 1 female (19.2 mm) (ZRC 2008.0869, paratype), Manado, northern Sulawesi, Indonesia, coll. P. K. L. Ng, 17 July 2003. *P. affine*: 1 male (29.6 mm) (NCHUZOOOL 15426), Danshuei, New Taipei City, Taiwan, 30 June 2006. *P. dumacense*: 1 male (20.4 mm) (ZRC 2008.0833), Kawasan Waterfall, Cebu, Philippines, coll. H.-C. Liu, 25 November 2001. *P. pictum*: 1 male (15.0 mm) (NCHUZOOOL 15427), Nangan, Matsu, Taiwan, coll. P.-Y. Hsu et al., 26 August, 2011. *P. tripectinis*: 1 male (9.7 mm) (NCHUZOOOL 15428), Dajia R. estuary, Taichung, Taiwan, 31 May 2014.

## RESULTS

### Morphology

The CWs of the holotypes of *Parasesarma liho* (SMF 36266) and *P. cognatum* (NMMBCD 3975), as well as the paratypes of *P. liho* (SMF 36269; MNHN B32312) used for description and/or drawing are 13.0 mm, 14.3 mm, 14.2 mm and 14.5 mm, respectively. Different sized specimens with CW from 4.8 to 16.7 mm of males ( $n = 19$ ) and 5.6 to 16.4 mm of females ( $n = 22$ ) were included in this study.

The distal part of the G1s of the specimens of *P. liho* and *P. cognatum*, with CW ranging from 11.1 to 16.7 mm, are quite similar in the form of the apical processes (Fig. 2A–E, G, H). The morphology of G1 of the holotype (SMF 36266) was examined and also agrees with other G1s shown in figure 2.

The length/width ratios of the P4 propodi are shown in table 2; the differences in the maximum and minimum ratios are large, ranging from 2.67 to 4.76, for different sizes of CW (7.0–16.7 mm), including the paratype of *P. liho* (SMF 36269 and MNHN B32312; ratios: 2.79 and 2.67, respectively; based on Koller et al. 2010: figs. 2f, 3c) and the holotype of *P. cognatum* (NMMBCD 3975; ratio: 2.79).

The chelar morphology of the types of *P. liho* and *P. cognatum* are similar, including the 11–12 elongate tubercles on the dorsal margins (Fig. 3A–D). The holotype of *P. cognatum* (NMMBCD 3975) was re-examined, confirming only a subdistal angle, not a spine, on the outer margin of the cheliped merus (Fig. 4).

For the coloration of *P. liho* in the field, the carapace is light brown with dark brown blotches, whereas the chelipeds and legs are uniform yellow to brownish yellow (Fig. 5).

The male paratype of *P. paucitorum* (ZRC 2019.0578) was also found to resemble other specimens of *P. liho* (and *P. cognatum*), but somewhat different

from the holotype of *P. paucitorum* (MZB Cru 2243), including the G1s (Fig. 2F, I), chelae (Fig. 3E–H), as well as in the fresh coloration of carapace and legs (Rahayu and Ng 2009: fig. 1).

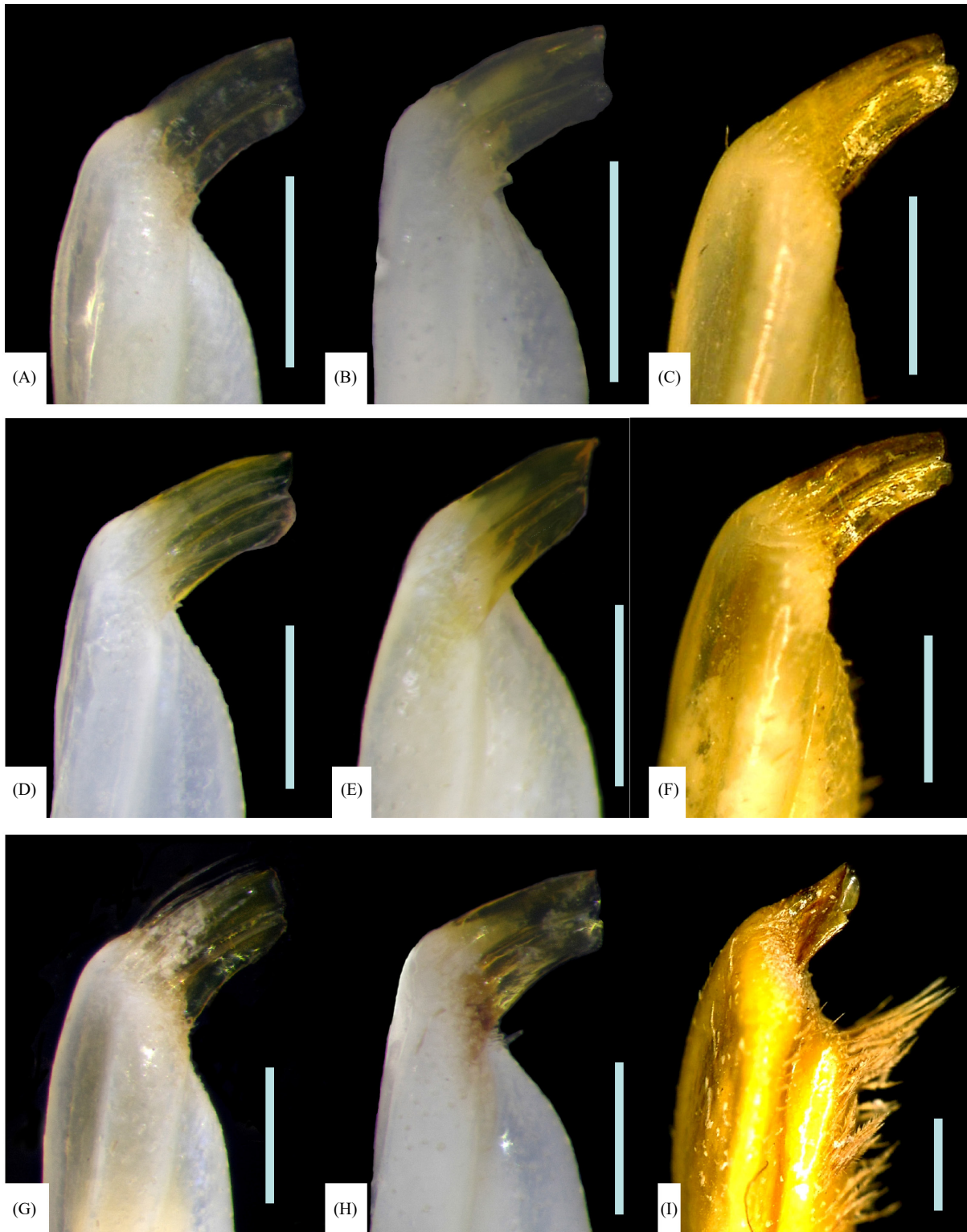
### DNA analysis

The molecular results comprised 22 *P. liho*-like and *P. cognatum*-like specimens, including the holotype and paratypes of *P. liho* and *P. cognatum* (Table 1). Available *COI* sequences for the holotype of *P. liho*, the paratype of *P. cognatum* and the male paratype of *P. paucitorum* were a bit shorter (614–635 bp see below), but otherwise identical to most other sequences with 658 bp. Therefore, they were omitted from further analyses. The male paratype of *P. paucitorum* (ZRC 2019.0578) has a similar genetic sequence as *P. liho* (see above) and is also referred to as being in the *P. liho* clade (see below). In total, three haplotypes of *P. liho* (including *P. cognatum*) are found from among the studied specimens (Table 1).

The reconstructed *COI* phylogenetic tree (Fig. 6) shows that the three haplotypes of *P. liho* (including the male paratype of *P. paucitorum*) form a distinct clade, sister to another clade including real *P. paucitorum* s. str. The mean pairwise nucleotide divergence with the K2P distances and bp differences of haplotypes of the two clades are shown in table 3. The intraspecific K2P nucleotide divergence within *P. liho* (including *P. cognatum*) is 0.05% (0–0.32%), and the interspecific K2P distance between *P. liho* and the *P. paucitorum* clade is 1.49% (1.46%–1.64%). Molecular data shows that the male paratype of *P. paucitorum* belongs to the *P. liho* clade and is different from the other types of *P. paucitorum* (Figs. 6, 7, Table 3). The haplotype network based on *COI* haplotypes (Fig. 7) further shows *P. liho* and *P. paucitorum* to be separated by 11 steps, and both species to be different from *P. kui* and *P. macaco* by 34–38 steps.

**Table 2.** The ratios of length/maximum width and length/minimum width of P4 propodi of specimens of *Parasesarma liho* with different size. The ratios of paratypes of *P. liho* was measured by Koller et al. (2010: figs. 2f, 3c). For abbreviations of museums and universities see MATERIALS AND METHODS

| CW (mm) | cat. no.                                      | Length / maximum width | Length / minimum width |
|---------|---|------------------------|------------------------|
| 7.0     | NCHUZOOL 15025                                | 3.19                   | 4.76                   |
| 11.1    | NCHUZOOL 15022                                | 2.89                   | 4.17                   |
| 11.6    | NCHUZOOL 15027                                | 2.81                   | 4.15                   |
| 11.6    | NCHUZOOL 15026                                | 2.67                   | 3.79                   |
| 14.2    | SMF 36269 (paratype of <i>P. liho</i> )       | 2.79                   | 4.01                   |
| 14.5    | MNHN B32312 (paratype of <i>P. liho</i> )     | 2.67                   | 3.88                   |
| 14.3    | NMMBCD 3975 (holotype of <i>P. cognatum</i> ) | 2.79                   | 4.17                   |
| 16.7    | NCHUZOOL 15031                                | 3.36                   | 4.53                   |



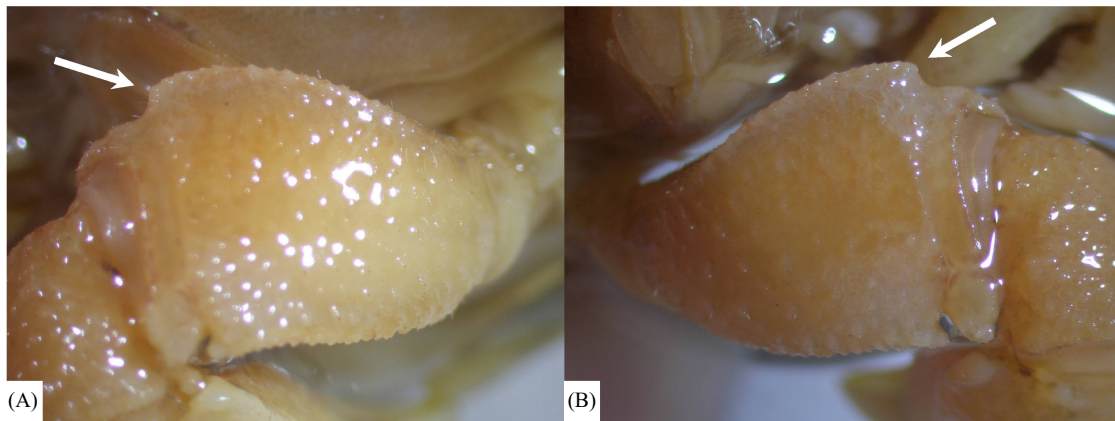
**Fig. 2.** The morphological variation of distal part the right G1s of *Parasesarma liho* (A–H), and *P. paucitorum* (I). A, CW 11.1 mm (NCHUZOO 15022), Pingtung, Taiwan; B, CW 11.6 mm (NCHUZOO 15027), Hualien, Taiwan; C, CW 12.7 mm (ZSM A20100040, paratype of *P. liho*), Hualien, Taiwan; D, CW 13.29 mm (NCHUZOO 15034), Cebu, Philippines; E, CW 14.3 mm (NMMBCD 3975, holotype of *P. cognatum*), Pingtung, Taiwan; F, CW 15.5 mm (ZRC 2019.0578, paratype of *P. paucitorum*), Sulawesi, Indonesia; G, CW 16.2 mm (NCHUZOO 15022), Pingtung, Taiwan; H, CW 16.7 mm (NCHUZOO 15031), Pingtung, Taiwan; I, CW 19.7 mm (MZB Cru 2243, holotype of *P. paucitorum*), Sulawesi, Indonesia. Scales bars = 0.5 mm.





**Fig. 3.** The morphology of left chela of types of *Parasesarma liho* (A–B), *P. cognatum* (C–D) and *P. paucitorum* (E–H). A, C, E, G, outer view; B, D, F, H, upper view. A, B, holotype of *P. liho* (CW 13.0 mm, SMF 36266); C, D, holotype of *P. cognatum* (CW 14.3 mm, NMMBCD 3975); E, F, paratype of *P. paucitorum* (CW 15.5 mm, ZRC 2019.0578); G, H, holotype of *P. paucitorum* (CW 19.7 mm, MZB Cru 2243). Scales bars = 2 mm.





**Fig. 4.** The outer surface of chelipedal meri of *Parasesarma cognatum* (holotype, NMMBCD 3975). A, left cheliped; B, right cheliped. Arrow indicates a subdistal angle on the upper margin of chelipedal merus.



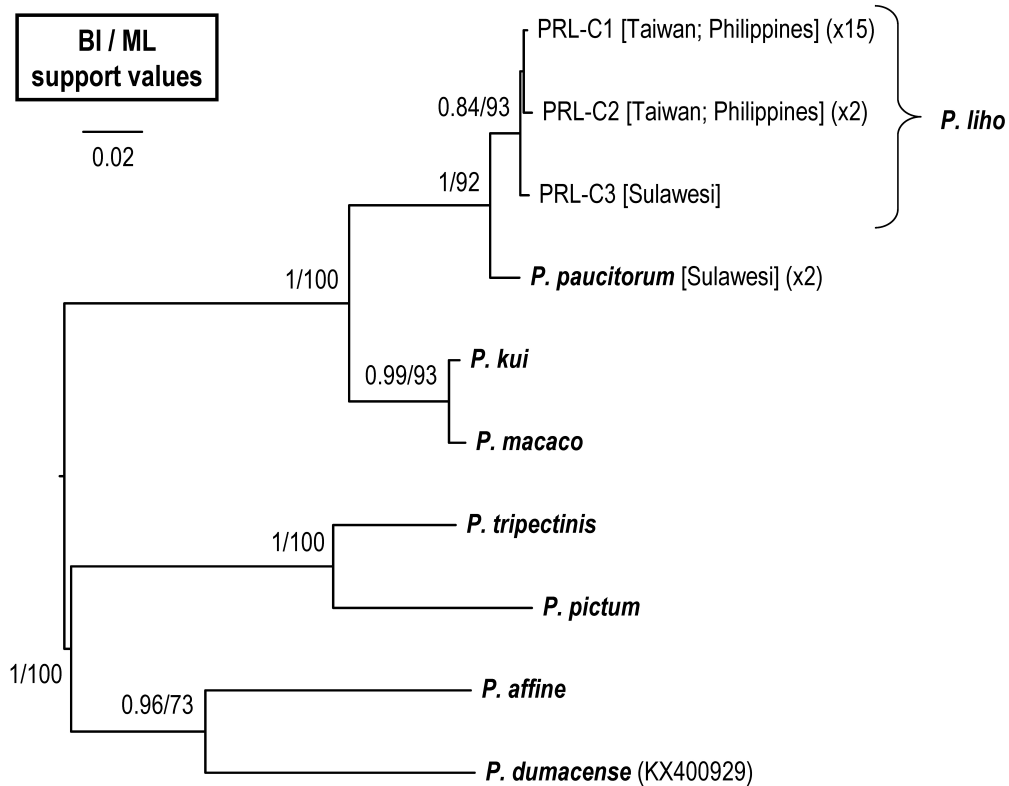
**Fig. 5.** The coloration of *Parasesarma liho* in the field in Taiwan. A, specimen (not captured) from Gangkou R. estuary, Pingtung; B, specimen (not captured) from Meilun R. estuary, Hualien.



**DISCUSSION**

In this study, we confirm that *Parasesarma cognatum* Rahayu & Li, 2013 is a synonym of *Parasesarma liho* Koller, Liu & Schubart, 2010 based on the evidence from morphology (Figs. 2–4, Table 2) and mitochondrial DNA (Figs. 6–7, Table 3).

The main differences between the two species was supposedly the morphology of G1 and the ratio of length/width of P4 propodus (Rahayu and Li 2013). The apical processes of the presently examined G1s are quite different from that of the paratype of *P. liho* (SMF 36269), which has a very tapering tip (Koller et al. 2010: fig. 2e). After comparing the line drawings of



**Fig. 6.** A Bayesian inference (BI) tree of *Parasesarma liho*, as well as the outgroups, based on the cytochrome oxidase subunit I genes (*COI*). Probability values at the nodes represent support values for BI and maximum likelihood (ML). For haplotype names, see table 1.

**Table 3.** Matrix of percentage pairwise K2P nucleotide divergences (lower left) and mean number of differences (upper right) based on *COI* within the clade of *Parasesarma liho* (including types of *P. cognatum* and the male paratype of *P. paucitorum*) and other related species (see Table 1). Values of range are shown in parentheses

|                      | Intraspecific         |                            | Interspecific       |                      |                     |                  |
|----------------------|-----------------------|----------------------------|---------------------|----------------------|---------------------|------------------|
|                      | Nucleotide divergence | Mean nucleotide difference | <i>P. liho</i>      | <i>P. paucitorum</i> | <i>P. kui</i>       | <i>P. macaco</i> |
| <i>P. liho</i>       | 0.05<br>(0-0.32)      | 0.32<br>(0-2)              |                     | 9.17<br>(9-10)       | 33.11<br>(33-34)    | 35.11<br>(35-36) |
| <i>P. paucitorum</i> | 0                     | 0                          | 1.49<br>(1.46-1.64) |                      | 33<br>(33-33)       | 33<br>(33-33)    |
| <i>P. kui</i>        |                       |                            | 5.26<br>(5.22-5.58) | 5.5<br>(5.5-5.5)     |                     | 4 (4-4)          |
| <i>P. macaco</i>     |                       |                            | 5.6<br>(5.56-5.94)  | 5.5<br>(5.5-5.5)     | 0.61<br>(0.61-0.61) |                  |

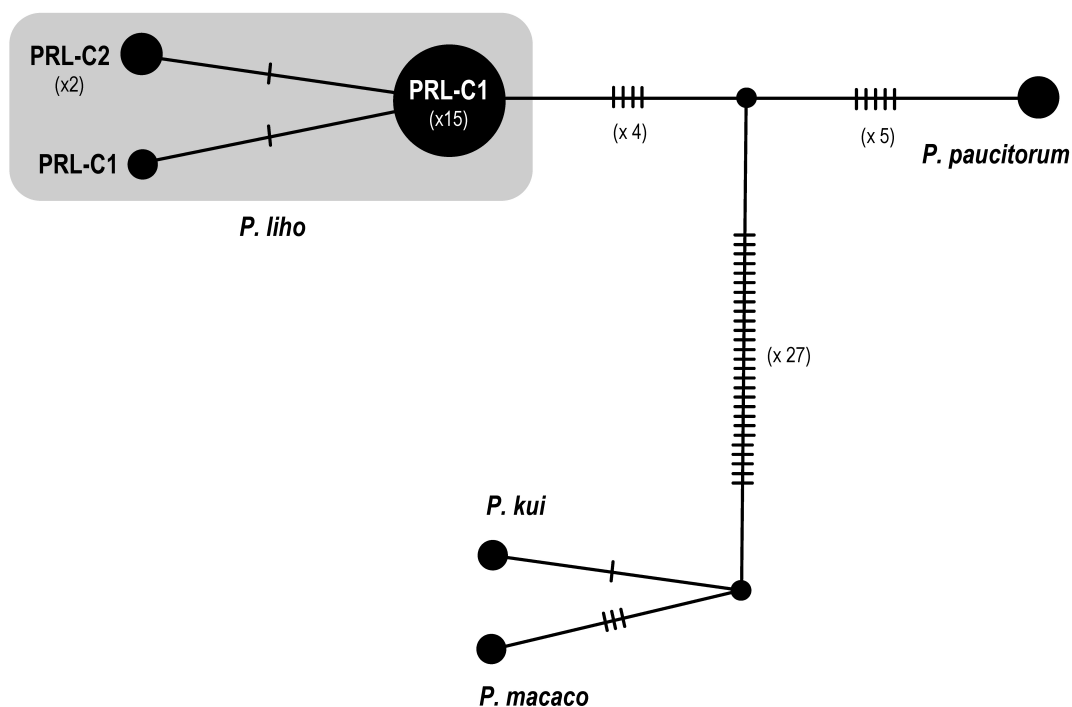
G1 in Koller et al. (2010), the tapering tip of the apical process in figure 2e (left) was likely due to breakage before or after it was denuded because it appeared broader when it was still with setae (Fig. 2d); which agrees with the specimens examined (Fig. 2) and the holotype of *P. cognatum* (Rahayu and Li 2013: fig. 7A-D). The morphology of both G1 structures of the holotype (SMF 36266) and the other paratypes (see Material examined) were also examined and agrees with the other G1s of this species (see Fig. 2).

Rahayu and Li (2013) mentioned the length/width ratio of P4 propodi in *P. liho* being 2.8 and that of *P. cognatum* 3.6, but our ratios for the paratype of *P. liho* are 2.79 and 2.67 (based on Koller et al. 2010: figs. 2f, 3c); and the holotype of *P. cognatum* is 2.79 (Table 2). The different values are probably caused by different positions used for measurement by different authors (see Shih and Do 2014), but the values are still variable, even if measured in a standardised manner (Table 2). The character of the length/width ratio of P4 propodi (Koller et al. 2010; Rahayu and Li 2013; Maenosono and Naruse 2015) is here considered not useful to distinguish the two species.

Rahayu and Li (2013: 637) described the merus of the chelipeds of *P. cognatum* as “outer margin tuberculate, with large subdistal spine”, but this character was not clearly shown in the corresponding figures. Maenosono and Naruse (2015: 22, fig. 7B)

identified the species in the Ryukyus as *P. liho* because the specimens did not have such a large subdistal spine on the upper (= outer) margin of the merus (Koller et al. 2010: fig. 2a). In our study, it is clear that only a subdistal angle (not really a spine) is present on the upper margin of the merus of the holotype of *P. cognatum* (Fig. 4), a character that agrees with other specimens of *P. liho* (see Koller et al. 2010; Maenosono and Naruse 2015).

In the phylogenetic tree and haplotype network (Figs. 5, 6), only three haplotypes with only 1 or 2 bp difference were found in the clade of *P. liho*, including the haplotypes identical to the shorter sequences of the types of *P. liho* and *P. cognatum*. This supports the hypotheses that the two species should now be considered as conspecific. The two trees (Figs. 5, 6) also show that the male paratype of *P. paucitorum* should be identified as *P. liho*. The minimum interspecific divergence between the *P. liho* clade and the sister species, *P. paucitorum*, is 1.49%, which is small compared with those of most families in the Thoracotremata (Varunidae, Mictyridae, Ocypodidae, Dotillidae and Sesarmidae; see Chu et al. 2015). The small interspecific divergence between *P. liho* and *P. paucitorum* implies that these species are quite young, with an estimated divergence time of 0.88 mya (with an uncorrected *p*-distance divergence of 1.47%, based on the substitution rate of 1.66% per millions of years



**Fig. 7.** Genealogical network for the *COI* haplotypes observed within the clades of *Parasesarma liho* and other related species. Unlabelled hatches indicate inferred haplotypes not found in the sampled population. For haplotype names, see table 1.

for *COI* for marine *Sesarma* in Schubart et al. 1998). The possibility remains that the small interspecific divergence could also be caused by mitochondrial introgression (e.g., Cannicci et al. 2017), but this needs further study by using nuclear evidence.

Koller et al. (2010) mentioned that *P. liho* is morphologically similar to *P. paucitorum* Rahayu & Ng, 2009 (type locality Manado, Sulawesi, Indonesia). The holotype and male paratype of *P. paucitorum* are, however, quite differently coloured (Rahayu and Ng 2009: figs. 1–2), with the colour of the paratype being very close to that of *P. liho* (Fig. 4; see Li and Chiu 2013: 82; Rahayu and Li 2013: fig. 4; Maenosono and Naruse 2015: figs. 2F–H, 8). Our study finds that the male paratype of *P. paucitorum* (ZRC 2019.0578) actually belongs to *P. liho* instead (Table 3, Figs. 5, 6). The holotype and paratype female of *P. paucitorum* remain genetically different.

Even if the interspecific genetic distance is small, adult *P. liho* and *P. paucitorum* can still be distinguished by the chelae (Fig. 3A–F vs. Fig. 3G–H), G1s (Fig. 2A–H vs. Fig. 2I), as well as the coloration of the adult carapace and ambulatory legs (Fig. 5 vs. Rahayu and Ng 2009: fig. 1A). That these two closely allied species occur together in one location in Sulawesi is somewhat surprising (all the specimens of *P. paucitorum* were collected together at the same time), but not without precedence in other sesarmids.

As *P. cognatum* is from now on synonymized with *P. liho*, and the male paratype of *P. paucitorum* also turned out to belong to *P. liho*, the updated distribution of *P. liho* is Japan (Okinawa, Miyako and Ishigaki), Taiwan (Hualien, Taitung, Pingtung), Philippines (Cebu) and Indonesia (Sulawesi).

## CONCLUSIONS

In our study, the type specimens of *Parasesarma liho* and *P. cognatum* were re-examined and their main characters were found to be identical, which are also supported by the evidence from *COI*. *Parasesarma cognatum* is treated as a junior subjective synonym of *P. liho* accordingly. In addition, the male paratype of *P. paucitorum* is confirmed to be conspecific with *P. liho*. As a result, the updated distribution of *P. liho* is Japan (Ryukyus), Taiwan, Philippines (Cebu) and Indonesia (Sulawesi).

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**Authors' contributions:** HTS conceived this study, the molecular analysis, and drafted the manuscript. PYH and AS performed the morphological comparison, measurements and the molecular work. CDS contributed to the discussion and drafted the manuscript. JJJ collected and processed the samples, performed the ecological observation, and drafted the manuscript. All authors read and approved the final manuscript.

**Competing interests:** The authors declare that they have no conflict of interest.

**Availability of data and materials:** Sequences generated in the study have been deposited into the DNA Data Bank of Japan (DDBJ) database (accession numbers in Table 1 in manuscript).

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**Ethics approval consent to participate:** Not applicable.

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