A newly recorded species of *Cheiracanthium* C. L. Koch, 1839 (Araneae, Cheiracanthiidae) from Taiwan

臺灣產紅螯蛛屬蜘蛛之新紀錄種

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Abstract

This paper provides a morphological description of a newly recorded species from Taiwan, *Cheiracanthium insigne* O. Pickard-Cambridge, 1874, based on newly collected materials. Both male and female *C. insigne* specimens are analysed, redescribed, and photographed. We applied DNA barcoding validation for matching sexes and reviewed taxonomic issues of *C. insigne* with other related species. The results of maximum likelihood tree and Kimura two-parameter genetic distance confirm the correct matching of male and female *C. insigne* specimens from Taiwan (mean intraspesific distance as 1.15%). In addition, the phylogenetic analysis suggests that previous female specimens of *Cheiracanthium triviale* from Assam, India, could potentially be conspecific to *C. insigne* from Taiwan.

Key words: *Cheiracanthium insigne*; Cheiracanthiidae; new record; Taiwan

摘要

本報告依新採集標本及形態檢視，報告台灣產紅螯蛛屬蜘蛛之新紀錄種－短突紅螯蛛(*Cheiracanthium insigne* O. Pickard-Cambridge, 1874)。雌、雄性皆重新描述及拍攝，同時運用基因條碼驗證本種正確之性別配對，並回顧與近緣種的分類問題。由maximum likelihood親緣關係樹及以K2P model計算之遺傳距離結果(平均種內遺傳距離為1.15%)皆驗證台灣產短突紅螯蛛的雌雄配對之正確性，並支持在過去研究中採集於印度阿薩姆的平庸紅螯蛛(*C. triviale*)雌蛛樣本應屬於本種。

關鍵詞：短突紅螯蛛、紅螯蛛科、新紀錄、台灣

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Introduction

Cheiracanthium C. L. Koch, 1839, the long-legged sac spiders, is the largest genus of Cheirachanthiidae Wagner, 1887 and distributed worldwide (World Spider Catalog 2020). They are small to medium-sized spiders and generally yellowish in body

coloration. Long-legged sac spiders can be identified by the following characters: the leg I is conspicuously the longest, the male palp with well-develop papal tibial apophysis and distinctive backward-direction basal retrolateral spur on the cymbium, and the female epigyne with a central or posterior depression (Chen and Huang 2012).

To date, there are 218 valid species of the genus *Cheiracanthium* reported globally. However, the actual diversity of the genus remains unclear, and several new species from Asia were described recently (Barrion *et al*. 2013; Wang and Zhang 2013; Marusik and Fomichev 2016; Zhang *et al*. 2018; Li and Zhang 2019; Zhang *et al*. 2020). Yaginuma (1970) first noted *C. lascivum* Karsch, 1879 in Taiwan. A total of nine valid species of long-legged sac spiders have been reported successively (Chen and Huang 2004; Chen *et al*. 2006; Chen and Huang 2012). Recently, a newly recorded species, *Cheiracanthium insigne* O. Pickard-Cambridge, 1874, was discovered in Taiwan. The male *C. insigne* is distinguishable from congeneric species using the structure of palp organs, which have extremely well-developed tegular flanges. However, the identification of female is still unconfirmed.

The morphology of female *C. insigne* was first reported by O. Pickard-Cambredge (1874) in India, then later noted in another study using samples from Myanmar (Gravely 1931). However, the delineation of epigyne was conspicuously inconsistent. Dankittipakul and Beccaloni (2012) examined the holotype of *C. truncatum* and supposed that the specimen of *C. insigne* examined by Gravely (1931) was actually *C. truncatum* because they could not find distinct differences between female genitalia of these two species. Therefore, the problem of female *C. insigne* identification remained unsolved. Due to cryptic habitat and the resembling appearances of *Cheiracanthium* species, to affirm correct sex-match is one of the challenges for taxonomic studies on the genus. For example, almost half of *Cheiracanthium* species are described from single sex: 38 species from males and 59 species from females (World Spider Catalog 2020). Therefore, the application of the molecular tools such as DNA barcoding is valuable for correct sex matching.

Hebert *et al*. (2003) proposed the common use of cytochrome c oxidase subunit 1 (*COI*) sequences on the mitochondrial gene in DNA barcoding techniques, which enable species identification in most animal phyla. The application of *COI* barcodes has been widely adapted in various animal groups and research purposes (Kress *et al*. 2015), such as species-level identification in immature

stages of rolled-leaf bettles (Garcia-Robledo *et al.* 2013), species identification in illegal trades or highly processed animal materials (Gonçalves *et al.* 2015; Janjua *et al.* 2017), the solution to certain taxonomic issues or the discovery of cryptic butterfly species (Hebert 2004), diet analysis for the ocean sunfish (Sousa *et al.* 2016), and the delimitation of species boundaries in Acridoidea (Huang *et al.* 2013). Similarly, DNA barcoding of spiders has been commonly used in taxonomic studies and relevant research in species identification or delimitation (Barrett and Hebert 2005; Candek and Kuntner 2015; Xu *et al.* 2015; Cao *et al.* 2016; Coddington *et al.* 2016). As morphological variation of spiders can be dependent on sex and life history stages, DNA barcode approach is effective to match sexes, life stages, or distinguish species with similar morphologies, which has been successfully used to rectify a previously mismatched male specimen in *Micrathena* genus in Brazil (Robinson *et al.* 2009; De Alvarenga *et al.* 2020). Also, it has ably overcome extent challenges of taxonomic and phylogenetic obstacles (Blagoev *et al.* 2016; Breitling 2019). Thus, the efficacy of DNA barcoding analysis illustrated its usefulness to correctly macth sexes and identify species even if there was a lack of information on diagnostic features for one sex or with few specimens avialable.

This study adds a new record of spider *Cheiracanthium insigne* for the Taiwanese spider fauna using both male and female specimens, assessed by morphological characeteristics and through *COI* gene-based DNA barcoding validation to match sexes.

Materials and Methods

Sample collection and morphological examination

Specimens were preserved in 75% ethanol and deposited in the collection of Taiwan Endemic Species Research Institute (TESRI). The selected specimens were dissected to examine the palpal organs of the males and the epigyne of the females. To dissect the genital organs of the females, epigyna and inner genital structures were cleaned in the protein removal solution. Photographs were taken with a digital camera (Nikon D850), using a stereo microscope (Leica M125). For each specimen, Helicon Focus version 6 (Helicon Soft Ltd) was used for focus stacking. Scale bars were labeled on the digital images using ImageJ version 1.52k (National Institutes of Health) and Photoshop version 19.1.2 (Adobe Systems Corporation). All measurements of morphological structures were given in millimeters (mm), obtained by

a micrometer mounted on the eyepiece of the stereo microscope. The measurements of the pedipalp and leg were given as the total length (pedipalp: femur, patella, tibia, and tarsus lengths; leg: femur, patella, tibia, metatarsus, and tarsus lengths). Abbreviations addressed in this paper include: **A**, atrium; **AER**, anterior eye row; **ALE**, anterior lateral eye; **AME**, anterior median eye; **AW**, anterior width of MOA; **C**, conductor; **CD**, copulatory duct; **CS**, cymbial spur, **E**, Embolus; **MOA**, median ocular area; **MOA-L**, length of MOA; **PER**, posterior eye row; **PLE**, posterior lateral eye; **PME**, posterior median eye; **PW**, posterior width of MOA; **RTA**, retrolateral tibial apophysis; **S**, spermatheca; **TF**, tegular flange.

DNA extraction, PCR amplification, and sequencing

Tissues collected from one to four legs of the specimens were preserved in 95% ethanol and deposited in -20°C refrigerator until DNA extraction. DNA barcodes were subsequently obtained for molecular analyses and sex matching of conspecific. The partial cytochrome c oxidase subunit I gene (*COI*) was amplified using the primer pairs of LCO 1490 (Folmer *et al*. 1994) and Chelicerate R2 (Barrett and Hebert 2005). The polymerase chain reaction (PCR) protocol was as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 sec, annealing at 45 °C for 30 sec, and elongation at 72 °C for 40 sec; and a final extension at 72 °C for 5 min. The 25-μL PCR reactions included 9.5 μL of double-distilled H_2O, 1 μL of each forward and reverse primer (10 μM), 1 μL of DNA template, and 12.5 μL *Taq* DNA Polymerase 2× Master Mix RED (with 1.5 mM $MgCl_2$ final concentration; Ampliqon, Herlev, Denmark). The PCR products were visualized by agarose gel electrophoresis (1% agarose). PCR products were sequenced by Genomics BioSci & Tech. Co., Ltd. (New Taipei City, Taiwan) using an ABI 3730xl DNA Analyzer.

Molecular data analysis and phylogenetic inference

The DNA sequences were trimmed and edited by Sequencher v.5.4.5 (DNA sequence analysis software, Gene Codes Corporation), then aligned with ClustalW in BioEdit v.7.0.5.3 (Hall 1999). For molecular analysis, the *COI* sequences of a closely related species, *Cheiracanthium triviale* (Thorell, 1895), were downloaded from GenBank (Benson *et al*. 2012) which were provided in Tyagi *et al.* (2019, 2020) as its epigynal characters were similar to *C. insigne* based on preliminary specimen

examination. To identify haplotypes and polymorphic sites, DnaSP v.6.12 (Rozas *et al*. 2017) was utilized, and the pairwise genetic distances (Kimura two-parameter [K2P]) were calculated using MEGA 7 (Kumar *et al*. 2016) to assess the genetic differences. Phylogenetic tree was reconstructed using maximum likelihood (ML) with GTR+G model, which was determined as the best-fit nucleotide substitution model using jModelTest v.2.1.7 (Darriba *et al.* 2012) under the Bayesian information criterion (BIC). Maximum likelihood analysis was conducted in RAxML v.8.2.10 (Stamatakis 2006), and the robustness was evaluated by 1000 bootstrap pseudo replicates. Ultimately, the obtained phylogenetic tree was visualized and edited using FigTree v.1.4.4 (Rambaut 2018) and Illustrator version 22.1 (Adobe Systems Corporation, San Jose, CA, USA).

Results

Taxonomy

Cheiracanthium insigne O. Pickard-Cambridge, 1874

(Figures 1–2)

Cheiracanthium insigne O. Pickard-Cambridge 1874: 408, Pl. 52, fig. 32; Gravel 1931: 266, fig. 17L; Tikader and Biswas 1981: 70, Pl. IX, fig. 122; Chen and Zhang 1991: 252, fig. 264; Majumder and Tikader 1991: 60, fig. 113-116; Song, Zhu and Chen 1999: 413, fig. 243K-L; Dankittipakul and Beccaloni 2012: fig. 1, 3-7; Dhali *et al* 2017: fig. 190-195.

Eutittha gracilipes Thorell, 1895: 47.

Examined Materials. All specimens were obtained from Taiwan. **Chiayi County**: one female (TESRI-LT047), Lantan Trail, 2018-03-17, Ying-Yuan Lo leg. **Kaohsiung City**: one female (TESRI-C020126), Shoushan National Nature Park, 2017-06-10, one male (TESRI-C020159), Shoushan National Nature Park, 2017-08-12, one female (TESRI-C020171), Shoushan National Nature Park, 2017-11-04, one male (TESRI-C030145), Shoushan National Nature Park, 2017-08-04, one male (TESRI-CS031), Shoushan National Nature Park, 2018-08-03, Guo-Yuan Wu leg. **Yilan County**: one male (TESRI-Ar4714), North Guanyin Tunnel Entrance, 2020-03-02, Chi Wei leg.; one female (TESRI-Ar4722), Dongyue, 2020-03-03, Chi Wei leg. **Taitung County:** one male (TESRI-Ar2540), 2017-09-20, Fuyuan Village, Ying-Yuan Lo leg. (Figure 3).

Diagnosis. *Cheiracanthium insigne* can be distinguished from other congeneric

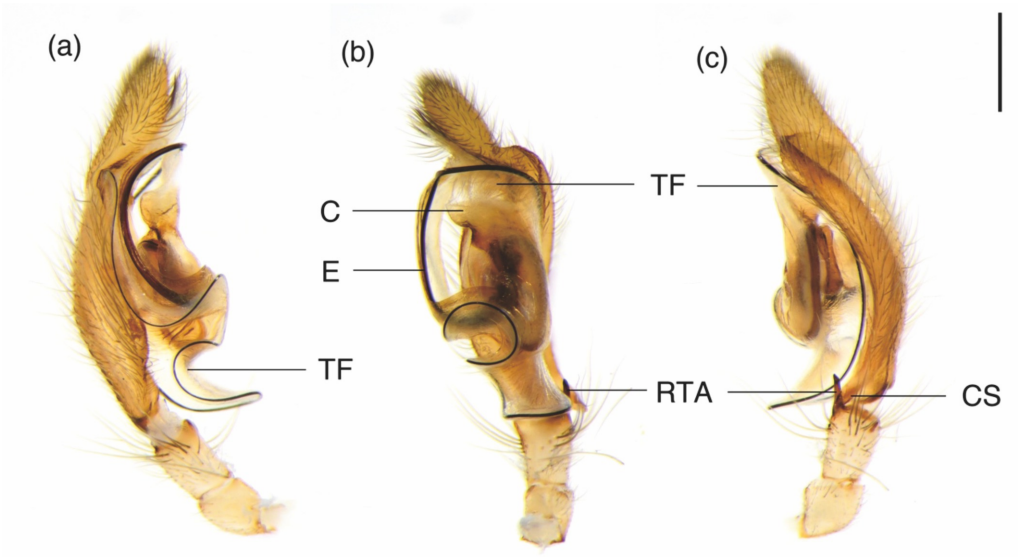

Fig. 1. *Cheiracanthium insigne*, male (TESRI-C030145). (a)–(c) are prolateral, ventral, and retrolateral views of palp organ, respectively. Scale bar: 0.5mm.

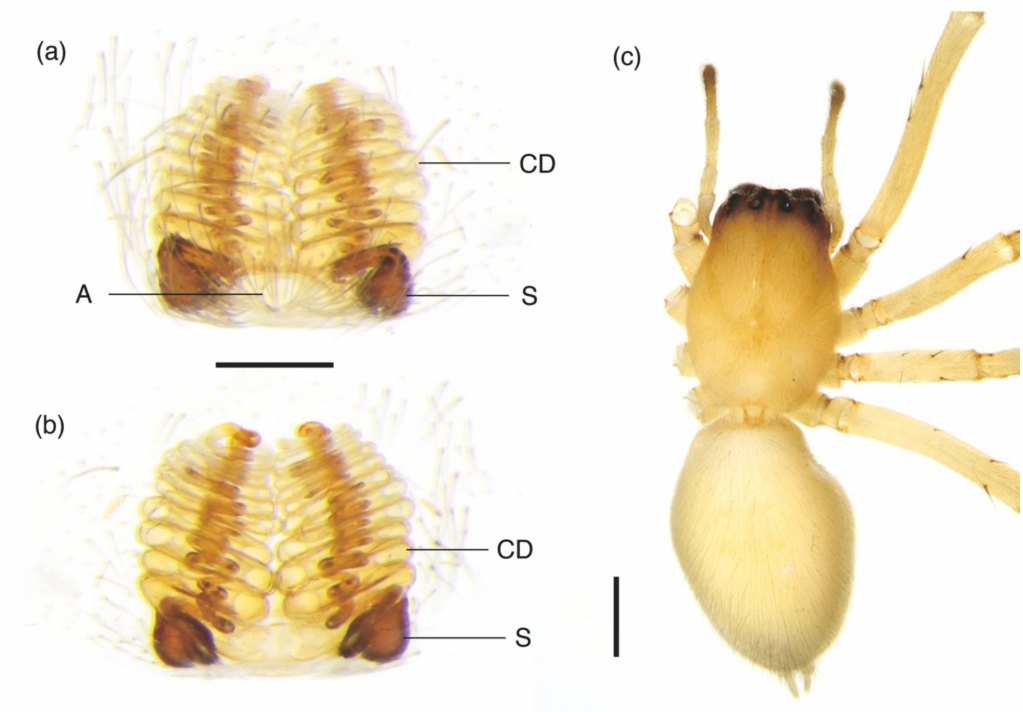

Fig. 2. *Cheiracanthium insigne*, female (TESRI-C020171). (a)–(b), ventral and dorsal view of epigynum. (c) dorsal view of appearance. Scale bars: (a)–(b) 0.2mm; (c) 1.0mm.

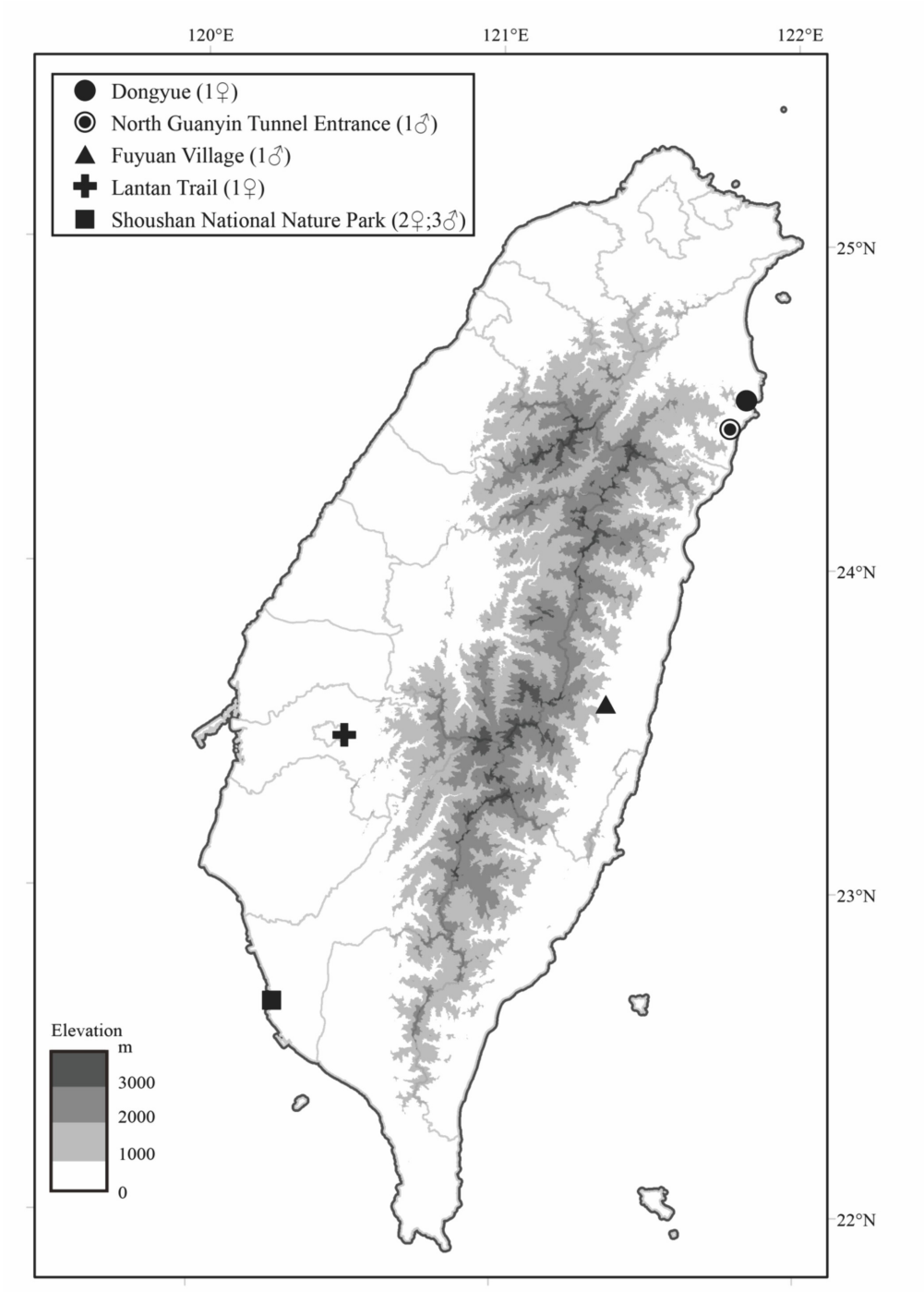

Fig. 3. Collection localities of *Cheiracanthium insigne* from Taiwan in the present study.

species by the following characteristics: (1) tegular with enormously developed and semi-transparent flange on the bulb; (2) embolus long and curved along with tegular flange; (3) basal retrolateral cymbial spur is rudimentary; (4) epigyne with a pair of elongated and entwined copulatory ducts on the anterior of spherical spermathecae.

Description. *Male* (TESRI-C030145). Total length 5.0; carapace length 2.5, width 1.8; abdomen length 2.5, width 1.4. Carapace yellowish with indistinct cervical and radical grooves. Ocular area light brown, eight eyes are arranged in two rows with AER slightly procurved and PER straight. Eye diameters and inter-distances (mm): AME = ALE = PME = PLE = 0.16; MOA-L 0.36, MOA-AW 0.40, MOA-PW 0.52; AME-I 0.14, PME-I 0.20, AML-I 0.10, PML-I 0.12; clypeus height 0.10. Chelicera light brown, with three promarginal teeth and three retromarginal teeth; endite longer than wide, and labium equal in length and width. Sternum yellowish and posterior margin slightly convex. Pedipalps and legs yellowish; the third and fourth posterior metatarsus bear a coarser, distal curve spine on the ventral side. Measurements of pedipalps and legs (mm): Measurements of pedipalps and legs (mm): palp 4.0 (1.3, 0.4, 0.4, 1.9), leg I 15.6 (3.7, 1.1, 4.2, 4.6, 2.0), leg II 9.6 (2.6, 0.9, 2.5, 2.7, 0.9), leg III 7.1 (1.7, 0.8, 1.6, 2.3, 0.7), leg IV 10.6 (2.6, 1.0, 2.7, 3.4, 0.9). Leg formula: I > IV > II > III. Abdomen yellowish and elongate-oval. Palp with a straight retrolateral tibia apophysis. Cymbium folded obviously in ventral and retrolateral view, and basal retrolateral cymbial spur indistinct. Tegulum with a well-developed, conspicuous, and translucent membranous flange. Tegulum apophysis absent. Embolus slender, curved, and elongated along the margin of the tegulum flange (Figure 2).

Female (TESRI-C020171). Total length 5.2; carapace length 2.7, width 1.9; abdomen length 3.5, width 2.3. Carapace yellowish with indistinct cervical and radical grooves. Ocular area dark brown, eight eyes are arranged in two rows with AER slight procurve and PER. Eye diameters and inter-distances (mm): AME = ALE = PME = PLE = 0.14; MOA-L 0.34, MOA-AW 0.48, MOA-PW 0.56; AME-I 0.16, PME-I 0.28, AML-I 0.18, PML-I 0.28; clypeus height 0.06. Chelicera dark brown, with three promarginal teeth and three retromarginal teeth; endite longer than wide, and labium equal in length and width. Sternum yellowish and the posterior margin slightly convex. Pedipalps and legs yellowish. Measurements of pedipalps and legs (mm): Measurements of pedipalps and legs (mm): palp 3.2 (1.1,

0.4, 0.6, 1.1), leg I 12.6 (3.2, 1.1, 3.3, 3.5, 1.5), leg II 7.8 (2.2, 0.9, 1.9, 2.1, 0.7), leg III 6.0 (1.6, 0.8, 1.3, 1.7, 0.6), leg IV 9.2 (2.5, 0.9, 2.3, 2.7, 0.8). Leg formula: I > IV > II > III. Abdomen yellowish and elongate-oval. Epigyne with an oval-shaped atrium. Spermatheca and copulatory ducts are visible through the cuticle. A pair of copulatory ducts elongated and entwined in ten loops, and situated anteriorly to round spermatheca (Figure 3).

Distribution. China, India, Laos, Myanmar, Sri Lanka, Thailand, Taiwan (new record).

DNA Barcode

The barcode dataset in this study contains 37 sequences of five putative species in genus *Cheiracanthium*, including *C. eutittha*, *C. insigne, C. falcatum*, *C. insulanum* and *C. taiwanicum*, which were obtained from Taiwan; *C. triviale* and three outgroups were acquired from open data source (Appendix 1). The *COI* database contains 573 base pairs (bp) with 168 variable sites, 126 parsimony informative sites, and 28 haplotypes.

The Maximum likelihood phylogenic tree agreed with our previous assumption that our collected male and female

Table 1. The COI divergence with K2P genetic distances of *Cheiracanthium*. N means sample size. Intraspecific genetic distances are shown as: percentage of mean distance (range of distance). Within the matrix of pairwise interspecific genetic distances, lower left indicates mean distance and upper right indicates the range of distance

Species	N	Intraspecific genetic distance (%)	Interspecific genetic distance (%)					
			C. insigne	*C. eutittha*	*C. falcatum*	*C. insulanum*	*C. taiwanicum*	*C. trivale*
C. insigne	9	1.15 (0.00–1.43)	–	13.40–15.10	11.02–11.48	10.78–13.15	7.91–9.22	0.71–4.67
C. eutittha	4	1.49 (0.53–2.17)	14.03	–	6.86–7.70	10.10–11.48	11.04–12.43	13.88–15.36
C. falcatum	2	0.18	10.40	7.22	–	9.22–11.01	9.47–10.13	11.49–12.89
C. insulanum	5	1.15 (0.18–2.00)	12.01	10.84	11.19	–	10.11–11.94	11.24–14.11
C. taiwanicum	7	0.33 (0.00–0.71)	8.52	11.71	9.83	11.18	–	8.34–9.88
C. trivale	7	1.95 (0.00–4.09)	3.32	14.67	12.37	13.01	9.28	–

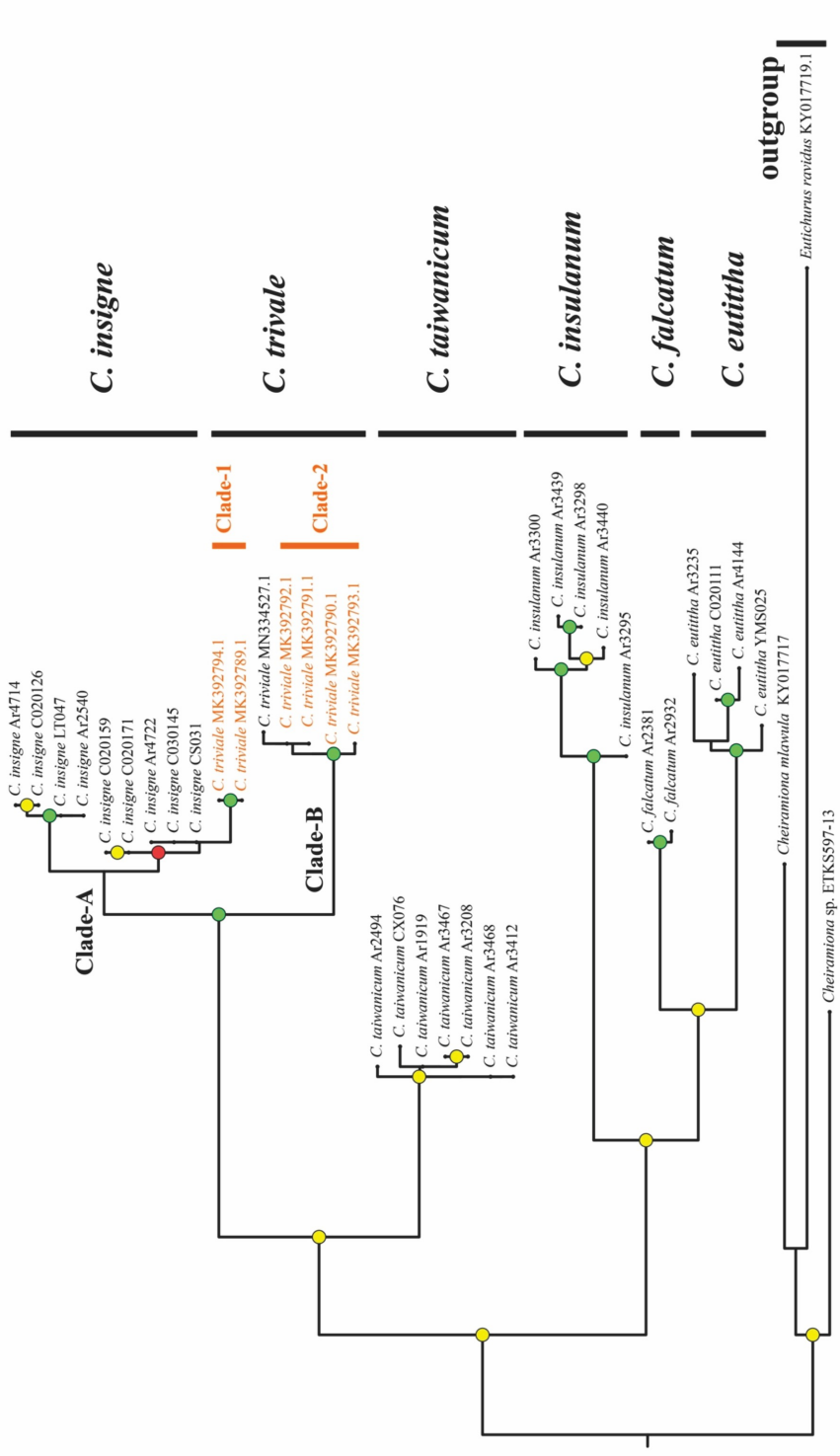

Fig. 4. Maximum likelihood (ML) phylogenetic tree of genus *Cheiracanthium* and outgroups based on the COI dataset. Colored circles at the nodes represent the bootstrap values with different levels of support: > 50% (red), >70% (yellow), and >90% (green). Organ color tips were samples collected from India that were separated into two distinct clades: clade-1 (Assam) and clade-2 (Gujarat) in Tyagi *et al*. (2019).

specimens, which we assumed as *C. insigne* from Taiwan, are conspecific. Moreover, the topology indicated that *C. insigne* and *C. triviale* belong to one clade with a high support value (bootstrap value = 100), which contains two main subclades: clade-A comprises *C. insigne* and two *C. triviale* samples, and clade-B comprises the remaining *C. triviale* samples. The mean intraspecific and interspecific *COI* genetic distances of *Cheiracanthium* in the present dataset are 0.33%–1.95% and 3.32%–14.67%, respectively (Table 1). Almost minimum interspecific distance is much greater than maximum intraspecific distance for all species. However, the mean interspecific distance between *C. insigne* and *C. triviale* is relatively lower (3.32%, ranging from 0.71%–4.67%).

Disccusion

Although the male *Cheiracanthium insigne* has been reported several times and can be distinguished from other congeneric species, the identification of female remains problematic (Dankittipakul and Beccaloni 2012). According to the examined samples and barcoding information, we clarified the identification of female *Cheiracanthium insigne*. The features of female genitalia organ of *C. insigne,* with a pair of spiral and entwisted insemination ducts on the anterior of spherical spermathecae, are incongruent with which was delineated by Gravely (1931), but fairly similar to that of *C. triviale* and *C. rupicola* (Gravely 1931; Dankittipakul and Beccaloni 2012; Liu *et al*. 2019; Tyagi *et al*. 2019). Contrary to *C. insigne*, there were only two reports which examined both sexes of *C. triviale* simultaneously (Gravely 1931; Majumder and Tikader 1991). Dankittipakul and Beccaloni (2012) examined both specimen types of female *C. triviale* and female *C. rupicola*, suggesting that *C. triviale* can be distinguished by insemination ducts with ten entwisted loops and epigynal atrium with clearly defined elongate-oval aperture, whereas insemination ducts with seven entwisted loops and poorly defined epigynal aperture in *C. rupicola*. They also proposed that the “Himalaya” form of female *C. triviale*, formerly illustrated by Gravely (1931), was *C. rupicola*. However, the female *C. insigne* was not examined in the report.

Tyagi *et al*. (2019) analyzed DNA barcoding of spiders from India and found that *C. triviale* could be separated into two distinct clades: clade-1 specimens (two females) from Assam and clade-2 specimens (four females) from Gujarat, India. In this study, we used the same sequences of *C.*

trivale and combined with the dataset of *Cheiracanthium* species from Taiwan. The ML tree shows that *C. insigne* and *C. triviale* belong to one clade with two subclades: clade-A comprises *C. insigne* and *C. triviale* samples from Assam, and clade-B comprises *C. triviale* samples from Gujarat (Figure 4). The mean genetic distance between these two subclades is 4.11%, which is much greater than the mean genetic distance within each subclade (0.91% in clade-A and 0.25% in clade-B). Our results agree with Tyagi *et al*. (2019) that *C. triviale* from two regions of Inida present a clear genetic differentiation, which was defined as two molecular operational taxonomic units (MOTUs) based on multiple species delimitation methods. Accordingly, we suspect that the previously collected specimens of *C. triviale* from Assam are conspecific to *C. insigne* from Taiwan; however, the original samples from Assam would be required for further confirmation of species determination.

On the other hand, both male *C. insigne* and *C. triviale* bear well-developed membranous tegular flange on the palpal organs, but male *C. insigne* can be differentiated by the former with downward elongate membranous flange and narrower anterior cymbium (Majumder and Tikader, 1991). Though male *C. insigne* is distinguishable with congeneric species, the identification of female has been problematic for decades. This study examined both sex specimens of *C. insigne* and applied DNA barcoding information to correct sex matching for the first time, and highlighting the significance of integrated morphological and molecular information for further taxonomic research in *Cheiracanthium* species.

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Reference

Barrett, R. D. H. and P. D. N. Hebert. 2005. "Identifying spiders through DNA barcodes". *Canadian Journal of Zoology* 83: 481–491.

Barrion, A. T., A. L. A. Barrion-Dupo, J. L. A. Catindig, S. C. Villareal, D. Cai, Q. Yuan and K. L. Heong. 2013. "New species of spiders (Araneae) from Hainan Island, China". *UPLB Museum Publications in Natural History* **3**:

1–103.

Benson, D. A., M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell and E. W. Sayers. 2012. GenBank. *Nucleic Acids Research* 41: D36–D42.

Blagoev, G. A., J. R. deWaard, S. Ratnasingham, S. L. deWaard, L. Lu, J. Robertson, A. C. Telfer and P. D. N. Hebert. 2016. "Untangling taxonomy: a DNA barcode reference library for Canadian spiders". *Molecular Ecology Resources* 16: 325–341.

Breitling, R. 2019. "A barcode-based phylogenetic scaffold for *Xysticus* and its relatives (Araneae: Thomisidae: Coriarachnini)". *Ecologica Montenegrina* 20: 198–206.

Čandek, K. and M. Kuntner. 2015. "DNA barcoding gap: reliable species identification over morphological and geographical scales". *Molecular Ecology Resources* 15: 268–277.

Cao, X., J. Liu, J. Chen, G. Zheng, M. Kuntner and I. Agnarsson. 2016. "Rapid dissemination of taxonomic discoveries based on DNA barcoding and morphology". *Scientific Reports* 6:37066.

Chen, S. H. and W. J. Huang. 2004. "A newly recorded spider of the genus *Cheiracanthium* (Araneae, Clubionidae) from Taiwan". *BioFormosa* 39: 55–59.

Chen, S. H. and W. J. Huang. 2012. *The spider fauna of Taiwan. Araneae. Miturgidae, Anyphaenidae, Clubionidae.* National Taiwan Normal University. Taipei. 130 pp.

Chen, S. H., W. J. Huang, S. C. Chen and Y. Wang. 2006. "Two new species and one newly recorded species of the genus *Cheiracanthium* (Araneae: Miturgidae) from Taiwan". *BioFormosa* 41: 9–18.

Chen, Z. F. and Z. H. Zhang. 1991. *Fauna of Zhejiang: Araneida.* Zhejiang Science and Technology Publishing House, 356 pp.

Coddington, J. A., I. Agnarsson, R.-C. Cheng, K. Čandek, A. Driskell, H. Frick, M. Gregorič, R. Kostanjšek, C. Kropf, M. Kweskin, T. Lokovšek, M. Pipan, N. Vidergar and M. Kuntner. 2016. "DNA barcode data accurately assign higher spider taxa". *PeerJ* 4: e2201.

Dankittipakul, P. and J. Beccaloni. 2012. "Validation and new synonymies proposed for *Cheiracanthium* species from South and Southeast Asia (Araneae, Clubionidae)". *Zootaxa* 3510: 77–86.

Darriba, D., G. L. Taboada, R. Doallo and D. Posada. 2012. "jModelTest 2: more models, new heuristics and parallel computing". *Nature Methods* 9: 772–772.

De Alvarenga, A. D. S., I. L. F. Magalhaes, R. N. Da Fonseca and A. Pérez-González. 2020. "Rectifying the identities of the males of two *Micrathena* species (Araneae: Araneidae), with report of the first case of intersexuality in the genus". *Zootaxa* 4808: 151–164.

Dhali, D. C., S. Saha and D. Raychaudhuri. 2017. "Litter and ground dwelling spiders (Araneae: Arachnida) of reserve forests of Dooars, West Bengal". *World Scientific News* 63: 1–242.

Díaz-Rodríguez, J., H. Gonçalves, F. Sequeira, T. Sousa-Neves, M. Tejedo, N. Ferrand and I. Martínez-Solano. 2015. "Molecular evidence for cryptic candidate species in Iberian *Pelodytes* (Anura, Pelodytidae)". *Molecular Phylogenetics and Evolution* 83: 224–241.

Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates'. *Molecular Marine Biology and Biotechnology* 3: 294–299.

García-Robledo, C., E. K. Kuprewicz, C. L. Staines, W. J. Kress and T. L. Erwin. 2013. "Using a comprehensive DNA barcode library to detect novel egg and larval host plant associations in a *Cephaloleia* rolled-leaf beetle (Coleoptera: Chrysomelidae)". *Biological Journal of the Linnean Society* 110: 189–198.

Gravely, F. H. 1931. "Some Indian spiders of the families Ctenidae, Sparassidae, Selenopidae and Clubionidae". *Records of the Indian Museum, Calcutta* 33: 211–282.

Hebert, P. D. N., A. Cywinska, S. L. Ball and J. R. deWaard. 2003. "Biological identifications through DNA barcodes". *Proceedings of the Royal Society B* 270: 313–321.

Hebert, P. D. N., M. Y. Stoeckle, T. S. Zemlak and C. M. Francis. 2004. "Identification of Birds through DNA Barcodes". *PLOS Biology* 2: e312.

Huang, J., A. Zhang, S. Mao and Y. Huang. 2013. "DNA Barcoding and Species Boundary Delimitation of Selected Species of Chinese Acridoidea (Orthoptera: Caelifera)". *PLoS ONE* 8: e82400.

Janjua, S., Fakhar-I-Abbas, K. William, I. U. Malik and J. Mehr. 2017. "DNA Mini-barcoding for wildlife trade control: a case study on identification of highly processed animal materials'. *Mitochondrial DNA Part A* 28: 544–546.

Kress, W. J., C. García-Robledo, M. Uriarte and D. L. Erickson. 2015. "DNA

barcodes for ecology, evolution, and conservation". *Trends in Ecology & Evolution* 30: 25–35.

Kumar, S., G. Stecher and K. Tamura. 2016. "MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets". *Molecular Biology and Evolution* 33: 1870–1874.

Hall, T.A. 1999. "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT". *Nucleic Acids Symposium Series* 41, 95–98.

Li, Z.-Y. and F. Zhang. 2019. "Two new species of *Cheiracanthium* C. L. Koch, 1839 (Araneae, Cheiracanthiidae) from Xizang, China". *Acta Arachnologica Sinica* 28(2): 87–95.

Liu, P., M. Irfan and X. J. Peng. 2019. "Redescription of *Cheiracanthium rupicola* (Thorell, 1897) (Araneae, Eutichuridae) with the first description of the male from Yunnan, China". *Journal of Asia-Pacific Biodiversity* 12(2): 316–319.

Marusik, Y. M. and A. A. Fomichev. 2016. "A new species of *Cheiracanthium* (Araneae: Cheiracanthiidae) from Mongolia". *Indian Journal of Arachnology* 5(1–2): 79–83.

Majumder, S. C. and B. K. Tikader. 1991. "Studies on some spiders of the family Clubionidae from India". *Records of the Zoological Survey of India* 102: 1–175.

Pickard-Cambridge, O. 1874. "On some new species of Drassides". *Proceedings of the Zoological Society of London* 42(3): 370–419.

Rambaut, A. 2018. FigTree v1.4.4, a graphical viewer of phylogenetic trees. Available from http://tree.bio.ed.ac.uk /software/figtree/.

Robinson, E., G. Blagoev, P. Hebert and S. Adamowicz. 2009. "Prospects for using DNA barcoding to identify spiders in species-rich genera". *ZooKeys* 16: 27–46.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S. E. Ramos-Onsins and A. Sánchez-Gracia. 2017. "DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets". *Molecular Biology and Evolution* 34: 3299–3302.

Song, D. X., M. S. Zhu and J. Chen. 1999. *The spiders of China.* Hebei Science and Technology Publishing House Shijiazhuang, 640 pp.

Sousa, L. L., R. Xavier, V. Costa, N. E. Humphries, C. Trueman, R. Rosa, D. W. Sims and N. Queiroz. 2016. "DNA barcoding identifies a cosmopolitan diet in the ocean sunfish". *Scientific Reports* 6: 28762.

Stamatakis, A. 2006. "RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models". *Bioinformatics* 22: 2688–2690.

Thorell, T. 1895. *Descriptive catalogue of the spiders of Burma, based upon the collection made by Eugene W. Oates and preserved in the British Museum*. *London*, 406 pp.

Tikader, B. K. and B. Biswas. 1981. "Spider fauna of Calcutta and vicinity: Part-I". *Records of the Zoological Survey of India* 30: 1–149.

Tyagi, K., V. Kumar, S. Kundu, A. Pakrashi, P. Prasad, J. T. D. Caleb and K. Chandra. 2019. "Identification of Indian Spiders through DNA barcoding: Cryptic species and species complex". *Scientific Reports* 9: 14033.

Tyagi, K., V. Kumar, N. Poddar, P. Prasad, I. Tyagi, S. Kundu and K. Chandra. 2020. "The gene arrangement and phylogeny using mitochondrial genomes in spiders (Arachnida: Araneae)". *International Journal of Biological Macromolecules* 146: 488–496.

Wang, Y.-N. and F. Zhang. 2013. "A new spider species of the genus *Cheiracanthium* (Araneae, Miturgidae) from Guangxi, China". *Acta Zootaxonomica Sinica* 38(1): 59–63.

World Spider Catalog. 2020. *World Spider Catalog. Version 21.5*. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on {Access on 25 Sep 2020}. doi: 10.24436/2.

Xu, X., F. Liu, J. Chen, D. Li and M. Kuntner. 2015. "Integrative taxonomy of the primitively segmented spider genus *Ganthela* (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology". *Zoological Journal of the Linnean Society* 175: 288–306.

Yaginuma, T. 1970. *The spider fauna of Japan*. Bulletin of the National Museum of Nature and Science Tokyo 13: 639–701.

Zhang, J., H. Yu and S. Li. 2020. "New cheiracanthiid spiders from Xishuangbanna rainforest, southwestern China (Araneae, Cheiracanthiidae)". *ZooKeys* 940: 51–77.

Zhang, J., G. Zhang, H. Yu. 2018. "Four species of spider genus *Cheiracanthium* C. L. Koch, 1839 (Araneae, Eutichuridae) from Jinggang Mountains, Jiangxi Province, China". *ZooKeys* 762: 33–45.