Revision of the taxonomic status of two coelotine species (Araneae: Agelenidae) from Japan

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Abstract — Taxonomic accounts of two species belonging to the subfamily Coelotinae from Japan are reviewed. *Iwogumoa portus* Nishikawa 2009 originally described from Osaka, Japan and *Coelotes plancyi* Simon 1880 described from Beijing, China are considered to be conspecific. Therefore the former is synonymized with the latter, and *C. plancyi* is transferred to the genus *Iwogumoa* Kishida 1955. *Coelotes inabaensis* Arita 1974 described from Tottori, Japan is placed under the genus *Dichodactylus* Okumura 2017.

Key words — Coelotinae, taxonomy, new synonymy, new combination, COI

Introduction

One hundred and seventeen species of the subfamily Coelotinae have been recorded in Japan to date (World Spider Catalog 2017). However, most of them have been placed under the genus *Coelotes* Blackwall 1841, although a phylogenetic study revealed that *Coelotes* is a polyphyletic taxon (Chen et al. 2016). Therefore, the precise taxonomic status of many Japanese species remains uncertain, and thus should be elucidated by morphological and molecular studies.

Coelotes plancyi Simon 1880 was described from Beijing, China, and has been considered to be indigenous to Japan as well (Bösenberg & Strand 1906; Saito 1941; Zhu & Zhang 2011). According to the original description (Simon 1880), this species is closely related to *Coelotes insidiosus* L. Koch 1878 (*Iwogumoa insidiosa* at present), which is the type species of the genus *Iwogumoa* Kishida 1955. However, reconsideration of the taxonomic status and proper revision of the genus name have not been made up to the present, and this species is thus still classified under *Coelotes*. Moreover, exact identification of this species is difficult because the figures by Simon (1880) provided only simple and partial drawings of its male palp. Therefore, this species has been treated as an unidentified species for a long time in Japan. Recently, one *Iwogumoa* species, *I. portus* Nishikawa 2009, was described by using the specimens collected from Osaka (type locality), Chiba, Tokyo, and Yamaguchi (Nishikawa 2009). Thus, *I. portus* shows discontinuous distribution and has been primarily collected from urban areas and near the seaport or airport,

hence the species might be an artificially introduced species. Moreover, morphological characteristics of *I. portus* are almost concordant with those of *C. plancyi* described by recent studies (e.g. Song et al. 2001; Zhu & Zhang 2011; Wang 2017). Therefore, we evaluate the taxonomic relation of both *C. plancyi* and *I. portus* by means of a molecular phylogeny inferred from mitochondrial cytochrome *c* oxidase subunit I (mt-COI) sequences and morphological examination of the type specimens of *I. portus*.

The other species, of which taxonomic account is revised in this study, is *Coelotes inabaensis* Arita 1974 originally described from Tottori, Japan (Arita 1974). By a careful examination of genital organs of both sexes, it became clear that this species has the morphological characteristics of the genus *Dichodactylus* Okumura 2017. Prior to this, the three Japanese ‘*Coelotes*’ species diagnosed by same characteristics are currently placed under *Dichodactylus*: *D. tarumii* (Arita 1976) (the type species), *D. satoi* (Nishikawa 2003), and *D. shinshuensis* Okumura 2017. Accordingly, we analyze mt-COI data of *Dichodactylus* specimens to test whether *C. inabaensis* could be treated as a fourth species under this genus.

The present study contributes to our knowledge about the species diversity of the genera *Iwogumoa* and *Dichodactylus* in Japan.

Materials and methods

Sampling and morphological examination

In total 19 coelotine specimens used for molecular phylogenetic analyses were collected from 17 localities, Japan (see Appendix): those were identified according to

Okumura et al. (2009), and have been deposited in the Zoological Collection of Kyoto University (KUZ). In addition to these specimens, morphological characteristics of the type specimens of *Iwogumoa portus* and the newly collected specimens identified with *Coelotes inabaensis* were examined. A part of the examined specimens of *C. inabaensis* have been deposited in the Tsukuba Collection Center, National Museum of Nature and Science, Tokyo (NSMT). Examination of the specimens was performed using an Olympus SZX-7 stereomicroscope. Photographs were taken using an Olympus E-620 digital camera attached to the microscope.

DNA extraction, polymerase chain reaction and sequencing

The third or fourth leg was dissected from the body of each specimen, and preserved in 99.5% ethanol at 4°C for DNA extraction. DNA was extracted from the muscle of the leg. The extraction of genomic DNA followed Okamoto et al. (2006) along with a minimally destructive method by Paquin & Vink (2009). Partial sequences of mt-COI were used for phylogenetic analysis. Primer sets for PCR and cycle sequencing (CS) reaction for COI were, COIARAF (5'-ACAAATCATAAAGATATTGC-3'; this study) and COIARAR (5'-ATAGCATAAATTATTCCTAA-3'; this study) (PCR and CS). All amplification reactions were performed in a GeneAmp PCR System 2700 (Applied Biosystems Ltd., ABI), or a MyCycler (Bio-Rad Laboratories). The reactions were initially heated to 94°C for 5 min, followed by 35 cycles of 94°C (10 sec), 40°C (20 sec), and 72°C (42 sec), and a final extension at 72°C for 6 min. PCR products were checked for the presence of bands and their size by using agarose gel (2%) electrophoresis, and then the amplified DNA fragments were purified using polyethylene glycol (20% PEG 6000) precipitation. All samples were sequenced in both directions. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI). Each sequencing reaction mixture was incubated at 96°C for 2 min, followed by 40 cycles of 96°C (10 sec), 50°C (5 min), and 60°C (42 sec). The products were collected by ethanol precipitation and sequenced on an ABI 3130xl Genetic Analyzer (ABI). The obtained sequences were edited using DNA Baser (Heracle Biosoft S.R.L.). The DNA sequences newly obtained in this study have been deposited with the International Nucleotide Sequence Database Collaboration (INSDC) through DNA Data Bank of Japan.

Molecular phylogenetic analyses

Six specimens classified into five *Iwogumoa* species were included in the phylogenetic analysis for the taxonomic accounts of *I. portus* and *C. planyci* (see Appendix): *I. acco* (Nishikawa 1987) (topotype), *I. insidiosa*, *I. interuna* (Nishikawa 1977) (topotype), *I. nagasakiensis* Okumura 2007 (topotype), and *I. portus* from Nagasaki Prefecture, Japan.

Along with the individual of *C. inabaensis* collected from Tottori Pref., the phylogenetic position of *C. inabaensis* was determined using five specimens of two *Dichodactylus* species, and seven specimens classified into five *Coelotes* species morphologically related to *Dichodactylus*: *D. satoi* (including topotype), *D. tarumii* (including topotypes), *C. kitazawai* Yaginuma 1972, *C. lamellatus* Nishikawa 2009 (topotype), *C. musashiensis* Nishikawa 1989, *C. yahagiensis* Nishikawa 2009 (topotype), and *C. yodoensis* Nishikawa 1977 (topotype).

The previously published mt-COI sequence of *C. planyci* in the name *Asiacoelotes planyci* collected from China was obtained from INSDC (KY778827). The mt-COI sequences were aligned using MEGA 7.0 (Kumar et al. 2016). Maximum likelihood (ML) phylogenies based on 763 bp alignment positions of mt-COI were constructed using MEGA with non-parametric bootstrapping (BS) conducted with 1000 replicates. In the analysis, Tamura-Nei Model was selected for ML analyses using MEGA.

Results and conclusions

Morphological characteristics

Iwogumoa portus: Male palp (Fig. 1A, B) has a short conductor with a broad proximal part and thin tip. Cymbial furrow is clearly recognized and the tip protrudes from the lateral side of the cymbium. Patellar apophysis is slender only at the tip. Epigyne (Fig. 1C) has small teeth situated close to each other in anterior part. Spermathecae of female internal genitalia (Fig. 1D) are kidney shaped and widely separated from each other. *C. planyci* also has above-mentioned characteristics without exception (Song et al. 2001; Zhu & Zhang 2011; Wang 2017). Therefore we consider that *I. portus* and *C. planyci* should be treated as the conspecific.

Coelotes inabaensis: This species is small-sized, and characterized by the possession of four retromarginal teeth on the chelicera. Male palp (Fig. 2A, B) has two patellar apophyses and no lateral tibial apophysis. Conductor is broad and strongly curved toward the tip of the cymbium. Epigyne (Fig. 2C) is oval-shaped and has no teeth. Female internal genitalia (Fig. 2D) has irregularly convolute and anteroposteriorly elongated spermathecae. Among the above morphological characteristics, having two patellar apophyses and no lateral tibial apophysis in male palp, and irregularly convolute and anteroposteriorly elongated spermathecae in the female internal genitalia can be enumerated as common points of the genus *Dichodactylus* (Okumura 2017). As mentioned above, morphological characteristics of this species strongly suggested a change in the taxonomic status.

Molecular phylogenetic relationships

The ML tree (ln $L = -1285.647$; Fig. 3) for estimating the phylogenetic positions of *I. portus* and *C. planyci* showed that these species formed a monophyletic lineage (BS=100%). The monophyly of three *Iwogumoa* species,

Fig. 1. *Iwogumoa plancyi* (Simon 1880). Holotype (A–B), allotype (C) and paratype (D) of *Iwogumoa portus* Nishikawa 2009. A, left male palp, ventral view; B, same, retrorateral view; C, epigyne, ventral view; D, internal female genitalia, dorsal view. Scales = 0.5 mm.

Fig. 2. *Dichodactylus inabaensis* (Arita 1974). Mt. Kenashi, Shinjo Village, Maniwa County Okayama Pref. (A–B), and Wakasugi Pass, Nishiwakura Village, Aida County, Okayama Pref. (C–D). A, left male palp, ventral view; B, same, retrorateral view; C, epigyne, ventral view; D, internal female genitalia, dorsal view. Scales = 0.5 mm.

Fig. 3. Maximum likelihood tree for 763 bp alignment positions of mt-COI marker of the genus *Iwogumoa* collected from Japan and *Coelotes plancyi* from China. Numbers on nodes indicating bootstrap values.

Fig. 4. Maximum-likelihood tree for 763 bp alignment positions of mt-COI marker of the genus *Dichodactylus* and small sized *Coelotes* species from Japan. Numbers on nodes indicating bootstrap values.

namely *I. acco*, *I. interuna* and *I. nagasakiensis*, was weakly supported (BS=61%). Two specimens of *I. insidiosa* different in collection locality formed a monophyletic lineage with the slight support value (BS=62%). The obtained phylogenetic relationship between *I. portus* and *C. plancyi* supported the results of the morphological analysis of the two species, and also suggested that *C. plancyi* belongs to the genus *Iwogumoa*.

The obtained ML tree ($\ln L = -2279.490$; Fig. 4) for estimating the phylogenetic position of *C. inabaensis* failed to recover the monophyly of *Dichodactylus* (BS=41%).

The sample identified as *D. satoi* collected from Mt. Ibuki, Gifu Prefecture, Japan was nested within the clade comprised *D. tarumii* samples (BS=100%). Although the support value was not high (BS=50%), *C. inabaensis* formed a monophyletic lineage with *D. tarumii* clade. According to the obtained phylogenetic characteristics, *C. inabaensis* can be treated as the fourth *Dichodactylus* species. The present mt-COI data obtained from the other small sized 'Coelotes' species will help elucidating their taxonomic status by a future study.

Taxonomic conclusions

Iwogumoa plancyi (Simon 1880) **comb. nov.**
(Fig. 1)

Coelotes plancyi Simon 1880, pp. 115–116, pl. 3, figs. 12–14; Song & Hubert 1983, p. 4, figs. 6–9; Song 1987, p. 194, fig. 152; Song et al. 1999, p. 377, fig. 223; Hu 2001, p. 143, fig. 53; Song, Zhu & Chen 2001, p. 293, fig. 186; Zhu & Zhang 2011, pp. 318–319, fig. 230.

Coras plancyi: Simon 1898, p. 258; Bösenberg & Strand 1906, p. 301; Saito 1941, pp. 50–51.

Coelotes insidiosus: Lehtinen 1967, p. 224. (Rejected syn.)

Iwogumoa portus Nishikawa 2009, p. 70, figs. 111–116; Okumura et al. 2009, p. 199, figs. 2-2-33-393–398. **syn. nov.**

Specimens examined. One male (holotype of *I. portus*; NSMT), Nippon-bashi, Naniwa-Ku, Osaka City, Osaka Prefecture, October 19, 1975, Y. Watanabe leg.; 2 females (allotype and paratype of *I. portus*; NSMT), Kōsei, Minato-Ku, Osaka City, Osaka Prefecture, January 6, 1973, H. Shirakawa leg.

Remarks. Judging from the distributional areas in Japan, the Japanese populations of this species are likely to be naturalized from China. The demographic history of the Japanese *I. plancyi* should be revealed on the basis of additional genetic data obtained from the sufficient specimens of the species.

Dichodactylus inabaensis (Arita 1974) **comb. nov.**
(Fig. 2)

Coelotes inabaensis Arita 1974, pp. 41–43, figs. 1–5; Nishikawa 1974, p. 176, figs. 12–14; Nishikawa 1976, p. 1060; Yaginuma 1986, p. 149, fig. 81; Okumura et al. 2009, p. 197, figs. 2-2-33-346–348.

Specimens examined. All the specimens were collected from Okayama Pref., Japan by K. Nojima leg. Takiyama, Nagi Town, Katsuta County, 1 male and 1 female, October 22, 1989; Wakasugi Pass, Nishiawakura Village, Aida County, 1 male and 1 female, November 12, 1989, 1 female, June 2, 1990; Hiruzen-shitao, Maniwa City, 2 males and 3 females, October 29, 1990; Kurami, Kamo Town, Tsuyama City, 2 males and 1 female, November 3, 1990, 1 male, May 28, 1991; Mt. Kenashi, Shinjo Village, Maniwa County, 2 males and 2 females, November 15, 1993; Higashiaono, Mimasaka City, 1 male, October 1, 2000. Two samples used for taking the photographs are deposited in NSMT.

Diagnosis. *Dichodactylus inabaensis* can be distinguished from the other congener by the following points. Male palp (Fig. 2A–B); conductor is broad and anteriorly bent, but that of the others is thin (*D. tarumii* and *D. shinshuensis*), or ventrally bent (*D. satoi*). Female genitalia (Fig. 2C–D); epigyne is oval-shaped and without epigynal

teeth, but that of the others is hourglass shaped (*D. tarumii* and *D. shinshuensis*), or hexagonal shaped (*D. satoi*). In addition, *D. tarumii* has epigynal teeth.

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Appendix. Samples with collection localities and INSDC accession numbers used for molecular phylogenetic analyses.

Species	Sex	Voucher	Collection localities	Accession No.
<i>Coelotes kitazawai</i>	Female	KUZ Z1889	Urabandai, Kitashiobara Village, Yama County, Fukushima Pref.	LC310804
<i>Coelotes kitazawai</i>	Male	KUZ Z1890	Ryusen-sindo cave, Iwaizumi, Iwaizumi Town, Simohei County, Iwate Pref.	LC310805
<i>Coelotes kitazawai</i>	Male	KUZ Z1891	Hiyamizu Pass, Okunai-Tsukushimori, Mutsu City, Aomori Pref.	LC310806
<i>Coelotes lamellatus</i>	Male	KUZ Z1894	Occhi, Ueno Village, Tano County, Gunma Pref.	LC310807
<i>Coelotes musashiensis</i>	Female	KUZ Z1905	Hirasawa, Suruga Ward, Shizuoka City, Shizuoka Pref.	LC310808
<i>Coelotes yahagiensis</i>	Female	KUZ Z1904	Mt. Sangane 260m alt. Higashi-hazu Town, Nishio City, Aichi Pref.	LC310809
<i>Coelotes yodoensis</i>	Female	KUZ Z1902	Minoo park, Minoo City, Osaka Pref.	LC310810
<i>Dichodactylus inabaensis</i>	Female	KUZ Z1899	Ashizu Valley, Chizu Town, Yazu County, Tottori Pref.	LC310811
<i>Dichodactylus satoi</i>	Male	KUZ Z1893	Mt. Ena, Nakatsugawa City, Gifu Pref.	LC310812
<i>Dichodactylus satoi</i>	Female	KUZ Z1892	Mt. Ibuki, Ibigawa Town, Ibi County, Gifu Pref.	LC310813
<i>Dichodactylus tarumii</i>	Female	KUZ Z1896	Iwato-ji Temple, Kunisaki City, Oita Pref.	LC310814
<i>Dichodactylus tarumii</i>	Female	KUZ Z1900	Mt. Daisen, Daisen Town, Saihaku County, Tottori Pref.	LC310815
<i>Dichodactylus tarumii</i>	Female	KUZ Z1901	Toyofusa, Daisen Town, Saihaku County, Tottori Pref.	LC310816
<i>Iwogumoa acco</i>	Female	KUZ Z1906	Kōmori-ana cave, Utobara, Futtsu City, Chiba Pref.	LC310817
<i>Iwogumoa insidiosa</i>	Female	KUZ Z1895	Takazawa, Kuma Village, Kuma County, Kumamoto Pref.	LC310818
<i>Iwogumoa insidiosa</i>	Female	KUZ Z1907	Mt. Osakadanyama, Izuhara Town, Tsushima City, Nagasaki Pref.	LC310819
<i>Iwogumoa interuna</i>	Female	KUZ Z1903	Minoo park, Minoo City, Osaka Pref.	LC310820
<i>Iwogumoa nagasakiensis</i>	Female	KUZ Z1897	Mt. Yasuman 500m alt., Hirado City, Nagasaki Pref.	LC310821
<i>Iwogumoa portus</i>	Male	KUZ Z1898	Ishida Town, Iki City, Nagasaki Pref.	LC310822