Notes on Several Japanese Species of *Iwogumoa* and *Coelotes* (Araneae: Agelenidae: Coelotinae)

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*Iwogumoa songminjae* (Paik and Yaginuma in Paik et al., 1969) is recorded for the first time from Japan on the basis of specimens collected in the Tsushima Islands, Kyushu and previously identified as *I. insidiosa* (L. Koch, 1878). The relationships among the Japanese species of *Iwogumoa* Kishida, 1955 are analyzed using mitochondrial cytochrome *c* oxidase subunit I with various Japanese species of *Coelotes* Blackwall, 1841 *sensu lato* as the outgroup. A new synonym is proposed through a comparison of morphological characteristics and the results of our molecular analysis: *Coelotes iyoensis* Nishikawa, 2009 (known only from the male) is a junior synonym of *Coelotes mohrii* Nishikawa, 2009 (known only from the female).

**Key Words:** Taxonomy, morphology, DNA barcoding, COI, coelotine spiders.

**Introduction**

Spiders of the subfamily Coelotinae F. O. P.-Cambridge, 1893 (Agelenidae C. L. Koch, 1837) are restricted to the Northern Hemisphere, and about 750 species have been described mainly from East Asia. Of these, 119 species are known in Japan (World Spider Catalog 2019). Although great progress on taxonomic revision of this subfamily has been made in recent years (e.g., Chen et al. 2015, 2016; Okumura et al. 2016; Zhao and Li 2016; Okumura 2017; Okumura et al. 2017; Li et al. 2018a, b, c, 2019), many problems still remain. The present paper presents the results of our taxonomic study of several Japanese species of *Iwogumoa* Kishida, 1955 and *Coelotes* Blackwall, 1841 *sensu lato*.

Many Japanese spider species currently classified in *Coelotes* likely are not *Coelotes* *sensu stricto*, [*i.e.*, based on the European type species, *Coelotes atropos* (Walckenaer, 1830) (Okumura, unpublished data)]. For example, an easily distinguishable character is the number of teeth on the cheliceral retromargin: *C. atropos* has three while many Japanese species of *Coelotes* have only two. In addition, characteristics of the genital organs in several species groups are clearly different from those of *C. atropos*. The Japanese coelotine fauna is diverse and complex, and determining the phylogenetic relationships of the Japanese species is beyond the scope of this paper. Therefore, we continue to use *Coelotes sensu lato* for the Japanese species of *Coelotes* considered in this paper, as in their original descriptions.

**Materials and Methods**

**Sampling and morphological examination.** Specimens other than those provided by Y. Ibara and N. Tsurusaki were collected by the first author. Examination of specimens was performed using an Olympus SZX-7 stereomicroscope. Measurements of body parts were made using a micrometer mounted in one ocular lens. All measurements are given in millimeters. Genitalic morphology and body size of specimens from the Tsushima Islands and the mainland of Kyushu identified as *Iwogumoa insidiosa* (L. Koch, 1878) were observed and photographs were taken using an Olympus E-620 digital camera attached to the microscope. Voucher specimens will be deposited in the collection of the Department of Zoology, National Museum of Nature and Science, Tsukuba, Japan (NSMT).

**Molecular phylogenetic analysis.** Three specimens of *I. insidiosa* (two from the Tsushima Islands, and one from the mainland of Kyushu), one specimen of *Coelotes mohrii* Nishikawa, 2009, and one of *C. iyoensis* Nishikawa, 2009 were used in the molecular analysis. Comparison of base sequences was carried out using sequences of other closely related species. Details of all specimens used in the molecular analysis are listed in Table 1. Previously published data for two *Iwogumoa* species (LC310817, LC310821) and two *Coelotes* species (LC382419, LC382466) were obtained from the International Nucleotide Sequence Database Collaboration (INSDC). Mitochondrial cytochrome *c* oxidase subunit I (mt-COI) was amplified using the primer combination COI-1628 and COI-Nancy (Vandergast et al. 2004). For details of the DNA extraction, polymerase chain reaction and sequencing, refer to Okumura et al. (2016). The DNA
sequences newly obtained in this study have been deposited with INSDC through the DNA Data Bank of Japan (Table 1).

### Results and Discussion

**Comparison between putative *Iwogumoa insidiosa* collected from the Tsushima Islands and mainland Kyushu.**

*Iwogumoa insidiosa* is widely distributed throughout Japan, from Hokkaido to Kyushu, but it has been suggested that the specimens from the Tsushima Islands in Nagasaki Prefecture are different from those on the mainland of Japan based on body size and male and female genitalic morphology (Okumura, unpublished data). We analyzed differences in the genitalic morphology, body sizes, and molecular data of specimens of *I. insidiosa* from the two localities (Tsushima Islands, n = 35; mainland Kyushu, n = 38).

Male palp: conductor, tip of the cymbium, and base of the embolus of the Tsushima specimens (Fig. 1a) are slightly broader than those of the mainland Kyushu specimens (Fig. 1b). In addition, the conductor lamella of the Tsushima specimens (Fig. 1a) is not as developed as it is in the mainland Kyushu specimens (Fig. 1b). Epigyne (Fig. 1c, d): no significant differences were noted between the Tsushima and mainland Kyushu specimens. Internal female genitalia: morphology of the spermatheca and fertilization duct is similar in specimens from the two localities but the general appearance of the internal female genitalia differs: slightly curved anteriorly and trapezoidal in the Tsushima specimens (Fig. 1e) while angular anteriorly and rectangular in the mainland specimens (Fig. 1f). The shape of the anterior-most portion of the spermathecal stalks also differs between specimens from the two localities: crescentic in Tsushima specimens (Fig. 1e) and nearly circular in mainland specimens (Fig. 1f).

In addition, body size is significantly different between specimens from the two localities: the Tsushima specimens are markedly smaller than those from mainland Kyushu (Mann-Whitney u-test, *p* < 0.01) (Fig. 2).

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**Table 1.** Samples with collection localities and INSDC accession numbers used for molecular phylogenetic analysis.

| Species             | sex    | Collection localities                                      | Accession No. |
|---------------------|--------|-----------------------------------------------------------|---------------|
| *Iwogumoa songminjiae* | female | Kamisaka 300 m alt., Izuhara Town, Tsushima City, Nagasaki Pref. | LC440631      |
| *Iwogumoa songminjiae* | female | Mikata, Mitsushima Town, Tsushima City, Nagasaki Pref.     | LC440632      |
| *Iwogumoa insidiosa*   | female | Mt. Konpira-san 250 m alt., Nagasaki City, Nagasaki Pref.  | LC440633      |
| Coelotes mohrii       | female | Kurose-Koh, Saijo City, Ehime Pref.                        | LC440634      |
| Coelotes iyoensis     | male   | Matsusegawa, To-on City, Ehime Pref.                       | LC440635      |
| Coelotes oxyacanthus  | female | Matsuyma, Kishiku Town, Goto City, Nagasaki Pref.          | LC440636      |
| Coelotes irici       | female | Kamamuta, Takahara Town, Nishimorokata County, Miyazaki Pref. | LC440637      |
| Coelotes sanoi       | female | Yamamuro, Ochi Town, Takaoka County, Kochi Pref.           | LC440638      |
| Coelotes unicatus     | female | Midorikawa, Yamato Town, Kamimashiki County, Kumamoto Pref. | LC440639      |
Finally, our ML tree obtained from molecular analysis showed that the specimens from the two localities are phylogenetically distinct (Fig. 3). Okumura et al. (2017) recently demonstrated the same result. The calculated p-distance (the number of base differences per total number of analyzed bases) between the Tsushima and mainland Kyushu specimens was 0.081.

The male and female genitalic morphology of the Tsushima specimens corresponds to that of I. songminjae (Paik and Yaginuma in Paik et al., 1969) previously known only from Korea (Paik et al. 1969; Paik 1971, 1978; Kim and Lee 2006; Zhu et al. 2017). Specimens of I. songminjae are smaller than those of I. insidiosa: carapace lengths of I. songminjae males and females are, respectively, 3.70 and 3.79 (Paik 1971), and of I. insidiosa from mainland Kyushu, respectively, 5.10 and 4.38 (this study). Based on these morphological results coupled with the phylogenetic difference demonstrated by the molecular data, we conclude that the specimens from Tsushima Islands previously identified as I. insidiosa were misidentified, and represent the first Japanese records of I. songminjae.

Comparison between Coelotes mohrii and Coelotes iyoensis. Coelotes mohrii and C. iyoensis are sympatric in Ehime Prefecture in the northwestern region of Shikoku, Japan. Each species was described based on a single sex: C. mohrii from females, and C. iyoensis from males. Nevertheless body size and coloration are quite similar in both species, and there is no difference in the number of the retro-marginal cheliceral teeth (Nishikawa 2009; Okumura et al. 2009). We tested the hypothesis that the two species are the same through analysis of body size, comparison with other closely related species, and DNA barcoding.

Both species have two retromarginal teeth on the chelicera, and their body lengths are similar: 10.0–10.5 in C. iyoensis and 10.0–11.0 in C. mohrii (Nishikawa 2009; Okumura et al. 2009). Morphological characteristics the two specimens used in this study were consistent with those reported in the original description. Analysis of mt-COI partial sequences of the two species showed a nearly complete match (p-distance=0.002). Therefore we conclude that C. mohrii and C. iyoensis are conspecific, and that C. iyoensis as a junior synonym of C. mohrii. In addition, the molecular analysis demonstrated that C. iyoensis is phylogenetically close to C. sanoi Nishikawa, 2009 and C. unicatus Yaginuma, 1977, both of which also have a relatively large, dark-colored body and two retromarginal teeth on their chelicera (Fig. 3).

Taxonomy

Family Agelenidae C. L. Koch, 1837
Subfamily Coelotineae F. O. P.-Cambridge, 1893
Iwogumoa songminjae
(Paik and Yaginuma in Paik et al., 1969)
[Japanese name: Kōrai-shimofuri-yachigumo] (Fig. 1)
2009; Kamisaka, 380 m alt., Izuhara Town, 3 females (29 December 2009), 1 male (30 December 2009), 2 males and 15 females (10 October 2010), 1 male and 2 females (6 October 2012), 1 male and 7 females (7 October 2012), 2 females (10 October 2015); Kashi, Izuhara Town, 1 female, 9 October 2011, Y. Ihara leg.; Mt. Sumo-shiratake, 250 m alt., Mitsushima Town, 1 male, 10 October 2010; Mt. Sumo-shiratake, 280 m alt., Mitsushima Town, 1 female, 30 December 2009; Kashi, Mitsushima Town, 1 female, 11 October 2015; Mikiata, Mitsushima Town, 1 female, 11 October 2015. The four specimens used for taking the photographs are deposited in NSMT (Ar-16979-16982).

**Distribution.** Korea, China, Russia and Japan (Tsushima Islands).

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**Fig. 4.** *Coelotes mohrii* Nishikawa, 2009 (NSMT-Ar 16983, 16984): a, ventral view of male palp; b, retrolateral view of male palp; c, ventral view of epigyne; d, dorsal view of internal female genitalia. Abbreviations: CO, conductor; EM, embolus; MA, median apophysis.

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**Fig. 5.** Distribution of *Coelotes mohrii* Nishikawa, 2009 and two related species from Shikoku. Closed circles denote *C. mohrii*; open circles, *C. unicatus* Yaginuma, 1977; closed triangles, *C. sanoi* Nishikawa, 2009.
Diagnosis. *Iwogumo songminjiae* can be distinguished from *I. insidiosa* by the shape of the male palp and internal genitalia (see results and discussion). Kim and Lee (2006) argued that there is a difference in the number of retromarginal teeth on the chelicerae of the two species, viz., female of *I. insidiosa* has three retromarginal teeth on the right chelicera, while the female of *I. songminjiae* has four. However, we believe this is simply intra-specific variation: in the course of this study we observed differences in the number of teeth between the left and right chelicerae in individual specimens. This variation (normally three, rarely four) was also reported by Paik (1971). Moreover, Okumura (2009) demonstrated that variation in the number of retromarginal teeth occurs with high probability in species of *Iwogumo* in comparison to other coelotine spiders (Okumura 2009).

**Coelotes mohrii** Nishikawa, 2009  
[Japanese name: Iyo-yachigumo]  
(Fig. 4)

**Coelotes mohrii** Nishikawa, 2009: 64, figs 2-1-N-80, 2-1-N-81; Okumura et al. 2009: 184, fig. 2-2-33-159.  
**Coelotes iyoensis** Nishikawa, 2009: 70, figs 2-1-N-108-2-1-N-110; Okumura et al. 2009: 197, figs 2-2-33-358, 2-2-33-359. New synonymy

**Material examined.** Ehime Prefecture, Japan. Narikawa Valley, Kihoku Town, Kitauwa County, 2 females, 25 April 1993, Y. Ihara leg.; Tengu Plateau, 1400 m alt, Nishidani, Kuma-kogen Town, Kamiyakena County, 2 males, 21 September 2009, Y. Ihara leg.; Senba, Tobe Town, Iyo County, 2 females, 31 December 2014; Takaichi, Tobe Town, Iyo County, 1 female, 31 December 2014; Saragamine, To-on City, 2 females, 6 April 1972, N. Tsurusaki leg.; Masekawa, 420 m alt, To-on City, 2 males and 1 female, 1 January 2015; Kuroseko, Saijo City, 1 female, 1 January 2015. The two specimens used for taking the photographs are deposited in NSMT (Ar-16983-16984).

**Distribution.** Ehime Prefecture, Japan. In the Shikoku district, this species shows a nearly parapatric distribution (Fig. 5) with two closely related species (see Fig. 3) *C. unicusatus* (Yaginuma, 1977) and *C. sanoi* (Nishikawa, 2009).

**Diagnosis.** The shape of the male palp of *Coelotes mohrii* is unique: the embolus extends in a counterclockwise direction (left palp) from the anterior end of the genital bulb, and the conductor and median apophysis are both located on the retrolateral side of tegulum (Fig. 4a, b).

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