A New Species of the Subfamily Coelotinae (Araneae: Agelenidae) from Shiga and Fukui Prefectures, Japan

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This study describes a new species of the subfamily Coelotinae (Agelenidae) from Kinki district, central Japan, under the name of \textit{Coelotes nojimai} sp. nov. It resembles \textit{C. yodoensis} Nishikawa, 1977 and \textit{C. nagaraensis} Nishikawa, 2009 but is characterized by a male palp with a large and greatly curved conductor and slender spermatothecae in the internal genitalia of female specimens. The morphological differences and distribution ranges of these three species have been discussed in detail. In addition, the mitochondrial cytochrome \textit{c} oxidase subunit I (mt-COI) partial sequences of the new species have been analyzed and documented for future use.

Key Words: Coelotine spider, taxonomy, morphology, DNA barcoding.

Introduction

In Japan, the spider fauna of the subfamily Coelotinae F. O. Pickard-Cambridge, 1893, is diverse despite its limited land area, because of its geomorphic complexity with many islands. On the Kyushu and Ryukyu islands in particular, 59 coelotine species have been reported to date (Okumura et al. 2009, 2020; World Spider Catalog 2021). Remarkable levels of speciation have also been observed in some areas on Honshu, which is the largest island in Japan. High levels of diversity have been reported in the Kinki district, which includes Lake Biwa and the Kii Peninsula, and \textit{Coelotes isensis} Okumura, 2019 has recently been described from this region. Among the subfamily Coelotinae, there is the genus \textit{Tegecoelotes} Ovtchinnikov, 1999, which mainly inhabits Japan. This genus includes three species that are endemic to this area, including one undescribed species (Nishikawa 2009; Okumura et al. 2009; Okumura, unpublished data). Furthermore, the \textit{Coelotes yodoensis} species group is found between the Kinki district and the east of the Chubu district. This species group includes three species: \textit{Coelotes yodoensis} Nishikawa 1977, \textit{C. nagaraensis} Nishikawa, 2009, and \textit{C. yahagiensis} Nishikawa, 2009. These three species have high morphological similarity and clearly have a close phylogenetic relationship (Nishikawa 2009). It is therefore important to ensure accurate identification, and the accurate distributional range of each species in this area.

As a result of comparing several specimens collected in this area, the study noted that the specimens found in the range from the area around Lake Biwa in Shiga Prefecture to Fukui Prefecture had large morphological differences in male palp compared to the above mentioned three species.

Fig. 1. Holotype female of \textit{Coelotes nojimai} sp. nov., dorsal view. Scale bar: 1.0 mm.
Neither the appearance of the morphological variation nor that of the intermediate characteristics with closely related species was confirmed. In this study, we conducted detailed morphological and genetic analysis of these specimens including female specimens, and confirmed that the unidentified specimens were different from the three species discussed above. Therefore, we describe these specimens as a new species and discuss the differences in its morphological characteristics in comparison with those of *C. yodoensis* and *C. nagaraensis*, which are distributed in adjacent areas.

### Materials and Methods

**Sampling and morphological examination.** Unidentified coelotine specimens were collected by several researchers and given to the authors. The detail information on the sampling localities and the collectors can be found in the sections “Type specimens” and “Other specimens examined”. Their examination and illustration were performed using an Olympus SZX-7 stereomicroscope. To examine the copulatory organs, the left male palp was cut as necessary, while for female vulva, the epigyne was dissected and treated in 10% KOH solution to remove the muscles. Photographs were taken using an Olympus E-620 digital camera attached to the stereomicroscope. Body parts and interdistsances were measured using a micrometer mounted on an ocular lens. All measurements are given in millimeters. Leg measurements are given as total length (femur length, patella and tibia length, metatarsus length, tarsus length). Abbreviations used in this study are as follows: ALE, anterior lateral eye; AME, anterior median eye; CO, conductor; MOA, median ocular area; PA, patellar apophysis; PLE, posterior lateral eye; PME, posterior median eye; RTA, retrolateral tibial apophysis; SP; spermatheca.

The type specimens of the new species have been deposited in the collection of the Department of Zoology, National Museum of Nature and Science (NSMT), Japan. Other specimens have been kept by the first author for future study.

**Molecular experiments.** DNA sequences of the holotype of the new species have been obtained for future study. Mitochondrial cytochrome *c* oxidase subunit I (mt-COI) DNA was amplified using the primer combination LCO1490: 5′-GGT CAACAAATCATAAAGATAAATG-3′ with HCO2198: 5′-TAAACTCTACGGTGAAGAAAATCA-3′ (Folmer et al. 1994). mt-COI partial sequences of 654 bp were obtained via DNA extraction, polymerase chain reaction, and sequencing; these methods have been described in more detail in Zhao et al. (2020).

The DNA sequences obtained in this study have been deposited in the International Nucleotide Sequence Database Collaboration (INSDC) through the DNA Data Bank of Japan under the accession number LC623770.

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**Fig. 2.** Copulatory organs of *Coelotes nojimai* sp. nov. holotype, NSMT-Ar 20961 (C), paratypes, NSMT-Ar 20962 (A, B), NSMT-Ar 20963 (D). A: Male palp (left), ventral view; B: male palp (left), retrolateral view; C: epigyne, ventral view; D: internal female genitalia. Scale bars: 0.5 mm. Abbreviations: CO, conductor; ET, epigynal tooth; FD, fertilization duct; MA, median apophysis; PA, patellar apophysis; RTA, retrolateral tibial apophysis; SH, spermathecal head; SP, spermatheca.
Fig. 3. Comparison of the male palp of three species inhabiting around Lake Biwa. *Coelotes nojimai* sp. nov. [1 paratype, NSMT-Ar 20962–6], *C. nagaraensis* [7 (topotype)–12], and *C. yodoensis* [13 (topotype)–18]. The photo numbers correspond to the numbers indicating the collection localities on the map. Scale bars: 0.5 mm.
Taxonomy

Family **Agelenidae** C. L. Koch, 1837
Subfamily **Coelotinae** F. O. Pickard-Cambridge, 1893

**Coelotes nojimai** sp. nov.
[Japanese name: Ōmi-yachigumo]
(Figs 1, 2, 3-1–3-6, 4A–C)

**Type specimens.** Holotype: female (NSMT-Ar 20961), Japan: Sanami, Imazu Town, Takashima City, Shiga Prefecture, 35.4329°N 136.0094°E, 12 November 2020, K. Kumada leg. Paratypes: 1 male (NSMT-Ar 20962), 1 female (NSMT-Ar 20963), Aiba, Shin-asahi Town, Takashima City, Shiga Prefecture, 35.3845°N 136.0198°E, 26 November 2020, K. Kumada leg.

**Other specimens examined.** SHIGA PREF.—Ritto City: Arahari (1♂1♀, 8 October 2005. Y. Ihara leg.). Higashi-omi City, Doki-cho (1♂, 22 March 2003. K. Nojima leg.). Gamogun, Hino-cho, Kaiga (2♂1♀, 9 November 2004, K. Nojima leg.). Nagahama City: Nishi-azai-cho, Yamakado (1♀, 11 October 2011, K. Kumada leg.). Kinomoto-cho, Kanei-hara (1♀, 18 July 2020, N. Tsurusaki leg.). FUKUI PREF.—Tsuruga City, Kashimagari, Nakaikemi (2♂, 20 March 2002,

Fig. 4. **Comparison of the copulatory organs of Coelotes nojimai** sp. nov. (holotype, NSMT-Ar 20961 and paratypes, NSMT-Ar 20962, 20963; A–C), *C. nagaraensis* (topotypes; D–F) and *C. yodoensis* (topotypes; G–I). A, D, G: Male palp, ventral view; B, E, H: epigyne, ventral view; C, F, I: internal female genitalia. Abbreviations: CO, conductor; PA, patellar apophysis; SP, spermatheca. Scale bars: 0.5 mm.
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K. Kumada leg.). Nanjo-gun, Minami-echizen-cho: Itadori (1♂, 17 April 2003, K. Kumada leg.); Furuki (1♂3♀, 6 November 2004). Yoshida-gun, Eiheiji-cho, Johoji (1♂1♀, 6 November 2004, K. Nojima leg.).

Distribution. Shiga and Fukui Prefectures, Japan (Figs 3, 5).

Etymology. The trivial name is dedicated to Mr. Koichi Nojima who collected and provided ten specimens of this new species.

Diagnosis. *Coelotes nojimai* sp. nov. resembles *C. yodoensis* and *C. nagaraensis* in body size and the form of the epigyne but can be distinguished by the form of the male palp and internal female genitalia (Figs 3, 4). In addition, no specimens having intermediate characteristics have been identified in the areas of boundary line for distribution of each species (Fig. 3). The CO in the ventral view is large and extremely curved in *C. nojimai* sp. nov., while in *C. yodoensis* and *C. nagaraensis* it is small and only slightly curved, and the PA of *C. nojimai* sp. nov. is markedly shorter than that of *C. nagaraensis*. In the female specimens, the SP of the new species were slender and elongated in the longitudinal direction, while they were thick and short in *C. yodoensis* and *C. nagaraensis* and were greatly curved in *C. yodoensis*.

Description of female (holotype). Total length 7.2, carapace 3.6 long, 2.2 wide; abdomen 3.6 long, 2.4 wide; sternum 1.7 long, 1.4 wide. Eye sizes and interdistances: AME 0.10, ALE 0.15, PME 0.13, PLE 0.14; AME–AME 0.05, AME–ALE 0.05, PME–PME 0.10, PME–PLE 0.15, AME–PME 0.15, ALE–PLE 0.06. MOA; anterior width 0.26, posterior width 0.36, length 0.37. Leg measurements: I: 7.6 (2.3, 2.6, 1.7, 1.0); II: 6.6 (2.0, 2.2, 1.5, 0.9); III: 6.2 (1.8, 1.9, 1.6, 0.9); IV: 8.7 (2.4, 2.8, 2.4, 1.1).

Chericeral promargin with three teeth and retromargin with four teeth.

Coloration: carapace yellowish brown with gray radial flecks; sternum yellowish brown; chelicerae and maxillae blackish brown; labium brown; dorsum of abdomen grayish brown with chevrons; venter yellowish brown with irregular flecks; legs yellowish brown with ring flecks.

Epigyne and internal genitalia (Figs 2C, D, 4B, C): epigynal plate almost hexagonal, twice as wide as its length, with deep color on the periphery; epigynal teeth slightly thin and situated in the anterior portions of the epigyne; SP slender, anteroposteriorly elongated, and situated close to each other; spermathecal heads situated on the anterior portion of the SP; copulatory ducts broad, covering the lateral regions of the genitalia.

Description of male (paratype). Total length 6.3, carapace 3.3 long, 2.2 wide; abdomen 3.0 long, 1.8 wide; sternum 1.7 long, 1.5 wide. Eye sizes and interdistances: AME 0.09, ALE 0.15, PME 0.13, PLE 0.14; AME–AME 0.05, AME–ALE 0.05, PME–PME 0.10, PME–PLE 0.15, AME–PME 0.15, ALE–PLE 0.06. MOA; anterior width 0.23, posterior width 0.36, length 0.37. Leg measurements: I: 8.2 (2.4, 2.8, 2.0, 1.0); II: 7.2 (2.1, 2.2, 1.7, 1.2); III: 7.0 (1.9, 2.1, 2.0, 1.0); IV: 8.9 (2.1, 2.9, 2.8, 1.1).

Chericeral promargin with three teeth and retromargin with four teeth.

Coloration: almost same as that of the holotype.

Palp (Figs 2A, B, 3-1–3-6, 4A): PA short and pointed from ventral view, but rounded from lateral view; lateral tibial apophysis absent; RTA large and broad; cymbial furrow indistinct and about one-fifth of the cymbial length; CO large and crescent-shaped; small CO dorsal apophysis present; median apophysis slightly crooked and spoon-shaped at the tip; embolus flagelliform and short. There are slight variations such as small PA and slender CO in the specimens from Fukui prefecture (Fig. 3-5, 3-6).

Remark. Many Japanese species of the subfamily Coelotinae have been classified under the genus *Coelotes*. However, recent studies using genetic data in addition to detailed morphological investigations have shown that these species in fact comprise several heterogeneric groups (Okumura et al. 2017; Zhao and Li 2017; Okumura 2020). All the species that are closely related to the new species described in this study are currently classified as *Coelotes*. Therefore, the new species has also been provisionally classified as *Coelotes*.

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