New synonymy of the species group of *Coelotes unicatus* Yaginuma 1977, 
with first description of the male of *Coelotes sanoii* Nishikawa 2009 
(Araneae: Agelenidae) from Shikoku District, Japan

Ken-ichi Okumura¹*, Mitsuwo Noine² & Shin-ya Ohba²

¹ Nagasaki Prefectural Nagasaki Kakuyo Senior High School, Sueishi-machi, 157–1, Nagasaki-shi, Nagasaki, 850–0991, Japan  
E-mail: coelotes@hor.bhiq.jp  
² Biological Laboratory, Faculty of Education, Nagasaki University, Bunkyo-machi 1–14, Nagasaki-shi, Nagasaki 852–8521, Japan  
*Corresponding author

Abstract — Three species of the coelotine spiders from the Shikoku and Kyushu Districts of Japan are reviewed. *Coelotes towaensis* Nishikawa 2009 and *Coelotes sanoii* Nishikawa 2009 have been described with only female specimens from Kōchi Prefecture. The species are considered to be conspecific, and the former is regarded as a junior synonym of the latter. Furthermore, male of this species is described for the first time, and morphological and molecular differences are shown in comparison with *Coelotes unicatus* Yaginuma 1977 which is the closely related species of *C. sanoii*.

Key words — Coelotinae, new synonymy, male description, molecular phylogenetic analysis

Introduction

The unicatus group of *Coelotes* is characterized by a large-sized body, dark coloration and two retromarginal teeth on chelicera. It is distributed in Western Japan (Okumura et al. 2009). The group has been known to show a remarkable geographical variation. Three species of the group, *Coelotes unicatus* Yaginuma 1977, *C. sanoii* Nishikawa 2009 and *C. towaensis* Nishikawa 2009 have been known from Shikoku District. However, only the shapes of female genital organs have been described of *C. sanoii* and *C. towaensis* from Kōchi Pref. Because these species are quite similar to each other, we surveyed in Shikoku district in 2014, and collected several female and one male specimens. To reveal the relationship between *C. sanoii* and *C. towaensis* we conducted detailed morphological observation and molecular phylogenetic analysis.

Materials and methods

Sampling, examination and illustration

All the materials including *Coelotes unicatus* and *C. sanoii* in this study were collected from Shikoku and Kyushu Districts, Japan. Examination and illustration were performed using an Olympus SZX-7 stereomicroscope. Photographs were taken using an Olympus E-620 digital camera attached to the microscope. Measurements of respective body parts were done using a micrometer mounted on an ocular lens. All measurements are given in millimeters. Leg measurements are given as total length (femur, patella & tibia, metatarsus, tarsus). The abbreviations used in this paper are as follows: ALE, anterior lateral eye; AME, anterior median eye; LTA, lateral tibial apophysis; MOA, median ocular area; PLE, posterior lateral eye; PME, posterior median eye; RTA, retroroteral tibial apophysis. Among the three taxa, *Coelotes sanoii* was identified by the comparison with the holotype.

DNA extraction, polymerase chain reaction and sequencing

Seven specimens of obtained samples were used in the molecular phylogenetic analysis. Collection locations and genital organs of these specimens are shown in Fig. 1. The details of the samples are shown in appendix. *Tegecoelotes corasides* was used as an outgroup. Forth leg was dissected from the body and preserved it in 99.5% ethanol at 4°C for DNA extraction. DNA was extracted from the muscle of leg. DNA extraction was mainly performed with REDE Extract-N-Amp Tissue PCR Kit (Sigma-Aldrich Japan Inc.) according to manufacturer’s protocol. In addition, DNeasy Blood & Tissue Kit (QIAGEN, Inc.) was used in some samples. Mitochondrial cytochrome oxidase subunit I (mt-COI) partial sequences were used for phylogenetic analysis. Mt-COI was amplified using the primer combination COI-1628: 5' - ATA ATG TAA TTG TTA CTG CTC ATG C - 3' (Vandergast et al. 2004) with COI-Nancy: 5' - CCC GGT AAA ATT AAA ATA TAA ACT TC - 3' (Vandergast et al. 2004). PCR reactions were performed in Gene Atlas S type programmable thermal cycler (ASTEC Co., Ltd.). The reactions were initially denatured for 2 min at 95°C, proceeded with 35 cycles of 30 sec at 95°C, 30 sec at 55°C, 45 sec at
72°C and extension for 7 min at 72°C. For a part of samples, the PCR reaction mixture was heated to 95°C for 2 min and then put through 20 cycles of PCR amplification: 95°C for 30 sec, 40°C for 30 sec, and 72°C for 45 sec, and then 20 cycles of PCR amplification: 95°C for 30 sec, 45°C for 30 sec, and 72°C for 45 sec, followed by 72°C for 7 min. PCR products were checked the presence of the bands and their size by agarose gel (2%) electrophoresis and purified with the ExSap-IT (Affymetrics Inc.) according to the manufacture’s protocols. Purified PCR products were sequenced using the primer combination COI-1628 and COI-Nancy, and analyzed on 3730 DNA Analyzer (Applied Biosystems Ltd.). As a result, mt-COI partial sequences of 453 bp (451 bp in two specimens) were obtained.

Results

After a careful examination of genital organs of these specimens, we concluded that Coelotes sanoi and C. towaensis are conspecific because these specimens show considerable morphological variation that is gradual between the two taxa and many intermediate specimens were recognized (Fig. 2). The characteristics of C. sanoi and C. towaensis are as follows; the longitudinal furrow of epigynum is narrow, rectilinear and dilated posteriorly in C. sanoi, while that of C. towaensis is slightly broad in anterior region, and narrow in central region. Epigynal teeth are small in C. sanoi, and large incurved in C. towaensis. However, it is hard to say that the characteristics of epigynal teeth are decisive attribute for classifying the species, judging from the figures of original description. Considering above, some specimens are identified with C. sanoi (Fig. 2F) or C. towaensis (Fig. 2C, D) by the type specimen and the original description. However, in the other specimens, it is difficult to classify them as one or the other. For

Fig. 1. Collection locations and morphological variation of the unicus group of Coelotes. A, Kirishima City, Kagoshima Pref.; B, Nobeoka City, Miyazaki Pref.; C, Saga City, Saga Pref.; D, Hata County, Kōchi Pref.; E, Nankoku City, Kōchi Pref.; F–G; Takaoka County, Kōchi Pref.
example, the following patterns were shown: the specimen having a wide and rectilinear longitudinal furrow (Fig. 2J), having a narrow furrow constricted in central region (Fig. 2A), having elongated hollows in both lateral sides of the longitudinal furrow (Fig. 2E), and having above characteristics compositely (Fig. 2B, E, G, H, I, K).

Furthermore, maximum-likelihood tree and bayesian tree were obtained on the basis of the result of the molecular phylogenetic analysis of mitochondrial cytochrome oxidase subunit I (mt-COI) partial sequences. The form of the phylogenetic tree was divided into two clades by both analysis, and the unknown male specimen was shown to belong in the sanoi clade (Figs. 3–4). Therefore it became clear that the unknown male specimen is C. sanoi. Coelotes unicus also lives in southwestern region of Kōchi Pref., and it became clear by our survey that twenty specimens of the species were collected and approximately shows parapatric distribution to C. sanoi. These two species are assumed to be different because of the following facts: 1) specimens that have intermediate characteristics of the two

---

**Fig. 2.** Variation of the epigynum of Coelotes sanoi Nishikawa 2009 (= Coelotes towaensis Nishikawa 2009). A–E, Yamamuro, Ochi Town, Takaoka County; F–I, Choya, 620 m alt., Ochi Town, Takaoka County; J–K, Shibamaki, 325 m alt., Kōchi City; L, Naro, 250 m alt., Nankoku City. The arrows indicate the portions of epigynal teeth lost.
species do not appear, 2) shape of the male palp is different between the two species, 3) two species are divided into two clades by molecular phylogenetic analysis (Figs. 3–4). In this paper, we regard *C. towaensis* as a junior synonym of *C. sanoii*, describe the male for the first time and show the phylogenetic relationship in comparison with *C. unicus* having a marked variation in Shikoku and Kyushu.

**Coelotes sanoii** Nishikawa 2009  
(Japanese name: Tosano-yachigumono)  
(Figs. 1E–G, 2, 5)

*Coelotes sanoii* Nishikawa 2009, p. 64, Fig. 2-1-N-82; Okumura et al. 2009, p. 187, Fig. 2-2-33-171.  
*Coelotes towaensis* Nishikawa 2009, p. 64, Fig. 2-1-N-83; Okumura et al. 2009, p. 187, Fig. 2-2-33-172.  
**New synonymy**

**Material examined.** Female holotype (NSMT-Ar 13507), 7 December 1993, Park of Kōchi-jō, Kōchi City, Kōchi Prefecture, Shikoku District, S. Sano leg. All the following specimens were collected in Kōchi Prefecture, Shikoku District by K. Okumura, and are in the first author’s private collection. Naro, 250 m alt., Nankoku City: 1 female, 28 December 2014. Shiamaki, 325 m alt., Kōchi City: 2 females, 28 December 2014. Yamamuro, Ochi Town, Takaoka County: 1 male and 5 females, 29 December 2014. Chōja, 620 m alt., Ochi Town, Takaoka County: 4 females, 29 December 2014.  

**Diagnosis.** *Coelotes sanoii* resembles *C. unicus* in external features viz. large body and two retromarginal teeth on chelicera, but can be distinguished from the latter species by the genital structure. The conductor of the male palp is elongated reaching to the lateral side of cymbium in *C. sanoii*, but that of *C. unicus* is elongated reaching to the base of cymbium obliquely (Figs. 1C, F, 5A). The atrium of the epigynum elongated in an anteroposterior direction, and the length is more than twice its width in *C. sanoii*, but elongated in a horizontal direction of less than twofold in *C. unicus* (Figs. 1A–B, D, 2, 5C). The spermatheca is elongated in anteroposterior direction in *C. sanoii* (Fig. 5D), but almost globular in *C. unicus*.

---

**Acta Arachnologica.** 65(2), December 2016 ©Arachnological Society of Japan
Description (one male from the above specimens). Total length 12.9, carapace 6.6 long, 4.2 wide; abdomen 6.3 long, 3.7 wide; sternum 3.2 long, 2.4 wide. Eye sizes; AME 0.18, ALE 0.26, PME 0.23, PLE 0.24. Distances between eyes; AME-AME 0.15, AME-ALE 0.88, PME-PME 0.15, PME-PLE 0.27, AME-PME 0.21, ALE-PLE 0.10. MOA; anterior width 0.51, posterior width 0.61, length 0.62. Leg measurements: I: 17.8 (4.9, 6.1, 4.4, 2.4); II: 15.8 (4.4, 5.2, 3.9, 2.3); III: 14.1 (3.8, 4.5, 3.9, 1.9); IV: 19.4 (5.1, 6.2, 5.6, 2.5).

Cheliceral promargin with three teeth and retromargin with two.

Male palp (Figs. 1F, 5A–B): patellar apophyses thick and robust, LTA small, RTA large and broad, cymbial furrow short and indistinct about one-fifth of cymbial length, embolus thick and short, conductor long, twisted at near the base and elongated to the lateral side of cymbium, conductor dorsal apophysis situated in a direction parallel with lines of the conductor, median apophysis spoon-shaped, looks like a F cleft from the ventral view.

Coloration: carapace brown with grayish brown radial flecks, dorsum of abdomen grayish brown with indistinct chevrons and venter yellowish brown, sternum brown, chelicerae reddish brown, maxillae and labium dark brown, legs brown without ring flecks.

Acknowledgments

We wish to express our sincere thanks to Dr. Akio Tanikawa, University of Tokyo, for giving constructive information on a technique of molecular phylogenetic analysis. This study is partly supported by JSPS KAKENHI Grant Number 15H00437.

References


Received January 28, 2016 /Accepted May 22, 2016
**Appendix.** Details of sampling data and DDBJ/EMBL/GenBank accession numbers of sequence data analyzed in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Collection locations</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coelotes sanoi</td>
<td>Male</td>
<td>Yamamuro, Ochi Town, Takaoka County, Kōchi Pref.</td>
<td>LC114263</td>
</tr>
<tr>
<td>Coelotes sanoi</td>
<td>Female</td>
<td>Naro, 250 m alt., Nankoku City, Kōchi Pref.</td>
<td>LC114264</td>
</tr>
<tr>
<td>Coelotes sanoi</td>
<td>Female</td>
<td>Yamamuro, Ochi Town, Takaoka County, Kōchi Pref.</td>
<td>LC114265</td>
</tr>
<tr>
<td>Coelotes unicusatus</td>
<td>Female</td>
<td>Kirishima-taguchi, 540 m alt., Kirishima City, Kagoshima Pref.</td>
<td>LC114266</td>
</tr>
<tr>
<td>Coelotes unicusatus</td>
<td>Female</td>
<td>Hirayama, Otsuki Town, Hata County, Kōchi Pref.</td>
<td>LC114267</td>
</tr>
<tr>
<td>Coelotes unicusatus</td>
<td>Female</td>
<td>Kono Town, Nobeoka City, Miyazaki Pref.</td>
<td>LC114268</td>
</tr>
<tr>
<td>Coelotes unicusatus</td>
<td>Male</td>
<td>Fukumaki, 710 m alt., Sefuri Town, Saga City, Saga Pref.</td>
<td>LC114269</td>
</tr>
<tr>
<td>Tegecoelotes coronides</td>
<td>Male</td>
<td>Mt. Tachibana, Fukuoka City, Fukuoka Pref.</td>
<td>LC114270</td>
</tr>
</tbody>
</table>