

***Cyclosa omonaga* (Araneae: Araneidae), an alternative host of the spider ectoparasitoid *Reclinervellus nielsenii* (Hymenoptera, Ichneumonidae), with notes on behavioural manipulation**

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Abstract

Parasitism upon *Cyclosa omonaga* (Araneidae) by the ichneumonid ectoparasitoid *Reclinervellus nielsenii* is reported for the first time, based on molecular identification, corroborating that *R. nielsenii* is a specialist parasitoid of members of the genus *Cyclosa*, having previously been recorded from *C. argenteoalba*, *C. conica* and *C. laticauda*. The parasitoid larva at the time of discovery (11 December) was still tiny in spite of the beginning of winter, indicating it was in its overwintering phase, and this is the first case of the overwintering *R. nielsenii* larva found in Japan. The host's cocoon web, with fibrous and fluffy thread decorations (FTD) resulting from the wasp's behavioural manipulation, produced by the parasitized *C. omonaga* is described and illustrated, and its potential role is discussed.

Keywords: cocoon web • COI • FTD • overwinter • *Polysphincta* group • resting web

Introduction

Among the megadiverse family of insect parasitoid, Ichneumonidae, a few groups interestingly have evolved to parasitize spiders, either the body or their egg mass (Fitton, Shaw & Austin 1987; Gauld 1988; Quicke 2015). The *Polysphincta* genus-group (Ichneumonidae, Pimplinae, Ephialtini; hereafter polysphinctines) is a monophyletic lineage of solitary koinobiont (i.e. allowing the host to recover normal activity for a time after being attacked) ectoparasitoids of spiders. Each clade or genus of polysphinctines attack a limited and closely related group of spiders, i.e. restricted to single host family or genus and each wasp species usually only utilizes one or a few closely related host species (Gauld & Dubois 2006; Matsumoto 2016), suggesting that polysphinctines have radiated by colonizing new hosts that are close relatives of existing ones. Any host records of polysphinctines are thus of value to trace their evolutionary path.

In this study, I found a polysphinctine larva of *Reclinervellus nielsenii* (Roman, 1923) parasitic upon a juvenile vertical orb weaver, *Cyclosa* sp. cf. *confusa* Bösenberg & Strand, 1906, *japonica* Bösenberg & Strand, 1906 or *omonaga* Tanikawa, 1992 (Araneae: Araneidae, Fig. 1) which is unidentifiable based on juvenile morphology (Tanikawa 1992). I thus tried to identify the host spider by means of a molecular method using the legs of the killed spider.

As many polysphinctine larvae in the final stage are known to manipulate web-building behaviour of their host spider to make the original fragile web more durable (the

cocoon web) to secure their cocoon (e.g. Eberhard 2000, 2013; Matsumoto 2009), *R. nielsenii* is known to manipulate its host spiders across several species exclusively in the genus *Cyclosa*: *C. argenteoalba* Bösenberg & Strand, 1906 (main host in Japan) (Matsumoto & Konishi 2007; Takasuka *et al.* 2015), *C. conica* (Pallas, 1772) (main host in Europe) (Nielsen 1923; Takasuka *et al.* 2017) and *C. laticauda* Bösenberg & Strand, 1906 (only one record in Japan) (Takasuka *et al.* 2017). Of note is that the cocoon webs induced in *C. argenteoalba* have fibrous and fluffy thread decorations (FTD) along radii of a simplified (with reduced number of radii) orb web. Unparasitized *C. argenteoalba* also build a simplified web with FTD before moulting (resting web), suggesting that *R. nielsenii* exploits pre-programmed behaviour of *C. argenteoalba* (Takasuka *et al.* 2015). On the other hand, cocoon webs induced in *C. conica* and *C. laticauda* were just simplified orb webs without any decoration but with pre-existing linear detritus or silk decoration left above and below the hub (Takasuka *et al.* 2017).

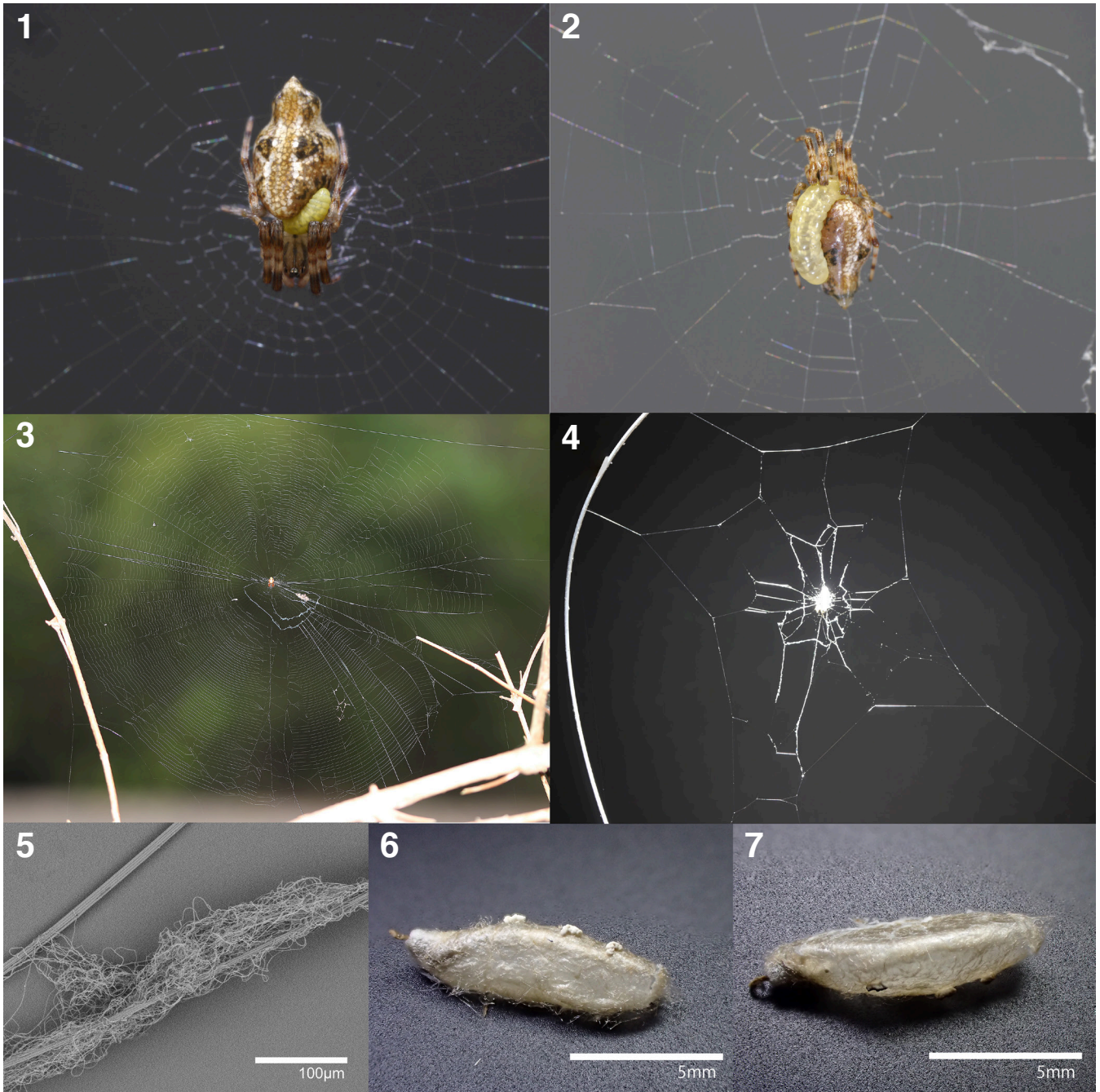
Rearing the parasitized *C. sp. cf. confusa, japonica*, or *omonaga* resulted in acquisition of a cocoon web as a result of behavioural manipulation by an *R. nielsenii* larva in its final larval stage. I report here a new host record of *R. nielsenii*, discuss the host range of *R. nielsenii*, and interspecific differences in host web manipulation among different host spiders. The natural history of *R. nielsenii* is inferred based on current knowledge and the new findings.

Materials and methods

Field investigation and rearing condition

The parasitized spider (Figs. 1–2) bearing a tiny parasitoid larva was found building a normal orb web (see an example of *C. japonica* in Fig. 3) in Kashifune jinja (a shrine), 34°57'49"N 135°35'32.1"E, 400 m a.s.l., located at Mt Myojingatake, Takatsuki-shi, Osaka prefecture, Japan on 11 December 2017. The parasitized spider was transferred to a rearing chamber (a greenhouse, 960 cm × 700 cm × 1110 cm) covered by black drapes with two LED lights (programmed as light:dark = 14:10) and two heaters (Dantotsu, Midori-Shokai Co. Ltd.) inside. Temperature was set at 25°C, controlled by a thermostat (Easy Glo Thermo, GEX Co. Ltd.) and relative humidity was kept high (>60%) by frequent spraying of water, in addition to placing a bowl filled with water in the chamber. A rounded wire hoop held on a wooden stand was also incorporated into the chamber to help the spider build its web. The spider was fed with *Drosophila* sp. and killed *Tetragnatha squamata* Karsch, 1879. The emerged wasp was identified by an ichneumonid taxonomist, Kyohei Watanabe, Kanagawa Prefectural Museum of Natural History (KPMNH). The specimen with a cocoon and the spider carcass is deposited in KPMNH under depository number KPM-NK 5006648.

Another specimen of *R. nielsenii* reared from *C. laticauda* recorded by Takasuka *et al.* (2017) was also deposited in KPMNH under depository number, KPM-NK 5006647.



Figs. 1–7: *Cyclosa omonaga* parasitized by *Reclinervellus nielsenii*, webs, and cocoon. **1** *C. omonaga* parasitized by a young instar larva of *R. nielsenii*, photographed on 30 December 2017; **2** *C. omonaga* parasitized and already manipulated by a penultimate instar larva of *R. nielsenii*, photographed on 1 January 2018; **3** example of a normal orb web of *Cyclosa japonica* in the field; **4** modified web under the manipulation by *R. nielsenii* (cocoon web); **5** SEM picture of FTD; **6** cocoon of *R. nielsenii* laid on the dorsum (obverse above) showing two (obverse and lateral) of three longitudinal ribs; **7** cocoon of *R. nielsenii* laid prone (obverse below) showing a lateral rib and a plain reverse side which was attached to the web.

The silk of the spider's cocoon web FTD was examined using scanning electron microscopy (FlexSEM 1000, Hitachi High-Technologies Corporation).

Molecular identification

To identify the species of the host spider, molecular identification was conducted by means of animal DNA barcode fragment of the mitochondrial cytochrome oxidase I gene (COI) performed by Arakawa Laboratory of IAB (Institute for Advanced Biosciences), Keio University. DNA was amplified from a crude extract of three legs of the host spider after

being killed by the wasp larva. The COI gene was sequenced using universal primers LCO1490 (5'-GGTCAACAAAT-CATAAAGATATTGG-3') and HCO2198 (5'-TAAACT-TCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Sequencing was performed by Eurofins Genomics K. K. (<https://www.eurofinsgenomics.jp/jp/home>). The obtained sequence was searched in an in-house sequence database of the 1000 Spiders Project (Arakawa 2018; Suzuki 2018). The sequence and collection data were uploaded to DNA Data Bank of Japan (DDBJ, <https://www.ddbj.nig.ac.jp/index-e.html>) under accession number LC387835.

Results

The parasitoid completed development to adulthood in the rearing chamber. It killed the juvenile host spider and started to spin its cocoon on 2 January 2018 and an adult male emerged on 12 January 2018. The parasitoid was identified morphologically as *R. nielsenii* meaning that this is the fourth host species record for the wasp. Identification was further confirmed by comparing the wasp with photographs of the lectotype and paralectotype of *R. nielsenii* deposited in the Swedish Museum of Natural History.

The final orb web made by the host two days before killing the spider (Fig. 4) was simplified in comparison to its previous ones. The reduced numbers of radii were covered with mats of FTDs and a scanning electron micrograph of the FTD is shown in Fig. 5. A cocoon of the parasitoid had three conspicuous longitudinal ribs along its top side and both lateral sides making the cocoon triangular in cross-section (Fig. 6); there was no rib on the reverse side (Fig. 7) where it was attached to the web.

The COI sequence of the host spider matched *C. omonaga* with 100% similarity, and unmatched *C. japonica* with 94.42% similarity and *C. confusa* with 94.11% similarity.

Discussion

The discovery of *R. nielsenii* parasitizing *C. omonaga* means that the parasitoid is now known exceptionally to utilize four host species: *C. argenteoalba* (Matsumoto & Konishi 2007; Takasuka *et al.* 2015), *C. conica* (Nielsen 1923; Fitton, Shaw & Gauld 1988; Fritzen 2005; Takasuka *et al.* 2017), *C. laticauda* (Takasuka *et al.* 2017), and *C. omonaga*, and thus appears exclusively to attack members of the genus *Cyclosa*, as do two congeneric parasitoids, *R. tuberculatus* (Uchida, 1932) and *R. masumotoi* (Matsumoto & Konishi, 2007, competitively parasitic on *C. octotuberculata* Karsch, 1879 (Iwata 1942; Matsumoto & Konishi 2007)). This indicates that the oviposition behaviour of *R. nielsenii* extraordinarily allows successful parasitization of several *Cyclosa* spp., which build orb webs with decorations made of silk and/or detritus, hence their common name of trashline orb-weavers (Levi 1977; Preston-Mafham & Preston-Mafham 1996; Preston-Mafham & Preston-Mafham 1984).

The longitudinal ribs on the parasitoid cocoon are unique to *R. nielsenii* among *Reclinervellus*, and have been recorded in three other individuals reared from three *Cyclosa* spp. This may suggest a visual signalling function towards flying natural enemies (Takasuka *et al.* 2017). However, unlike the cocoons of *R. nielsenii* reared from other *Cyclosa* spp. which all have four longitudinal ribs, the cocoon of the individual reared from *C. omonaga* lacks the rib on the reverse side of the cocoon (Fig. 7) for unknown reasons.

Cyclosa omonaga is the third known host species of *R. nielsenii* in Japan and the parasitoid larva at the time of discovery (11 December) was still tiny in spite of the beginning of winter (average temperature was 7.4°C that day), indicating it was in its overwintering phase. This is the first case of an overwintering *R. nielsenii* larva found in Japan. All records of *R. nielsenii* larvae in Japan were from April to

May and in August (Matsumoto & Konishi 2007; Takasuka *et al.* 2017; Takasuka *et al.* 2015). In Finland, Fritzen (2005) found three parasitized *C. conica* on 14 January 2005.

The FTD expressed in the resting and cocoon webs built by *C. argenteoalba* reflects UV light, which is thought to deter potential web destroyers such as flying insects and birds from bumping into the webs (Takasuka *et al.* 2015). The conformity of this specific phenotype between two types of web strongly supports the hypothesis that *R. nielsenii* exploits pre-programmed behaviour of the host spiders. FTD was also expressed in the cocoon web built by *C. omonaga* (Figs. 4–5) structurally almost identical to that by *C. argenteoalba* (see Takasuka *et al.* 2015 fig. 2B). This indicates that the same mechanism of manipulation by *R. nielsenii* affects two different species (*C. argenteoalba* and *C. omonaga*) in an identical way *in vivo* to release behaviours of web simplification and FTD spinning. However, two other host spiders (*C. conica* and *C. laticauda*) do not spin FTD at all (Takasuka *et al.* 2017).

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