

## Scientific Note

# The northernmost record of *Eriostethus rufus* (Uchida, 1932) (Hymenoptera, Ichneumonidae) with an indication of new host, *Trichonephila clavata* (Koch, 1878) (Araneae, Araneidae) and its web manipulation

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**Abstract.** *Eriostethus rufus* (Uchida, 1932) is a polysphinctine ectoparasitoid of araneid spiders (*Neoscona* spp.) and is endemic to Japan. An individual was collected in Yamagata Prefecture (38°46' N), northern Japan, the northernmost record of the species and also the genus. Its identification was confirmed by morphology and by DNA barcoding. The cocoon was found in a large modified web, which is unique in that the web structure is shaped like an inverted triangle extending to over 50 cm with the cocoon hanging from an ill-defined part of the cocoon web without any organized structure surrounding the cocoon. The host spider of this individual appears to be *Trichonephila clavata* (Koch, 1878) (Araneidae, Nephilinae) based on several circumstantial evidences. The structure of the modified web suggests that the pre-existing web was partly reused, the orb web was completely removed, and sustaining threads of the barrier web would be newly moored to the substrates. This record means that *E. rufus* parasitises host spiders of two subfamilies, which is unusual for the group.

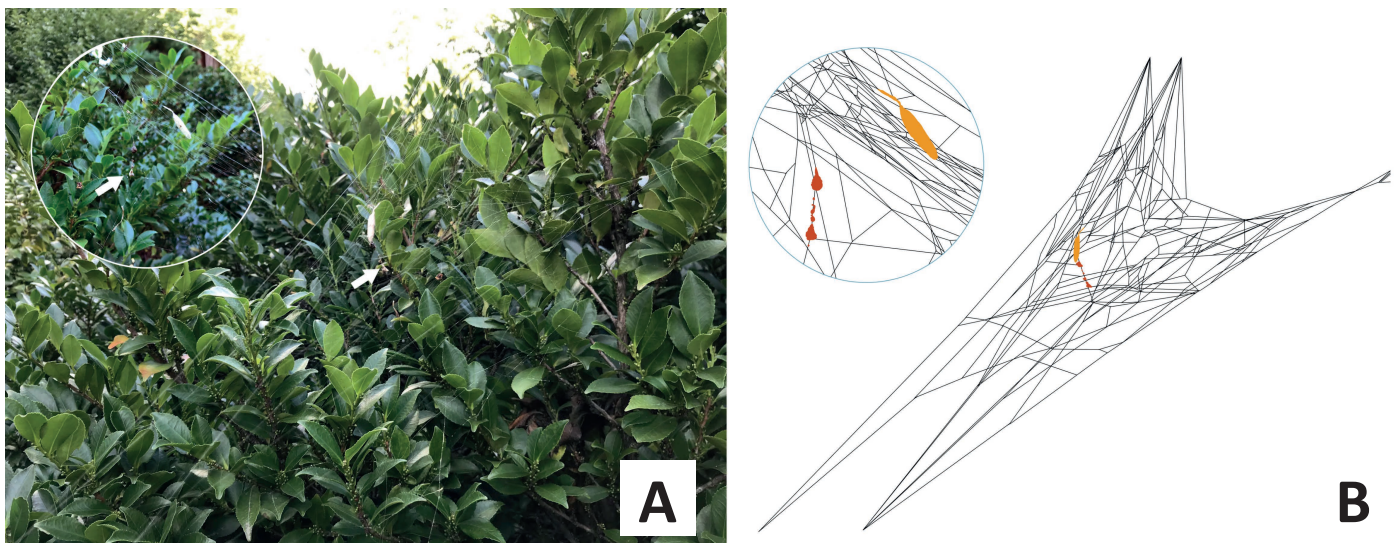
**Keywords:** Darwin wasp, host spider, Nephilinae, the *Polysphincta* genus-group.

*Eriostethus* Morley, 1914 is one of the genera in the *Polysphincta* genus-group (Pimplinae, Ephialtini), which is a monophyletic group comprised, with no known exceptions, of solitary koinobiont ectoparasitoids of spiders (Gauld & Dubois 2006; Matsumoto 2016). *Eriostethus* is a typical Indo-Australian genus consisting of 18 species to date, mainly found in the Australasian region, and with one endemic to Japan, *E. rufus* (Uchida, 1932). This is the only Palearctic species of *Eriostethus*.

Host records for *Eriostethus* are scarce, but two families have been recorded as host spiders so far; Araneidae (orb web weavers) by *E. perkinsi* (Baltazar, 1964) and *E. rufus*, and Theridiidae (three-dimensional cobweb weavers) by *E. minimus* Gauld, 1984 (Iwata 1942; Korenko et al. 2018a; Masumoto et al. 2002; Matsumoto 2016). Known host spiders of *E. rufus* are *Neoscona mellottei* (Simon, 1895) (Matsumoto

2016) and *N. scylloides* (Bösenberg & Strand, 1906) (Masumoto et al. 2002), and a record from *Araneus* sp. (Iwata 1942) which requires confirmation (Masumoto et al. 2002). Web manipulation, which is manifested by penultimate instar larvae behaviourally controlling a host spider, resulting in a “cocoon web” (Eberhard 2000), is known in *Eriostethus perkinsi* on *Eriophora* sp. (Korenko et al. 2018a) and *E. rufus* on *N. mellottei* (Matsumoto 2016). In both cases, orb webs are tri-dimensionalized into a roughly hemispherical or spherical structure, respectively, surrounding the wasp cocoon.

In this study, I found and collected a cocoon hung from a large modified web that was most probably spun by *Trichonephila clavata* (Koch, 1878), constructed high on an evergreen bush, *Eurya japonica* Thunb. (Pentaphragaceae) (Fig. 1) at 38°46'22.1"N 139°46'03.7"E, 30 m a.s.l., in the Zempoji temple, Tsuruoka city, Yamagata Prefecture,



**Figure 1.** The cocoon web of most probably *Trichonephila clavata* with several suspended residua (arrows in A and emphasized by red in B) under the influence of a larva of *Eriostethus rufus*: (A) An actual photo with an inset showing the same web from the opposite side; (B) A line drawing with an inset same as A (drawn by Kotaro Iwata). The web is at least 50 cm in maximal length and the cocoon is 16 mm in length.

Japan, on 19 September 2020. The cocoon web is a slightly three-dimensional but more or less one-directional structure resulting in an inverted triangle extending to over 50 cm (Fig. 1) without any vestige of the original orb construction (Fig. 2). The female wasp (Fig. 3), which emerged on 23 Sep. was identified by morphology and a DNA barcode fragment of the mitochondrial cytochrome oxidase I gene (COI), following the method described in Takasuka (2018) and using a crude extract of the right mid-tarsi immersed in DNAzol® Direct (Molecular Research Center, Inc). The sequence of 667 bp and collection data were uploaded to DNA Data Bank of Japan (DDBJ, <https://www.ddbj.nig.ac.jp/index-e.html>) under accession number LC604891. The COI sequence matched an unrecorded *E. rufus* (LC145362.1) collected in Yamatokouriyama city, Nara Prefecture, Japan (Matsumoto 2016) with 100% similarity to 541 bp (81% query coverage). This is the northernmost record of both the species (Fig. 4; Table 1) and also the genus. The specimen with its cocoon (Fig. 3) is deposited in Kanagawa Prefectural Museum of Natural History (KPMNH) under depository number KPM-NK55264.



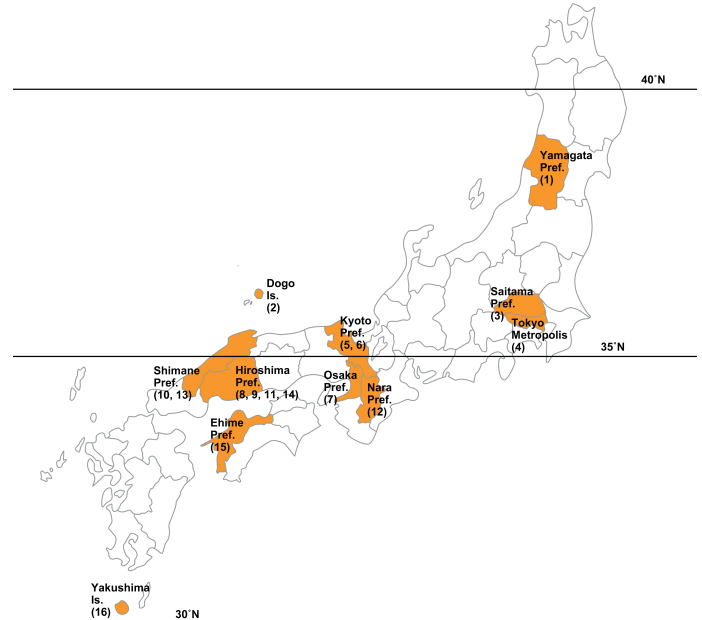
**Figure 2.** An adult *Trichonephila clavata* resting on its orb web 15m away from the cocoon web in question. It consists of a horseshoe-like vertical orb web with a three-dimensional barrier tangle (arrow 1) dorsally above the spider, suspended residua (arrow 2) from the barrier web, and long sustaining threads (arrow 3) perpendicular or parallel to the orb. Note that a smaller individual builds an orb web at the opposite side utilizing the frame thread of the larger one. It is uncertain whether a long thread (arrow 4) is the original frame thread of the smaller one or a sustaining thread of the larger one. The average size of the orb web of adult female *T. clavata* is 42 cm in length and 40 cm in width (N = 13) (Shinkai 1985).



**Figure 3.** Habitus and the cocoon of *Eriostethus rufus*. The accurate body length of the wasp is 12.26 mm by scaling four split parts using the open source software ImageJ.

*Trichonephila clavata* is widely distributed and a very common spider throughout Japan (excluding Hokkaido) (Shinkai et al. 2020) and quite conspicuous spider in terms of size of both body and web, body coloration, and abundance, but nevertheless it has never been recorded as host spider of any polysphinctines. If this host record is correct, it is a first utilization case of *T. clavata* by polysphinctines and *E. rufus* thus utilizes hosts of two different subfamily-level clades, i.e. Nephilinae and the 'ARA-clade' (sensu Scharff et al. (2019), who

informally defined the ARA-clade consisting of typical araneids i.e. all Araneidae excluding Zygellinae, Nephilinae, and *Paraplectanoides*). Utilization of nephiline spiders by polysphinctine wasps has been known in two species of the genus *Hymenoepimecis* Viereck, 1912, parasitoids of *Trichonephila clavipes* (Linnaeus, 1767) in the Neotropics (Fincke et al. 1990; Gonzaga et al. 2010) and a species of the genus *Zatypota* Forster, 1869, a parasitoid of *Trichonephila plumipes* (Latreille, 1804) in Australia (Korenko et al. 2018b). Therefore, *Eriostethus* is the third genus known or suspected to parasitise Nephilinae.



**Figure 4.** A map of *Eriostethus rufus* distribution recorded so far. The numbers behind each region correspond to the region number in Table 1.

**Table 1.** Detailed distribution of *Eriostethus rufus* and references.

Distribution	Latitude	Reference
1. Zempoji 善宝寺 (a temple), Tsuruoka city, Yamagata Pref.	38°46' N	this study
2. Dogo Is. 島後島, Oki archipelago, Shimane Pref.	36°09-21' N	Hayashi & Kadowaki (2019)
3. Yorii town 寄居町 and Kinsho-zan 金勝山 (Mt.), Saitama Pref.	36°04-06' N	Konishi & Nambu (1997)
4. Takao-san 高尾山 (Mt.), Tokyo	35°37' N	Uchida (1932)
5. Kyoto Pref.	34°42'-35°47' N	Uchida (1932)
6. Kyoto city, Kyoto Pref.	35°19'-34°52' N	Masumoto et al. (2002)
7. Kiyotani-mura 清溪村, Settsu 摂津国 (currently the area across Izuhara 泉原, Ibaraki city and Takayama 高山, Toyono town 豊能町, Osaka Pref.)	34°52-54' N	Iwata (1942)
8. Hommura town 本村町, Shobara city, Hiroshima Pref.	34°49-53' N	Enoki (1995)
9. Mihara town 三原町, Mitsugi city, Hiroshima Pref.	34°49-50' N	Enoki (1995)
10. Takatsu town 高津町, Masuda city, Shimane Pref.	34°40-41' N	Nakamura & Matsuda (2005)
11. Nukui dam 温井ダム, Akiolta town 安芸太田町, Hiroshima Pref.	34°37-38' N	Konishi & Nakamura (2010)
12. Yamatokouriyama city, Nara Pref.	34°35-39' N	Matsumoto (2016)
13. Mukaiyokota town 向横田町, Masuda city, Shimane Pref.	34°34-37' N	Konishi & Nakamura (2010); Nakamura & Matsuda (2005)
14. Fukagawa 深川, Asakita ward, Hiroshima Pref.	34°29' N	Konishi & Nakamura (2010)
15. Odamiyama (a valley) 小田深山, Ehime Pref.	33°32-33' N	Konishi & Yamamoto (2000)
16. Aikodake (Mt.) 愛子岳, Yakushima (Is.), Kagoshima Pref.	30°21-23' N	Watanabe & Yamauchi (2014)

Although the host spider carcass was not found despite searching for it on both the web and the ground beneath the web, circumstantial evidence supports *T. clavata* being the host of this *E. rufus* individual: (1) there were many *T. clavata* constructing orb webs in the studied temple at a similar high surface of adjoining evergreen trees (Fig. 2); (2) there was no other orb-weaving spider species constructing large aerial webs between trees like *T. clavata* in the precincts (although searching was limited to daytime); (3) the inverted triangular long threads across two distant plant substrates (Fig. 1) are similar to the sustaining threads of a barrier web of *T. clavata* perpendicularly or radially extending from an orb/barrier web to adjacent substrates (Fig. 2); (4) the spider's residua are still suspended from the cocoon web (Fig. 1), a common habit of unparasitised *T. clavata*, which usually hang residua from the barrier web (Fig. 2; Ono & Ogata 2018). The residua convincingly suggest that the cocoon web used to be the pre-existing barrier web and its sustaining threads that were reused and modified. Therefore, when the spider's behaviour is manipulated, the original orb web must be completely removed and the pre-existing sustaining threads of the barrier web would be newly moored to the substrates, and multiplied/reinforced by repeated long silk dragging.

Adult female *T. clavata* frequently host several argyrodine kleptoparasitic spiders (Theridiidae), especially *Argyrodes bonadea* (Karsch, 1881) in the mainland of Japan (Shinkai 2007). However, the possibility that one of them is a potential host of *E. rufus* can be rejected, because they are too small (e.g. 2.5-3.3 mm in length of adult female *A. bonadea* (Ono & Ogata 2018)) to be a host of *E. rufus* and because the wasp cannot manipulate kleptoparasitic spider to modify the owner's web with the owner spider (*T. clavata*) still being live.

The modified cocoon web (Fig. 1) is unique in comparison to other cocoon webs previously reported in polysphinctines including species parasitic on nephilines (Gonzaga et al. 2010; Korenko et al. 2018b) in that the web structure is composed of multiple long threads spun between two perpendicular twigs, forming a triangle. This structure is clearly different from the spherical cocoon web of *N. mellottei* induced by the same species (Matsumoto 2016) and graphically illustrates that manipulated web-building behaviour can differ greatly within hosts of the same polysphinctine species. To build a spherical web, the behavioural configuration would consist of a bunch of short-distance silk dragging between two silk attachments, whereas to build or reinforce the inverted triangular web, it needs long-distance movements. This suggests that the identical manipulative mechanism of *E. rufus* induces different behavioural configuration depending on the host spider species, resulting in such heterogeneous cocoon webs. Because 10 *T. clavata* which were artificially removed their barrier webs immediately rebuilt barrier webs (Tanikawa 1998), the barrier web construction (probably including sustaining threads) is an independent behavioural unit, indicating that *E. rufus* can exploit the innate behavioural unit of barrier web construction plus the orb web removal in its manipulation of the host.

The location of the cocoon and surrounding structure are also characteristic (Fig. 1). In most cocoon webs, the cocoon is located at the hub of organized silk structures. In the case of *E. rufus* manipulating *N. mellottei*, the cocoon hung from the central portion of the spherical cocoon web (Matsumoto 2016) as if the web surrounds the cocoon. On the other hand, the cocoon of *Hymenoepimecis bicolor* (Brullé, 1846) and *Hymenoepimecis robertsae* Gauld, 1991 in the cocoon web of *T. clavipes* was attached to the hub of a disk-like structure similar to the core structure of an orb web (Gonzaga et al. 2010). The cocoon of *Zatypota kauros* Gauld, 1984 in the cocoon web of *T. plumipes* was hung from the hub of three-dimensional structure with a seemingly radial plane converging centrally (Korenko, et al. 2018b). However, the cocoon observed in this study hangs from an ill-defined part of the cocoon web of *T. clavata* without any organized structure surrounding the cocoon (Fig. 1).

Barrier webs of *T. clavata* are constructed on both sides of the orb web during the spider's immature stages and decrease in intricacy as the spider matures; however, they do not completely disappear even in the adult stage as the barrier web to the dorsal side of the spider is almost always retained (Fig. 2; Shinkai 1985). Although whether the

host spider in question was adult or juvenile is uncertain, the collecting season (19th September), the scale of the cocoon web (over 50 cm in length), and the size of the wasp (12.26 mm in length) suggest that the spider would have been an adult or at least subadult. Body length of adult female *T. clavata* widely ranges from 11 to 32 mm (Uemura 1967).

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