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New Distribution Records of Two Species of *Arge* and *Spinarge* (Hymenoptera, Argidae) from Kyushu, Japan

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Abstract Two argid sawflies, *Spinarge fulvicornis* (Mocsáry, 1909) and *Arge naokoae* Shinohara and Hara, 2013, are newly recorded from Kyushu based on the discovery of the larvae in Fukuoka Prefecture and Oita Prefecture, respectively. The larvae were identified by the molecular analysis using the mitochondrial cytochrome oxidase subunit 1 (CO1) gene sequences.
Key words: Symphyta, Argidae, new distribution records, Kyushu, CO1 sequences.

Introduction

The argid sawfly subfamily Arginae in Kyushu has not been well investigated. A total of eleven species of the genus *Arge* Schrank, 1802, and two species of the genus *Spinarge* Wei, 1998, are known to occur in this island (Yoshida, 2016) but the material so far available for study is quite limited. An extensive sampling over the island will probably reveal the occurrence of several more species of the subfamily.

In the course of a sawfly survey in northern Kyushu in October, 2014, A. Shinohara found larvae of two argine species, which were apparently new to the fauna of Kyushu. One of them was discovered feeding on the leaves of *Pourthiaea villosa* (Thunb.) Decne. var. *villosa* [Rosaceae] on Mt. Hikosan, Fukuoka Prefecture and it closely resembled *Spinarge fulvicornis* (Mocsáry, 1909), a widely distributed species known from Japan (Hokkaido, Honshu and Shikoku), Russian Far East (Sakhalin), Korea and China (Jilin) (Hara and Shinohara, 2006). Another species, whose larvae were found on the leaves of *Spiraea dasyantha* Bunge [Rosaceae] in Usa, Oita Prefecture, resembled *Arge naokoae* Shinohara and Hara, 2013, which was known to occur in Japan (Honshu and Shikoku) (Shinohara and Hara, 2013). Because the specific identification of *Arge* and *Spinarge* based only on the larval stage is not decisive, attempts were made to compare the mitochondrial cytochrome oxidase subunit 1 (CO1) gene sequences between the larvae and correctly identified adults and larvae obtained from other areas, and to obtain the adults by rearing. We did not try to rear the larvae associated with *Spiraea* in Kyushu, because only two individuals were available.

Here we give the results of molecular analysis and rearing, which clearly showed that the *Pourthiaea*-feeding larvae belonged to *S. fulvicornis* and the *Spiraea*-feeding larvae belonged to *A. naokoae*. This is the first record of *S. fulvicornis* and *A. naokoae* from Kyushu.

Materials and Methods

Rearings were made in an air-conditioned room in Tokyo by A. Shinohara. In the rearing room, the temperature was usually 22–25°C and

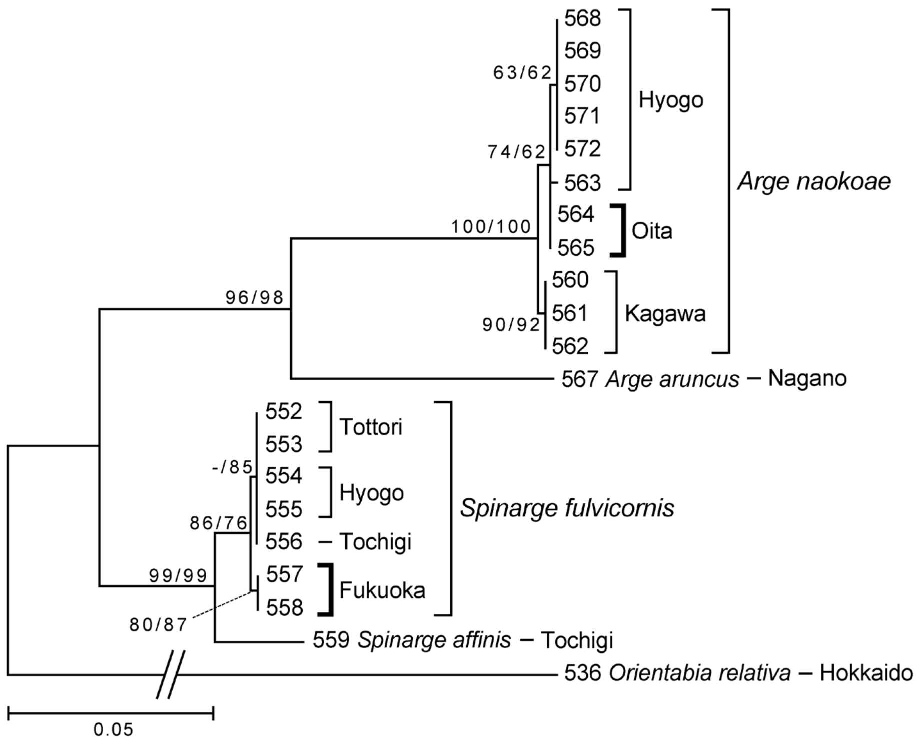

Fig. 1. The maximum likelihood tree showing phylogenetic relationships among *Arge* and *Spinarge* species (-ln likelihood = 1755.5787). Numbers on branches indicate bootstrap values for MP and ML analyses (shown only for higher nodes with >50). The number of each terminal label represents the sample ID for the individual.

the light was usually on for about 16 hours a day. Images of the larvae were taken with digital cameras, Canon EOS Kiss Digital X (Fig. 2) and Ricoh Caplio GX100 (Fig. 3), and the digital images were processed and arranged with Adobe Photoshop Elements 12.0 software. Scientific names of the host plants follow Yonekura and Kajita (2016).

To identify the obtained larvae by nucleotide sequences, we sequenced a partial region of mitochondrial CO1 gene, which is generally used for DNA barcoding in various insects. DNA extraction, PCR amplification, and sequencing were performed following the previously described method (Shinohara *et al.*, 2016). The obtained sequences have been deposited in the DDBJ/EMBL/GenBank database under accession numbers LC133470–LC133490.

The alignment of the CO1 gene required no gaps. Phylogenetic trees were obtained by maximum parsimony (MP) and maximum likelihood (ML) methods implemented in MEGA6 (Tamura *et al.*, 2013). For MP analyses, heuristic searches were conducted with 10 random addition analyses using equal character weights and tree-bisection-reconnection (TBR) branch swapping. Prior to ML analysis, we determined an appropriate model of sequence evolution and model parameters using MEGA. As a result, GTR + I model was selected. Based on the selected model, ML analysis was performed with heuristic searches with subtree-pruning-regrafting-extensive (SPR) branch swapping. Nodal support for the MP and ML analyses was assessed using bootstrap analyses with 1000 replications.

Table 1 shows the samples used. As outgroups, *Orientabia relativa* (Rohwer, 1910), a Cimbicidae, and two Argidae were selected. One of the argids is *Spinarge affinis* Hara and Shinohara, 2006, a member of the *S. fulvicornis* group (Hara

Figs. 2, 3. Larvae of *Spinarge* and *Arge* species.—2, *Spinarge fulvicornis*, mature larva, photographed on October 18, 2014; 3, *Arge naokoae* on *Spiraea dasyantha*, photographed on October 16, 2014.

and Shinohara, 2006) and the other is *Arge aruncus* Hara and Shinohara, 2012, which resembles *A. naokoae* most among the Japanese congeners (Shinohara and Hara, 2013). These samples and all the reared adult specimens referred to below are kept in the National Museum of Nature and Science, Tsukuba (NSMT).

Results and discussion

Rearing records

On October 4, 2014, A. Shinohara found six argid larvae solitarily feeding on *Pourthiaea villosa* var. *villosa* on Mt. Hikosan at an altitude of 750 m (33.49N 130.92E), Soeda, Fukuoka Prefecture. Four of these matured and made cocoons on October 8. Two others matured on October 18 (one of them shown in Fig. 2) and they are fixed in ethanol for molecular studies. One female adult emerged on March 8, one male and one female emerged on March 23, and one female emerged on March 25, 2015. These adults have been identified as *S. fulvicornis*.

On October 16 of the same year, A. Shinohara found two argid larvae feeding on *Spiraea dasyantha* in Hinotake at an altitude of 400 m (33.42N 131.26E), Innai, Usa, Oita Prefecture (Fig. 3). One of them matured on October 17 but failed to make a cocoon. The two larvae were fixed in ethanol for molecular works on October 18 and 27, respectively.

Molecular analysis

The CO1 data matrix consisted of 669 nucleotide sites, of which 74 were parsimony-informative. The MP analysis resulted in 10 most parsimonious trees (210 steps, consistency index excluding uninformative sites [CI] = 0.8350, retention index [RI] = 0.9669). The result of ML analysis was consistent with the MP analysis.

In the obtained tree (Fig. 1), the two larvae collected on *Pourthiaea* in Kyushu (557 and 558) belonged to the same clade with the samples of *Spinarge fulvicornis* from Honshu (552–556) with 86% MP and 76% ML bootstrap supports. Though the samples from the two islands show small differences (two base differences), we regard the Kyushu specimens as *S. fulvicornis*. The two larvae feeding on *Spiraea* in Kyushu form a clade with the samples of *Arge naokoae* from Honshu and Shikoku with 100% MP and ML bootstrap supports. The Kyushu larvae doubtless belong to *A. naokoae*.

Concluding remarks

Based on the results of rearing and molecular analyses, we record *Spinarge fulvicornis* and *Arge naokoae* from Kyushu for the first time. The occur-

Table 1. Material used for molecular analysis. All collected and reared by A. Shinohara.

	Species		Island	Prefecture	Locality	Host plant	Collecting and Rearing data
552	*Spinarge fulvicornis*	larva	Honshu	Tottori	Yokotemichi, Mt. Daisen	*Sorbus commixta*	collected 2. X. 2010, dead and fixed 10. X.
553	*Spinarge fulvicornis*	larva	Honshu	Tottori	Yokotemichi, Mt. Daisen	*Sorbus commixta*	collected 2. X. 2010, dead and fixed 10. X.
554	*Spinarge fulvicornis*	larva	Honshu	Hyogo	Shiawase-no-mura, Kobe	*Pourthiaea villosa* var. *villosa*	collected 20. X. 2010, fixed 22. X.
555	*Spinarge fulvicornis*	larva	Honshu	Hyogo	Shiawase-no-mura, Kobe	*Pourthiaea villosa* var. *villosa*	collected 20. X. 2010, fixed 22. X.
556	*Spinarge fulvicornis*	♀	Honshu	Tochigi	Hanaishi-cho, Nikko	*Pourthiaea villosa* var. *villosa*	collected 4. IX. 2014, matured 5. IX., emerged 29. IX. 2014
557	*Spinarge fulvicornis*	larva	Kyushu	Fukuoka	Mt. Hikosan	*Pourthiaea villosa* var. *villosa*	collected 4. X. 2014, matured and fixed 18. X.
558	*Spinarge fulvicornis*	larva	Kyushu	Fukuoka	Mt. Hikosan	*Pourthiaea villosa* var. *villosa*	collected 4. X. 2014, matured and fixed 18. X.
560	*Arge naokoae*	♀	Shikoku	Kagawa	Mt. Shippozan	*Spiraea dasyantha*	collected 9. X. 2012, matured 10. X., emerged 17. IV. 2013
561	*Arge naokoae*	♀	Shikoku	Kagawa	Mt. Shippozan	*Spiraea dasyantha*	collected 9. X. 2012, matured 11. X., emerged 28. IV. 2013
562	*Arge naokoae*	♀	Shikoku	Kagawa	Mt. Shippozan	*Spiraea dasyantha*	collected 9. X. 2012, matured 14. X., emerged 2. XI. 2012
563	*Arge naokoae*	♂	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 16. IX. 2014, matured 20. IX., emerged 7. X. 2014
564	*Arge naokoae*	larva	Kyushu	Oita	Higatake	*Spiraea dasyantha*	collected 16. X. 2014, fixed 18. X.
565	*Arge naokoae*	larva	Kyushu	Oita	Higatake	*Spiraea dasyantha*	collected 16. X. 2014, matured 17. X., fixed 27. X.
568	*Arge naokoae*	larva	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 9. VI. 2015, fixed 11–14. VI.
569	*Arge naokoae*	larva	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 9. VI. 2015, fixed 11–14. VI.
570	*Arge naokoae*	larva	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 9. VI. 2015, fixed 11–14. VI.
571	*Arge naokoae*	larva	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 9. VI. 2015, fixed 11–14. VI.
572	*Arge naokoae*	larva	Honshu	Hyogo	Takedao	*Spiraea dasyantha*	collected 9. VI. 2015, fixed 11–14. VI.
559	*Spinarge affinis*	larva	Honshu	Tochigi	Hanaishi-cho, Nikko	*Rhododendron quinquefolium*	collected 24. VII. 2010, fixed 5. VIII.
567	*Arge aruncus*	larva	Honshu	Nagano	Shiga-kogen	*Aruncus dioicus*	collected 9. IX. 2009, fixed 17. IX.
536	*Orientabia relativa*	♀	Hokkaido		Tokachi-shimizu		collected 24. VI. 2013

rence of the two species in Kyushu was expected but no evidence had been available so far.

Spinarge fulvicornis is very similar to *S. affinis* Hara and Shinohara, 2006, *S. prunivora* Hara and Shinohara, 2006, and *S. pumila* Hara and Shinohara, 2006. The four species, composing the *S. fulvicornis* group (Hara and Shinohara, 2006), are barely distinguishable by the shape of the lancet in the adult morphology, but their larvae are quite different in the host preference and behavior (Shinohara and Hara, 2010, 2011). The molecular analysis (Fig. 1) also suggested that *S. affinis* is closely related to *S. fulvicornis* but is certainly different from the latter at the species level. Within *S. fulvicornis*, two specimens from Kyushu and five specimens from Honshu formed different clusters, suggesting some geographical divergence at the molecular level.

Arge naokoae was described from Hyogo Prefecture, western Honshu, and Kagawa Prefecture, Shikoku. Only one female adult has been collected in the field. All the other material was obtained as larvae feeding on *Spiraea dasyantha* in the field, though the larvae also feed on *S. cantoniensis* under rearing conditions (Shinohara and Hara, 2013). It is interesting that the Kyushu samples were grouped with Honshu specimens, not with the Shikoku samples, in our tree (Fig. 1). The two Kyushu samples and the six Honshu samples examined were not clearly separable in our analysis.

The adult argids are not easy to collect in the field, because they are active only during the daytime and under favorable weather conditions. We can find the larvae more easily, because they always stay on the leaf of the host plant clinging to the leaf margin under all weather conditions. Employing molecular methods for identifying the larvae, as we did in the present work, is quite useful for investigating local fauna of the Argidae.

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