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<Original Article>

Molecular Phylogenetic Affinities of Japanese Xenid and Stylopid Strepsipterans (Strepsiptera : Xenidae, Stylopidae) Parasitizing Wasps and Bees

Yuichi ISAKA[1], Souhei UEDA[2] and Takao ITINO[2,3]

Abstract : Strepsiptera is an insect order comprising 11 families with about 600 described obligate endoparasitoid species. In Strepsiptera, Xenidae and Stylopidae are the most diverse, Hymenoptera-parasitizing families. Here, we reconstructed a phylogenetic relationships of xenid and stylopid strepsipterans in Japan by using molecular data sets of the mitochondrial cytochrome oxidase subunit I gene (*COI*). The results of our molecular phylogenetic analysis agree well with the results of the latest morphologically based phylogenetic aspect. In addition, we identified two distinct mitochondrial lineages in *Xenos moutoni*, which parasitizes different host species.

Key words : *COI*, cryptic host race, host specificity

Introduction

Strepsiptera (twisted-wing parasites) are an insect order comprising 11 families with about 600 described species (Pohl and Beutel, 2005). Although most entomologists have probably heard or read about this group of unusual, obligate endoparasitoids, it is likely that very few have actually seen live specimens (Kathirithamby, 2009). They have very complex life cycle : males spend the major part of their life cycle as larval endoparasitoids, but have a short, free-living adult stage, whereas the females are ovoviviparous and neotenic (larviform) (except in Mengenillidae) and spend their entire life in the host. The first-instar larva of males and females is a free-living, host-seeking stage (Kathirithamby, 1989). The array of hosts includes various insect taxa, such as Zygentoma, Blattaria, Mantodea, Orthoptera, Hemiptera, Hymenoptera and Diptera (Pohl and Beutel, 2005).

The Hymenoptera-parasitizing families Xenidae and Stylopidae are the most diverse groups in Strepsiptera. Xenidae (with about 110 described species) are characterized (and distinguished from Stylopidae) mainly by their exclusive use of various species of wasps (Crabronidae, Sphecidae and Vespidae) as hosts. Stylopidae (with about 160 described species) exclusively parasitize bees (Apidae) (Pohl and Beutel, 2008).

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In Japan, eight xenid (belonging to three genera) and 29 stylopid (two genera) species have been recorded. Most host species of them are parasitized by only one xenid or stylopid species; *i.e.*, most Xenidae and Stylopidae species are specific to a single host species (Kifune, 1992). However, some species parasitize several host species among all described Xenidae and Stylopidae species (Kathirithamby, 1989; Maeta and Kifune, 1990). For example, *Xenos moutoni* has been recorded from six host species in Japan (Kifune, 1992; Kifune and Maeta, 1990). Kathirithamby (2009) suggested that xenid and stylopid apparently polyphagous species might actually comprise several cryptic species.

In the latest study (Pohl and Beutel, 2005) suggested the phylogenetic affinity of strepsipterans based on 189 morphological characters of 37 genera. In addition, Kathirithamby (2009) reconstructed phylogenetic relationships among seven families by using molecular data sets.

However, no molecular study has described the phylogenetic relationships among genera. Therefore, in this study, we reconstructed a phylogenetic relationship of Japanese xenid and stylopid genera based on the mitochondrial cytochrome oxidase subunit I gene (*COI*), and investigated whether the apparently polyphagous species *X. moutoni* in fact comprised several cryptic species.

Materials and Methods

Collecting samples and DNA extraction, amplification and sequencing

A total of 27 twisted-winged insects were collected and used for the molecular phylogenetic analyses (Table 1). For an outgroup, we used *Elenchus japonicus* (Elenchidae), which belongs to Stylopiformia, the superfamily to which Xenidae and Stylopidae also belong (Pohl and Beutel, 2005). All samples were fixed in 99.5% ethanol and preserved in that solution until dissection.

Total genomic DNA was extracted from the tissue of each specimen with a DNeasy Tissue Kit (Qiagen), following the manufacturer's protocol. A partial *COI* gene (819 bp) corresponding to positions 2195-3014 in the *Drosophila yakuba* mtDNA genome was amplified by polymerase chain reaction (PCR) with the primers C1-J-2195 (5'-TTGATT TTTTGGTCATCCAGAAGT-

Table 1 Strepsiptera specimens analyzed in this study.

Sample No.	Strepsipterans	Hosts		Localities		GenBank
	Specimens	Species	Family	Locality	Prefecture	accession No.
	Xenidae					
1	*Xenos moutoni*	*Vespa mandarinia*	Vespidae	Matsumoto	Nagano	AB705463
2	*Xenos moutoni*	*Vespa ducalis*	Vespidae	Nagano	Nagano	AB705464
3	*Xenos moutoni*	*Vespa simillima*	Vespidae	Matsumoto	Nagano	AB705465
4-10	*Xenos moutoni*	*Vespa analis*	Vespidae	Matsumoto	Nagano	AB705466-AB705472
11	*Xenos japonicus*	*Vespula shidai*	Vespidae	Gero	Gifu	AB705473
12	*Pseudoxenos iwatai*	*Anterhynchium flavomarginatum*	Eumenidae	Matsumoto	Nagano	AB705474
13-17	*Paraxenos nagatomii*	*Bembicinus bimaculatus*	Sphecidae	Iriomote Is.	Okinawa	AB705475-AB705479
	Stylopidae					
18	*Stylops kaguyae*	*Andrena kaguya*	Andrenidae	Matsumoto	Nagano	AB705480
19	*Stylops kaguyae*	*Andrena kaguya*	Andrenidae	Sakuragawa	Ibaraki	AB705481
20	*Stylops collinus*	*Andrena nawai*	Andrenidae	Matsumoto	Nagano	AB705482
21	*Halictoxenos japonicus*	*Halictus aerarius*	Halictidae	Matsumoto	Nagano	AB705483
22	*Halictoxenos hondonis*	*Lasioglossum exiliceps*	Halictidae	Matsumoto	Nagano	AB705484
	Elenchidae					
23	*Elencus japonicus*	Gen. sp.	Delphacidae	Matsumoto	Nagano	AB705485

3') and TL2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Simon *et al.*, 1994), and using the following PCR temperature profile : 95°C for 10 min ; 35 cycles of 95°C for 30s, 45°C for 30s, and 72°C for 40s ; and 72°C for 8 min. After amplification, PCR products were purified with Qiagen PCR purification kits. Cycle sequencing reactions for both strands were performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI 377 automated sequencer (Applied Biosystems).

Phylogenetic analysis

DNA sequences were aligned using Clustal X (Thompson *et al.*, 1997) by using the default parameter settings, and ambiguously aligned regions were eliminated before further analysis. Substitution saturation in the *COI* dataset was checked by a satulation test (Xia *et al.*, 2003) implemented in the program DAMBE (Xia and Lemey, 2009). A saturation plot of the *COI* did not show signs of substitution saturation, which was confirmed by statistical test for saturation (*COI* ; Iss=0.3951, Iss. cSym=0.7225, $P<0.00001$).

We analyzed the data set with MrModeltest version 2.3 (Nylander, 2004), which uses the hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC) to identify the simplest substitution model in which the addition of parameters does not result in significant improvement (Posada and Crandall, 1998). Both the hLRT and the AIC indicated that a General Time Reversible (Tavaré, 1986) plus Gamma (GTR+G) model were optimal for our data set. We then performed a Bayesian phylogenetic analysis with MrBayes version 3.1.2 (Ronquest and Huelsenbeck, 2003). We used a default prior analysis, running one cold and three incrementally heated chains for five million generations while sampling trees from the current cold chain every 100 generations. The first 125 sampled trees were discarded as a burn-in, and the last 375 trees were used to calculate a Bayesian majority-rule consensus tree, in which the proportion of times that a clade was observed was used as to estimate its posterior probability.

Results and Discussion

A total of 434 nucleotide sites of the *COI* gene were available for the phylogenetic analyses. Bayesian phylogenetic analysis resolved relationships within and between Xenidae and Stylopidae (Fig. 1). Most of the nodes were supported by high posterior probability values. The topology obtained by the Bayesian analysis was the following : Xenidae ((*Xenos*+*Pseudoxenos*)+(*Paraxenos*+*Xenos*)), Stylopidae (*Stylops*+*Halictoxenos*). Xenidae and Stylopidae, respectively, each formed a basically monophyletic group. Pohl and Beutel (2005) carried out a cladistic analysis based on broad morphological data sets, and they found the following branching pattern for Xenidae and Stylopidae relationships: Xenidae (*Paraxenos*+(*Xenos*+*Pseudoxenos*))+ Stylopidae (*Hylecthrus, Crawfordia*+(*Halictoxenos*+*Stylops*)). The topology of the tree resulting from the present study was similar to those of Pohl and Beutel (2005). In Japan, Xenidae and Stylopidae comprise five genera (*Xenos, Pseudoxenos, Paraxenos, Stylops* and *Halictoxenos*), *Stylops* and *Halictoxenos* were revealed as monophyletic groups in this study supported by high posterior probabilities. Whether *Paraxenos* and *Pseudoxenos* were monophyletic or not could not be established because each group was represented by only one species. While, *Xenos* was polyphyletic,

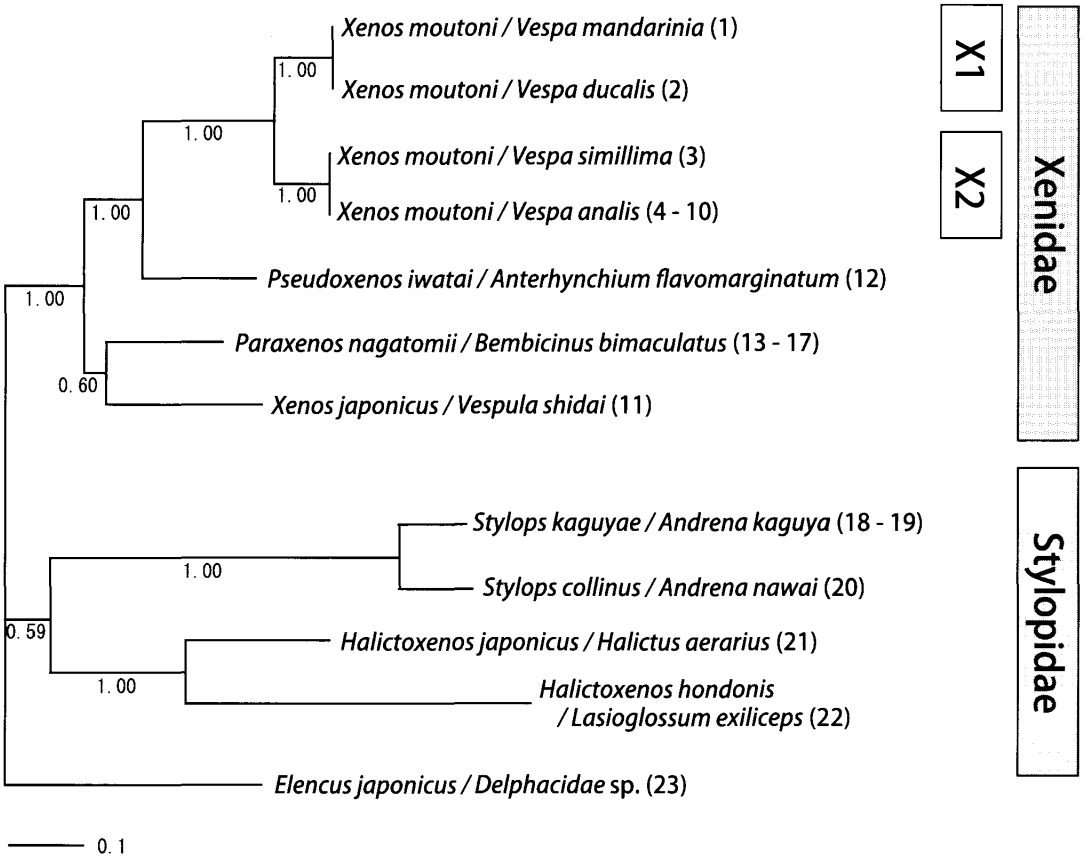

Fig. 1 Bayesian tree based on the mitochondrial *COI* gene sequences of Japanese Xenidae and Stylopidae. The number on the branches indicate Bayesian posterior probabilities. Both the Strepsiptera species (left of slash) and their host species (right of slash) are shown, along with the sample number (see Table1).

with *X. japonicus* belonging to independent clade (Fig. 1). Although in general *X. japonicus* is morphologically similar to other *Xenos* species, the morphological structure of the male aedeagus is remarkably different (Kifune and Maeta, 1975). In fact, for *X. japonicus*, Kifune and Maeta (1975) proposed a new subgenus establishment, which they called *Nipponxenos*.

In general, Xenidae parasitizes hunting wasps (Crabronidae, Sphecidae and Vespidae) and Stylopidae parasitizes bees (Apidae) (Pohl and Beutel, 2008). The specificities of strepsipteran genera to host families recognized in this study are consistent with those described by Kifune and Maeta (1990) : namely, *Xenos*-Vespidae, *Pseudoxenos*-Eumenidae, *Paraxenos*-Sphecidae, *Stylops*-Andrenidae and *Halictoxenos*-Halictidae (Table 1).

In this study, it is revealed that *X. moutoni* comprised two lineages (X1 and X2 ; Fig. 1). X1 parasitized *V. ducalis* and *V. mandarinia*, and X2 parasitized *V. analis* and *V. simillima*. Although Kifune (1992) reported that *X. moutoni* is a monotypic species, our molecular data suggest that *X. moutoni* comprises at least two cryptic host races, and support that in general the diversity of Strepsiptera species that parasitize several host species may be underestimated, as Hayward *et al.* (2011) proposed.

As hosts of *X. moutoni*, Kifune (1992) recorded six species of *Vespa* (*V. analis, V. crabro, V. ducalis, V. dybowskii, V. mandarinia* and *V. simillima*). Further molecular analysis of *X. moutoni* parasitizing *V. crabro* and *V. dybowskii* (not sampled in this study) should shed additional light on the host relationships of this species. As the hosts of X1 and X2 (X1, *V. ducalis* and *V. mandarinia*; X2, *V. analis* and *V. simillima*) are all sympatric in Japan, X1 and X2 might be reproductively isolated. In this regard, Kathirithamby and Johnston (2004) argued that *Caenocholax fenyesi* (Myrmecolacidae; Strepsiptera) included cryptic species, based on the large molecular divergence between morphologically similar males from distant populations.

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