Appl Entomol Zool (2012) 47:319–329
DOI 10.1007/s13355-012-0122-x

ORIGINAL RESEARCH PAPER

Occurrence of two acarivorous species of the genus *Feltiella* (Diptera: Cecidomyiidae) in Okinawa, southern Japan, and redescription of *F. acarivora* (Zehntner)

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Received: 8 June 2012 / Accepted: 8 July 2012 / Published online: 29 July 2012

Abstract Based on morphological features and molecular information, we record the occurrence of two acarivorous species of the genus *Feltiella* (Diptera: Cecidomyiidae) on some islands of Okinawa Prefecture, southern Japan. They are *Feltiella acarivora* (Zehntner), which is new to Japan, and *Feltiella acarisuga* (Vallot). *F. acarivora* is redescribed because the original description was incomplete. *Feltiella acarivora* was distinguished from *F. acarisuga* by having the following characteristics of male terminalia: hypoproct a little shorter than aedeagus, slightly tapering to a blunt apex with an apical ligule and gonocoxite with a hairy mediobasal lobe. For each species, we provide information on its geographic distribution, possible prey mites, and plant species on which the mites and *Feltiella* larvae or pupae were found. Through our surveys, we observed *F. acarivora* coexisting with at least eight species of tetranychid mites on various plant species, including fruit trees and vegetable crops. These observations suggest that *F. acarivora* and *F. acarisuga* contribute to the control of pest tetranychid mites in Okinawa.

Keywords *Feltiella* · Acarivorous cecidomyiid · Tetranychid mite · Distribution · Okinawa

Introduction

The genus *Feltiella* (Diptera: Cecidomyiidae) contains ten nominal species worldwide. The larvae of all known species of this genus attack tetranychid mites (Gagné 2010), and some of them have been regarded as important natural enemies of pest tetranychids (e.g., Barnes 1933; Nijveldt 1969; Gagné 1995). *Feltiella acarisuga* (Vallot) is now commercially available as a biological control agent for use against tetranychid mites (Zhang 2003).

Abe et al. (2011) reported that *F. acarisuga* is widely distributed in Japan from Hokkaido (north) to Okinawa (south). In that paper, the authors suspected the occurrence of one or two additional species of *Feltiella* in Kochi and Okinawa prefectures, but these species were left unidentified because of a lack of male specimens. Independently from Abe et al. (2011), we had opportunities to collect larvae of acarivorous cecidomyiids from some islands of Okinawa Prefecture during the course of faunistic surveys of tetranychid mites in 2007–2011 (Ohno et al. 2009, 2010, 2011). Our preliminary genetic analysis of these cecidomyiids indicates the possible occurrence in Okinawa of a *Feltiella* species other than *F. acarisuga*.

The aims of the work described in this paper were (1) to classify the acarivorous cecidomyiids found in Okinawa based on morphological features and molecular sequencing data, (2) to redescribe *Feltiella acarivora*, and (3) to provide information on their geographic distribution, possible

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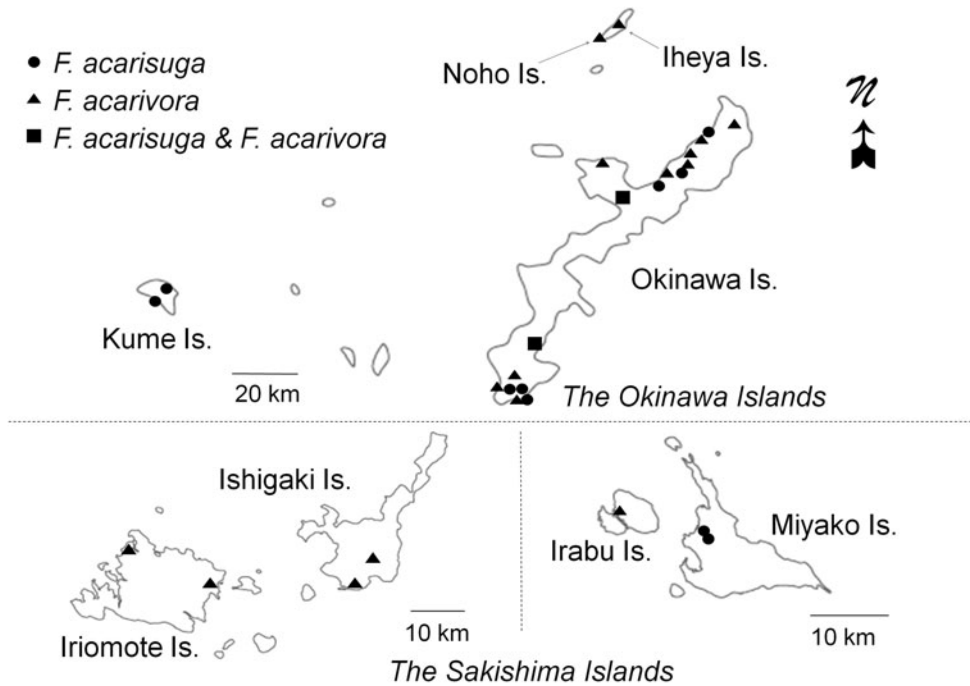

Fig. 1 Map of major islands in Okinawa Prefecture. Islands with names were surveyed. *Each black mark* shows a locality from which *Feltiella* species were collected

prey tetranychids, and plant species on which the mites and *Feltiella* larvae or pupae were found. We also report on parasitoids that attack the *Feltiella* species. The discovery of an additional *Feltiella* species in Japan will facilitate the use of indigenous natural enemies of tetranychids for pest management, particularly in Okinawa, where the tetranychid fauna is notably different from that of the mainland of Japan (Ohno et al. 2009, 2010, 2011).

Materials and methods

Collection, identification, and morphological description

In 2007–2009, leaves of crops and wild plants bearing tetranychid mites were collected from greenhouses or open fields on eight islands (four of the Okinawa Islands and four of the Sakishima Islands) in Okinawa Prefecture, Japan (Fig. 1). The leaves collected were examined under a binocular microscope to find *Feltiella* larvae or their cocoons. When mature larvae or cocoons were found, they were kept in plastic bags or rearing containers together with host leaves of tetranychid mites and paper towels to absorb moisture. The emergence of adults was monitored almost daily. Some of the mature larvae and all emerging adults and pupal exuviae were preserved in 70 or 99 % ethanol. For microscopic observations, the ethanol-preserved specimens were macerated in a solution of 5 % KOH, transferred successively to water, ethanol, and xylene, and slide mounted in Canada balsam. Species identification was based on Gagné (1995). To combine the morphological identification results with DNA sequencing data, the wings and terminalia of some male specimens were mounted on slides, and the remaining body parts were subjected to genetic analysis. Females were considered to be identical to the associated males when only one species of *Feltiella* was obtained from a particular locality on a particular occasion. Voucher specimens of *Feltiella* are deposited in the collection of the Entomological Laboratory, Kyushu University, Fukuoka City, Japan.

For tetranychid mites, living adults or specimens preserved in 70 % ethanol were mounted on slides with Hoyer's medium. Identification was based on Ehara and Gotoh (2009). Voucher specimens of the tetranychid mites are deposited in the collection of the Okinawa Prefectural Agricultural Research Center, Itoman City, Japan.

For morphological descriptions, drawings were made with the aid of a drawing tube. Adult morphological terminology follows McAlpine (1981), except for thoracic plates, the terminology of which follows Yukawa and Ohsaki (1988), and the terminology of pupae and cuticular spinules on the pupal abdomen, which follows Gagné (1994). Larvae were not described because they were all used to obtain adults. Pupae were described based on pupal exuviae that had been kept in 70 % ethanol after adult emergence.

Genetic analysis

In this paper, genetic analysis was only used to show genetic differences between the species treated in this paper and to contribute to the DNA barcoding project (Hebert et al. 2003) by registering the sequencing data to GenBank, etc. for future larval identification. We used fresh material from acarivorous gall midges for genetic analysis (see Tables 1, 2). For some individuals, the wings and male terminalia were removed for slide-mounted specimens, and the total DNA was extracted from the remaining body parts with the DNeasy blood and tissue kit (Qiagen, Tokyo, Japan), following the manufacturer's instructions. For other individuals, the total DNA was extracted from the whole bodies of larvae, pupae or adults, and sequencing data for them were compared with those for individuals that were identified based on the removed male terminalia.

A region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA (mtDNA) was amplified according to the PCR method described in Yukawa et al. (2003) and purified using a QIAquick PCR purification kit (Qiagen). The primers used in the analysis were: forward, LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ (Folmer et al. 1994); reverse, C1-N-2329 5′-ACT GTA AAT ATA TGA TGA GCT CA-3′ (Simon et al. 1994). The estimated length of the amplified region is 814 bp (Yukawa et al. 2011), including a partial COI region employed for the DNA barcoding project (658 bp from the 5′ end) (Hebert et al. 2003), and a region used for the molecular phylogenetic analysis of Cecidomyiidae (439 bp in the middle) (e.g., Yukawa et al. 2003; Uechi et al. 2003; Tokuda et al. 2004, 2008). The sequencing reaction was performed using PCR primers and the BigDye Terminator cycle sequencing reaction kit (Applied Biosystems, Foster City, CA, USA), and was electrophorased on an ABI 3100 sequencer (Applied Biosystems). The nucleotide sequence data reported in this paper were deposited into the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory, and the GenBank nucleotide sequence databases with the accession numbers shown in Tables 1 and 2.

The sequence data were analyzed with the neighbor-joining (NJ) and maximum-parsimony (MP) methods using PAUP* 4.0b10 (Swofford 2002). Evolutionary distances for the NJ method were computed by Kimura's two-parameter distances (Kimura 1980). In the MP method, the most parsimonious trees were determined under the heuristic search procedure with a TBR branch swapping algorithm. The resulting trees were subjected to bootstrap analysis (Efron 1982; Felsenstein 1985) with 1,000 pseudoreplications. When constructing phylogenetic trees, three cecidomyiid species—*Resseliella odai* (Inouye) (DDBJ accession no. AB5060017), *Dasineura rosae* (Bremi) (DDBJ accession no. AB505981), and *Pitydiplosis puerariae* Yukawa, Ikenaga and Sato (DDBJ accession no. AB614600 and AB614619)—were employed as outgroup taxa.

Results

Identification and redescription of *F. acarivora*

Based on morphological studies, we identified specimens of acarivorous gall midges from various localities in Okinawa as *F. acarisuga* and *F. acarivora* (Zehntner), among which the latter is new to Japan. Because *F. acarisuga* was redescribed in Abe et al. (2011), we provide here only some data on the collection of *F. acarisuga* in Okinawa. The original description of *F. acarivora* (Zehntner 1901) was incomplete for species comparison (Gagné 1995). Therefore, we redescribe *F. acarivora* in this paper using collection data. Genetic analysis also supported the occurrence of two *Feltiella* species in Okinawa.

Feltiella acarisuga (Vallot, 1827)

See Gagné (1995, 2004) for the synonym list of *F. acarisuga.*

Japanese name. Hadani Tamabae.

Specimens examined See Table 1 for the slide-mounted specimens examined.

Distribution Japan (Abe et al. 2011), Australia, widespread Europe, India, Israel, Morocco, New Zealand, Sri Lanka, Taiwan (Gagné 1995), and South Korea (Lee et al. 2004). In Okinawa, Japan, we found *F. acarisuga* on Kume Island for the first time, in addition to Okinawa and Miyako, from where it was previously known (Fig. 1).

Possible prey mites found in Okinawa and their host plants In addition to *Tetranychus piercei* McGregor that was previously found in Okinawa (Abe et al. 2011), we record for the first time the following tetranychid species that coexisted on the same plants with *F. acarisuga*: *Oligonychus biharensis* (Hirst), *Tetranychus kanzawai* Kishida, *Tetranychus neocaledonicus* Andre, *Tetranychus okinawanus* Ehara, *Tetranychus parakanzawai* Ehara, and *Tetranychus urticae* Koch (green form) (Table 1). In Okinawa, *Feltiella acarisuga* was found on nine plant species across six plant families (Table 1).

Feltiella acarivora (Zehntner 1901)

See Gagné (1995, 2004) for the synonym list of *F. acarivora.*

Japanese name: Minami Hadani Tamabae.

Male Eye bridge 6–7 facets long. Occiput with a short dorsal protuberance. Frons with usually 6, occasionally 7 clypeal setae. Palpus three-segmented; first palpal segment 1.0–1.3 times as long as wide; second 1.8–2.8 times as long as first; third 0.8–1.1 times as long as second.

Table 1 Specimens of *Feltiella acarisuga* that were used for genetic analysis or mounted on slides

Specimens used	Collection site	Collecting date of larvae or pupae	Date of adult emergence	Coexisting tetranychid mite	Plant name (family)	Collector(s)[a]	Accession and slide numbers[b]
3 ♂	Kawaraya, Utsunomiya City, Tochigi Pref.	12 Jun. 2002	Unknown	*Tetranychus kanzawai*	*Pueraria lobata* (Fabaceae)	MM	AB698978-80 (A035-037) AKJ012001-03
2 L	Hakozaki, Fukuoka City	21 Nov. 2007	–	*Tetranychus evansi*	*Solanum nigrum* (Solanaceae)	SO, TO	AB698981-82 (H013, 021)
1 ♂	Hakozaki, Fukuoka City	21 Nov. 2007	30 Nov. 2007	*Tetranychus evansi*	*Solanum nigrum* (Solanaceae)	SO, TO	KS07201
1 P	Koorimoto, Kagoshima City	3–6 Feb. 2008	–	*Tetranychus evansi*	*Lycopersicon esculentum* (Solanaceae)	YS	AB698983 (H027)
5 ♂♂, 2 ♀♀	Koorimoto, Kagoshima City	3–6 Feb. 2008	15–25 Feb. 2008	*Tetranychus evansi*	*Lycopersicon esculentum* (Solanaceae)	YS	AKJ015001-07
1 ♂	Hentona, Kunigami Okinawa Is.	31 Jul. 2008	14 Aug. 2008	*Tetranychus parakanzawai*	*Melanolepis multiglandulosa* (Euphorbiaceae)	SO	AB698984 (H035) KS37401
3 ♂♂	Oshikawa, Oogimi, Okinawa Is.	8 Aug. 2009	25–26 Aug. 2009	*Tetranychus okinawanus*	*Bidens pilosa* (Asteraceae)	SO, TGK	KS94901-03
1 ♂	Tsuha, Oogimi, Okinawa Is.	7 Aug. 2008	11–16 Aug. 2008	*Tetranychus kanzawai* *T. okinawanus*	*Morus australis* (Moraceae)	SO, TGK	AB698985 (H033)
3 ♂♂	Tsuha, Oogimi, Okinawa Is.	7 Aug. 2008	11–16 Aug. 2008	*Tetranychus kanzawai* *T. okinawanus*	*Morus australis* (Moraceae)	SO, TGK	KS40302-04
1 ♀	Ookita, Nago, Okinawa Is.	26 Sep. 2008	2 Oct. 2008	*Tetranychus okinawanus*	*Solanum melongena* (Solanaceae)	SO, KF, TGK	AB698986 (H005)
2 ♂♂	Senbaru, Nishihara, Okinawa Is.	22 Jul. 2008	29 Jul. 2008	*Oligonychus biharensis* *Tetranychus piercei*	*Pueraria montana* (Fabaceae)	SO	AB698987 (H034) KS35001-02
11 ♂♂, 2 ♀♀	Makabe, Itoman, Okinawa Is.	6 Jun. 2008	9 Jun. 2008	*Tetranychus neocaledonicus, T. okinawanus, T. urticae* (green form)	*Citrullus lanatus* (Cucurbitaceae)	KK, SO, TGK	KS26301-13
1 ♂	Makabe, Itoman, Okinawa Is.	20 Apr. 2009	21 Apr. 2009	*Tetranychus urticae* (green form)	*Solanum americanum* (Solanaceae)	TGK	KS78401
1 ♂	Yoza, Yaese, Okinawa Is.	15 Aug. 2008	24 Aug. 2008	*Tetranychus okinawanus* *T. urticae* (green form)	*Phaseolus vulgaris* (Fabaceae)	SO	AB698988 (H037)
1 L	Shirase River, Kumejima, Kumejima Is.	28 Jan. 2008	6 Feb. 2008	*Tetranychus okinawanus*	*Solanum melongena* (Solanaceae)	SO, TO	AB698989 (H026)
1 ♂	Shirase River, Kumejima, Kumejima Is.	28 Jan. 2008	6 Feb. 2008	*Tetranychus okinawanus*	*Solanum melongena* (Solanaceae)	SO, TO	KS12401
1 L	Hiyajo, Kumejima, Kumejima Is.	29 Jan. 2008	–	Unidentified	*Phaseolus vulgaris* (Fabaceae)	SO, TO	AB698990 (H025)

Table 1 continued

Specimens used	Collection site	Collecting date of larvae or pupae	Date of adult emergence	Coexisting tetranychid mite	Plant name (family)	Collector(s)[a]	Accession and slide numbers[b]
3 ♂	Hirara, Miyakojima, Miyako Is.	22 Feb. 2002	26 Feb. 2002	Unidentified	*Phaseolus vulgaris* (Fabaceae)	TO	AB698991-93 (A038-040) AKJ0101-03
1 ♂	Hiraranishizato, Miyakojima, Miyako Is.	9 Aug. 2008	12 Aug. 2008	*Tetranychus piercei*	*Mucuna pruriens* (Fabaceae)	AM	AB698994 (H040) KA09301

[a] Names of collectors. *AM* Akiko Miyagi, *KF* Kazuyasu Futagami, *MM* Mutsuko Miya, *SO* Suguru Ohno, *TGK* Tomoko Ganaha-Kikumura, *TO* Tsuyoshi Ooishi, *YS* Yoshitaka Sakamaki

[b] Accession and slide numbers are given to specimens used for genetic analysis and slide-mounted specimens, respectively. Specimens with both numbers indicate that the wings and male genitalia were mounted on slides and the remaining body parts were used for genetic analysis. Each number in parentheses after an accession number shows the individual number used in the NJ tree (Fig. 3)

Antenna: scape ventrally with a few setae; pedicel ventrally with several setae; first and second flagellomeres fused; basal node of fifth flagellomere 0.7–0.9 times as long as wide; distal node 1.0–1.2 times as long as wide, distal neck 1.2–1.6 times as long as internode neck; basal node with 1, distal node with 2 circumfila. Wing length 0.88–1.18 mm, 2.19–2.53 times as long as wide; R_5 joining costa slightly anterior to wing apex. Foreclaws toothed, mid- and hindclaws simple; empodia reaching bend in claws on all legs. See Table 3 for detailed data on setal counts and measurements. For abdomen, see generic synopsis in Gagné (1995), because there is no clear diagnostic difference from the generic description. Male terminalia (Fig. 2a, b): cerci rounded apically; hypoproct a little shorter than aedeagus, parallel sided basally, slightly tapering to blunt apex, with minute setulae, the apex of hypoproct with an apical ligule (Fig. 2b); aedeagus evenly cylindrical, broad, the distal margin of aedeagus slightly concave at apex; mediobasal lobe of gonocoxite barely swollen, with strong hairs; gonostylus slender, weakly arched, with minute setae sparsely and a comb of teeth apically.

Female Antenna with 12 flagellomeres; each flagellomere with 2 appressed horizontal circumfila and 2 vertical connectives; basal node of fifth flagellomere 1.5–1.7 times as long as wide; neck relatively short, about 0.27–0.29 times as long as length of basal node. Other pertinent characters as in male.

Pupa No clear morphological differences except for the arrangement of abdominal spiracles could be found to distinguish between *F. acarivora* and *F. acarisuga* (see Fig. 2d in Abe et al. 2011). Base of antenna short, slightly angular apically; cephalic pair of setae 80–95 μm long; frons smooth; lower and lateral facial papillae not apparent; prothoracic spiracle short, 45–55 μm long; abdominal spiracles short, present on second and third abdominal segments; first to eighth abdominal segments with several rows of very short spines on the anterior portion of dorsal surface; dorsal papillae not apparent.

Specimens examined See Table 2 for the slide-mounted specimens examined.

Distribution Japan (Iheya, Noho, Okinawa, Irabu, Ishigaki and Iriomote Islands, Okinawa Prefecture) (Table 2), Indonesia (Java), and Australia (Northern Territory) (Gagné 2010).

Possible prey mites and their host plants *Tetranychus exsiccator* (Acarina: Tetranychidae) (Gagné 2010). In Okinawa, we newly found *F. acarivora* coexisting on the same plants with the following tetranychid species: *Eutetranychus africanus* (Tucker), *Oligonychus biharensis* (Hirst), *T. kanzawai*, *T. neocaledonicus*, *T. okinawanus*, *T. parakanzawai*, *Tetranychus pueraricola* Ehara, and *T. urticae* (green form) (Table 2). *Feltiella acarivora* was found on 12 plant species across eight plant families (Table 2).

Parasitoid No parasitoids have been obtained from the cocoons of *F. acarivora*, but an unidentified wasp (Hymenoptera: Ceraphronidae) was frequently observed walking on the leaves on which *F. acarisuga* and *F. acarivora* coexisted.

Genetic analysis

The two maximum parsimony trees obtained from the analysis (tree length 363, consistency index 0.782, retention index 0.908, rescaled consistency index 0.711) were not shown in this paper because their topologies were fundamentally the same as the neighbor-joining tree

Table 2 Specimens of *Feltiella acarivora* used for genetic analysis or mounted on slides

Specimens used	Collection site in Okinawa Prefecture	Collecting date of larvae or pupae	Date of adult emergence	Coexisting tetranychid mite	Plant name (family)	Collector(s)[a]	Accession and slide numbers[b]
1 L	Dana, Iheya, Iheya Is.	14 Nov. 2007	22–27 Nov. 2007	Unidentified	*Carica papaya* (Caricaceae)	SO	AB698995 (H020)
5 ♂♂	Dana, Iheya, Iheya Is.	14 Nov. 2007	22–27 Nov. 2007	Unidentified	*Carica papaya* (Caricaceae)	SO	K2S05201-06
1 P	Noho, Iheya, Noho Is.	14 Nov. 2007	Unknown	Unidentified	*Bauhinia* sp. (Caesalpiniaceae)	SO	AB698996 (H018)
1 ♂, 2 P	Oku, Kunigami, Okinawa Is.	1 Aug. 2008	Unknown	*Oligonychus biharensis*	*Mucuna macrocarpa* (Fabaceae)	SO, TGK	K2S36801-03
1 ♂	Okuma, Kunigami, Okinawa Is.	31 Jul. 2008	Unknown	*Tetranychus pueraricola*	*Pueraria montana* (Fabaceae)	SO, SY	AB698997 (H045) K2S36601
1 ♂	Takasato, Oogimi, Okinawa Is.	6 Aug. 2008	17–19 Aug. 2008	*Tetranychus parakanzawai*	*Mallotus japonicus* (Euphorbiaceae)	SO	AB698998 (H044) K2S39501
8 ♂♂	Takasato, Oogimi, Okinawa Is.	6 Aug. 2008	17–19 Aug. 2008	*Tetranychus parakanzawai*	*Mallotus japonicus* (Euphorbiaceae)	SO	K2S39503-06, 08-11
1 ♂, 3 ♀♀, 1 P	Kijoka, Oogimi, Okinawa Is.	16 Oct. 2008	Unknown	*Tetranychus kanzawai* *T. piercei*	*Morus australis* (Moraceae)	SO	K2S53101-05
3 ♂♂	Shioya, Oogimi, Okinawa Is.	1 Feb. 2008	5–6 Feb. 2008	*Tetranychus kanzawai*	*Carica papaya* (Caricaceae)	SO, KTr, KTk, TGK	K2S13401-03
1 L	Imadomari, Nakijin, Okinawa Is.	11 Dec. 2007	17 Dec. 2007	*Tetranychus neocaledonicus* *Eutetranychus africanus*	*Carica papaya* (Caricaceae)	SO	AB698999 (H003)
4 ♂♂	Imadomari, Nakijin, Okinawa Is.	11 Dec. 2007	17 Dec. 2007	*Tetranychus neocaledonicus* *Eutetranychus africanus*	*Carica papaya* (Caricaceae)	SO	K2S08401-05
1 ♂	Senbaru, Nishihara, Okinawa Is.	18 Jul. 2008	25 Jul. 2008	*Oligonychus biharensis* *Tetranychus parakanzawai* *T. pueraricola*	*Pueraria montana* (Fabaceae)	TGK	K2S34801
2 ♂♂	Kakazu, Itoman, Okinawa Is.	15 Oct. 2008	Unknown	*Tetranychus neocaledonicus*	*Bauhinia* sp. (Caesalpiniaceae)	SO	K2S52401-02
3 ♂♂	Nashiro, Itoman, Okinawa Is.	21 Aug. 2008	31 Aug. 2008	*Tetranychus parakanzawai*	*Clerodendrum trochotomum* (Verbenaceae)	SO	K2S44401-03
1 ♂	Mabuni, Itoman, Okinawa Is.	12 Sep. 2007	15–21 Sep. 2007	*Tetranychus okinawanus, T. parakanzawai, T. pueraricola, T. urticae* (green form)	*Vigna angularis* (Fabaceae)	SO, TO, KTr. TGK	K2S03501
1 ♂	Shiratori-misaki, Miyakojima, Irabu Is.	2 Nov. 2008	17 Nov. 2008	*Tetranychus piercei*	*Canavalia lineata* (Fabaceae)	AM	K2A14802

Table 2 continued

Specimens used	Collection site in Okinawa Prefecture	Collecting date of larvae or pupae	Date of adult emergence	Coexisting tetranychid mite	Plant name (family)	Collector(s)[a]	Accession and slide numbers[b]
1 ♂	Ohama, Ishigaki, Ishigaki Is.	25 Jun. 2008	30 Jun. 2008	*Tetranychus okinawanus*	*Passiflora edulis* (Passifloraceae)	TA	AB699000 (H046) K2S32201
1 ♂	Hirae, Ishigaki, Ishigaki Is.	9 July 2008	Unknown	*Tetranychus piercei*	*Annona atemoya* (Annonaceae)	KK, RU, KY	AB699001 (H047) K2A06601
1 ♂	Mihara, Taketomi, Iriomote Is.	11 Jun. 2008	22 Jun. 2008	*Tetranychus parakanzawai*	*Melanolepis multiglandulosa* (Euphorbiaceae)	SO	AB699002 (H048) K2S30901
1 ♂	Hoshidate, Taketomi, Iriomote Is.	12 Jun. 2008	16 Jun. 2008	*Tetranychus parakanzawai*	*Melanolepis multiglandulosa* (Euphorbiaceae)	SO, TGK	AB699003 (H049) K2S30401

[a] Names of collectors. *AM* Akiko Miyagi, *KK* Keisuke Kijima, *KTk* Ken Takahashi, *KTr* Kazuhiko Tarora, *KY* Kaname Yonamine, *RU* Rie Ukuda, *SY* Seiei Yamauchi, *SO* Suguru Ohno, *TA* Tsunaki Ando, *TGK* Tomoko Ganaha-Kikumura, *TO* Tsuyoshi Ooishi

[b] Accession and slide numbers are given to specimens used for genetic analysis and slide-mounted specimens, respectively. Specimens with both numbers indicate that the wings and male genitalia were mounted on slides and the remaining body parts were used for genetic analysis. Each number in parentheses after an accession number shows the individual number used in the NJ tree (Fig. 3)

(Fig. 3), except for the relationship among outgroup taxa (*D. rosae* and *R. odai* became a sister group in one tree, and *D. rosae and P. puerariae* in another). The NJ tree clearly indicated the existence of two clades among individuals of *Feltiella* examined (Fig. 3). One was the clade of *F. acarisuga* and the other was that of *F. acarivora*. Both clades were supported by a 100 % bootstrap value, respectively. Seven haplotypes were recognized among individuals of *F. acarisuga* and were further divided into two subclades, of which one includes individuals collected only from islands of Okinawa Prefecture, and the other includes those from the mainland of Japan, except for one individual from Okinawa Island. The monophyly of each subclade was supported by a 100 % bootstrap value. The maximum sequence divergence was 34 (4.18 %) bp between the haplotypes belonging to the two subclades. In the clade of *F. acarivora*, we found two haplotypes, between which the sequence divergence was only 1 (0.123 %) bp.

Discussion

Taxonomic remarks

The original description of *F. acarivora* (Zehntner 1901) was incomplete and the type specimen is probably lost, but Zehntner's sketch of the male terminalia is adequate for species identification. Zehntner (1901) emphasized the setae at the base of the gonocoxites, indicating the presence of a spiny mediobasal lobe, which is one of the important common characters of *F. acarivora* and the *Feltiella* species from Okinawa. The lengths and shapes of the hypoproct and aedeagus of the male terminalia of these species are also quite similar. Based on these morphological features, we identified the *Feltiella* species from Okinawa as *F. acarivora.*

The original description of *F. acarivora* does not mention whether any claws were toothed (Zehntner 1901), but Gagné (1995) pointed out that he did not believe that the tarsal claws were entirely toothless, otherwise *F. acarivora* would be the only species of *Feltiella* with no teeth. Gagné supposed that either Zehntner did not examine the forelegs or did not see them closely enough. In this study, we found that foreclaws were toothed and mid- and hindclaws were simple. Thus, we confirmed that there are no *Feltiella* species with toothless tarsal claws.

Feltiella acarivora is characterized and distinguished from *F. acarisuga* and other congeners by the following combination of characters: hypoproct a little shorter than aedeagus, parallel sided basally, slightly tapering to a blunt apex; apex of hypoproct with an apical ligule; gonocoxite with a spiny mediobasal lobe. In addition, female flagellomeres have appressed circumfila, whereas those of *F. acarisuga* have slightly looping circumfila (Gagné 1995; Abe et al. 2011). Abdominal spiracles of the pupa are present only on the second and third abdominal segments, whereas these of *F. acarisuga* are present on the second through fifth abdominal segments.

Table 3 *Feltiella acarivora*: frontoclypeal and mesepimeral setal counts, and measurements of wing (mm), palpus (μm), fifth flagellomere (μm), and legs (mm)

Body part	Male of *F. acarivora*		
	n	Mean ± SD	Range
Frontoclypeal setae	7	6.14 ± 0.38	6–7
Mesepimeral setae	13	2.77 ± 0.44	2–3
Wing length	16	1.04 ± 0.10	0.89–1.12
Wing width	16	0.44 ± 0.05	0.37–0.53
Length/width	16	2.37 ± 0.01	2.19–2.53
Palpus			
1st segment	5	14.0 ± 1.4	12.5–15.0
2nd segment	6	34.2 ± 4.1	27.5–40.0
3rd segment	5	30.5 ± 1.1	30.0–32.5
5th flagellomere			
Length of basal node	19	23.2 ± 1.8	20.0–25.0
Width of basal node	19	29.5 ± 1.7	27.5–32.5
Length of intermediate neck	19	23.2 ± 2.1	20.0–27.5
Length of distal node	19	32.4 ± 2.0	30.0–36.3
Width of distal node	19	29.7 ± 1.8	27.5–33.8
Length of distal neck	19	31.6 ± 1.0	30.0–32.5
Fore leg			
Femur	17	0.38 ± 0.04	0.32–0.44
Tibia	18	0.41 ± 0.05	0.33–0.50
Tarsomere 2	10	0.41 ± 0.03	0.36–0.47
Tarsomere 3	8	0.18 ± 0.01	0.16–0.20
Tarsomere 4	8	0.10 ± 0.01	0.08–0.12
Tarsomere 5	8	0.07 ± 0.01	0.06–0.08
Mid leg			
Femur	17	0.36 ± 0.04	0.29–0.43
Tibia	17	0.37 ± 0.04	0.31–0.45
Tarsomere 2	9	0.39 ± 0.04	0.35–0.45
Tarsomere 3	9	0.18 ± 0.02	0.16–0.20
Tarsomere 4	9	0.09 ± 0.01	0.09–0.11
Tarsomere 5	8	0.07 ± 0.01	0.06–0.09
Hind leg			
Femur	17	0.36 ± 0.04	0.30–0.42
Tibia	17	0.34 ± 0.04	0.27–0.41
Tarsomere 2	7	0.41 ± 0.05	0.32–0.48
Tarsomere 3	7	0.19 ± 0.01	0.18–0.22
Tarsomere 4	7	0.10 ± 0.01	0.08–0.11
Tarsomere 5	7	0.07 ± 0.004	0.06–0.07

Distribution of *Feltiella* species

Feltiella acarisuga has a widespread, almost cosmopolitan, distribution and has been recorded in Australia, Europe, India, Israel, Morocco, New Zealand, North America, Sri Lanka, Taiwan (Gagné 1995), South Korea (Lee et al. 2004), and Japan (Abe et al. 2011), whereas *F. acarivora* was known only from Java, Indonesia (Gagné 1995). Our finding of *F. acarivora* in Okinawa (southern Japan) is not surprising, because *Feltiella* species usually have wide distribution ranges (Gagné 1995, 2010) and Okinawa is warm enough for *F. acarivora* to have established long before. It is remarkable that Abe et al. (2011) did not find *F. acarivora* in Japan north of Okinawa Prefecture, but that is probably because of the lower temperatures there than in Okinawa. At the moment, there is no evidence suggesting that *F. acarivora* is a recent arrival in Okinawa due to trading.

In Okinawa Prefecture, larvae or pupae of *Feltiella* species were collected from eight surveyed islands (Fig. 1). Both *F. acarisuga* and *F. acarivora* were commonly found on Okinawa Island, and the two species sometimes coexisted on the same plant. These observations may suggest that *F. acarisuga* and *F. acarivora* do not exhibit different habitat preferences in Okinawa.

Possible prey mites and their host plants

Many species of Tetranychidae (Acarina) and *Acaphylla theavagrans* Kadono (Acarina: Eriophyoidea) have been reported as prey of *F. acarisuga* in Japan (Abe et al. 2011). Through our field surveys, eight tetranychid species were found with larvae or cocoons of *F. acarisuga* (Table 1). Among them, *Oligonychus biharensis*, *Tetranychus neocaledonicus*, *T. okinawanus*, and *T. parakanzawai* were newly recognized as possible prey of *F. acarisuga*. *Tetranychus exsiccator* was the only known prey of *F. acarivora* (Gagné 2010), but the current survey revealed that *F. acarivora* is possibly polyphagous (as is *F. acarisuga*), coexisting on the same leaf with at least eight tetranychid species in Okinawa Prefecture. Most of them are common prey of the two *Feltiella* species. *Feltiella acarisuga* and *F. acarivora* were found on many plant species across a wide range of plant families (Tables 1, 2). We have not seen any distinct difference between them in their preferred plant species. Because *F. acarivora* was found not only on wild plants but also on fruit trees and vegetable crops, as observed for *F. acarisuga*, this species may contribute to the control of pest tetranychids.

Parasitoids of *Feltiella* species found in Okinawa

Aphanogmus floridanus Ashmead and *A. fulmeki* Szelényi (including *A. parvulus* Roberti; Dessart 1992) (Hymenoptera: Ceraphronidae) have been known to emerge from cocoons of *F. acarisuga* in other parts of the world (Roberti 1954; Dessart 1992; Osborne et al. 2002) but not in Japan (Abe et al. 2011; the current data). The reason for this absence is unknown. In Japan, an unidentified species of endoparasitic wasp (Hymenoptera: Ceraphronidae) has

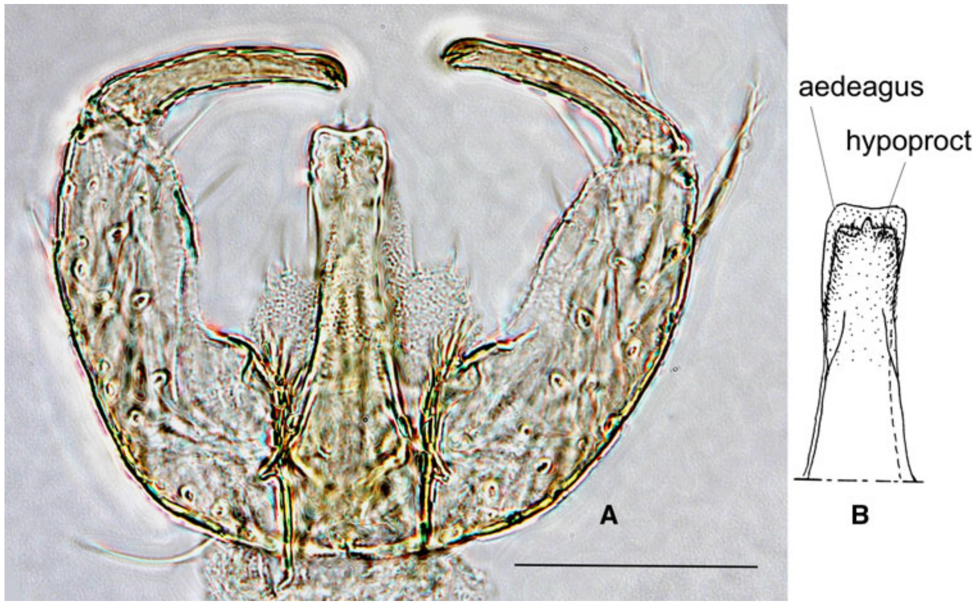

Fig. 2 **a** Male terminalia of *Feltiella acarivora* (dorsal view), **b** distal part of hypoproct and aedeagus (dorsal view). *Scale bar* 0.05 mm

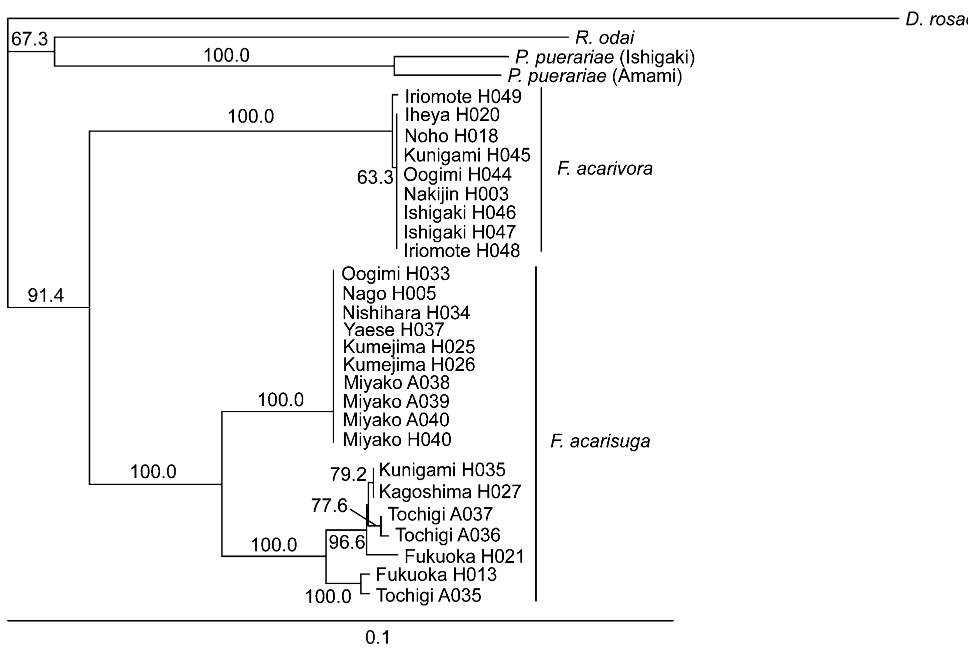

Fig. 3 Neighbor-joining tree for *Feltiella* species based on 814 bp of the mitochondrial cytochrome oxidase subunit I gene. Bootstrap values are indicated for nodes gaining more than 50 % support in 1,000 pseudoreplications. *Dasineura rosae*, *Resseliella odai*, and *Pitydiplosis puerariae* were used as outgroup species. DDBJ accession numbers are shown in Tables 1 and 2

been reared from cocoons of *F. acarisuga* (Abe et al. 2011). The finding of this wasp on the leaves on which *F. acarisuga* and *F. acarivora* coexisted may imply that the wasp also attacks *F. acarivora*.

Genetic analysis

Phylogenetic analyses supported the morphological identification of *F. acarisuga* and *F. acarivora*, indicating the occurrence of the *Feltiella* species in Okinawa (Fig. 3). Unlike most cecidomyiids that induce galls from which we can obtain all mature and immature specimens, species identification of these particular predaceous cecidomyiids is relatively difficult when based solely on larval specimens. Larval morphological differences between these two species are indistinct, and larvae are barely associated with males, which are more easily identified by differences in the male terminalia. Therefore, the current molecular sequencing data will aid in the identification of *Feltiella* species, particularly when only larval specimens are available. Such data for *Feltiella* species were not previously registered.

In the phylogenetic analyses, the individuals of *F. acarisuga* were divided into two subclades, roughly representing their collecting sites (Fig. 3). Although an exceptional individual from Okinawa was included in the individuals from the mainland of Japan, the two clades seem to show possible genetic isolation between *F. acarisuga* populations in Okinawa and the mainland of Japan.

A slight sequence divergence among individuals of *F. acarivora* (the current data), together with an absence of records from the mainland of Japan (Abe et al. 2011), may indicate that Okinawa is the northern distributional limit of *F. acarivora* in Japan. To confirm this, we need to collect further specimens from various localities in Asia, including its type locality, Indonesia, for future phylogenetic and biogeographic studies.

Acknowledgements We wish to thank Dr. R.J. Gagné (Systematic Entomology Laboratory, Plant Science Institute, USDA, USA, retired) and Dr. K.M. Harris (former Director of the International Institute of Entomology, UK, retired) for their comments on an early draft. The original description of *F. acarivora* written in Dutch (Zehntner 1901) was translated into English by Dr. W.C. Nijveldt (former staff member of IPO, Wageningen, The Netherlands, retired), to whom we are indebted. We are grateful to workers at the Okinawa Prefectural Plant Protection Center, Okinawa Prefectural Agricultural Development Center, Okinawa Prefectural Agricultural Research Center, and Ryukyu Sankei Co., Ltd. for providing us with plant samples bearing tetranychid mites. Our thanks are also due to Prof. T. Gotoh (Ibaraki Univ.) for identifying tetranychid mites, Mr. K. Matsuo (Kyushu Univ.) for his information on parasitoid species that attack *F. acarivora*, and Dr. S. Kamitani (Kyushu Univ.) for taking multi-layer photographs of the male terminalia of *F. acarivora*.

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