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https://doi.org/10.5109/2679

出版情報：ESAKIA. 44, pp.13-26, 2004-03-31. Entomological Laboratory, Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

ESAKIA, (44): 13-26. March 10, 2004

Redescription of *Hartigiola faggalli* (Monzen) comb. n. (Diptera: Cecidomyiidae) Inducing Leaf Galls on *Fagus crenata* (Fagaceae) in Japan*

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Abstract. *Hartigiola faggalli* (Monzen) (Diptera: Cecidomyiidae) comb. n. that induces bivalve-shaped galls on the leaves of *Fagus crenata* Linnaeus (Fagaceae) in Japan was newly transferred from the genus *Oligotrophus* Latreille and redescribed based on the adult, larval, and pupal stages of the species, together with biological notes. DNA analysis indicated that there were sequential differences (8-10 bp/439) in the partial COI region of mtDNA between individuals obtained from the galls induced on upper and under surfaces of the host leaves, although they were morphologically identical. The generic definition of *Hartigiola,* which was a monotypic genus, is somewhat broadened to contain *H. faggalli.*

Key words: *Fagus crenata*, Cecidomyiidae, *Hartigiola*, new combination, redescription, leaf gall.

Introduction

Fagus crenata Blume and *Fagus japonica* Maximowicz (Fagaceae) are deciduous

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* Contribution from the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka (Ser. 5, No. 107).

trees distributed widely in Japan, except Hokkaido for the latter (Horikawa, 1972). Yukawa & Masuda (1996) listed 26 sorts of midge gall on *Fagus* leaves in Japan and considered that they are induced by different gall midge species (Diptera: Cecidomyiidae), respectively. However, most gall midges have been left unnamed except for *Mikiola fagi* (Hartig, 1839), *Phegomyia tokunagai* Sasakawa et Koyama, 1953, *Oligotrophus faggalli* Monzen, 1955, and *Janetiella infrafoli* Monzen, 1955. Among the 4 genera recorded from Japan, *Mikiola* and *Phegomyia* are known to occur on *Fagus sylvatica* Linnaeus in Europe (Skuhravá, 1997). The generic position of *O. faggalli* is, however, doubtful, because all described species of the genus *Oligotrophus* Latreille, 1805, are responsible for galls on the species of *Juniperus* (Cupressaceae) (Gagné, 2004). Gall midges are mostly mono- or oligophagous and the gall midge-host plant linkage is frequently evident at the generic level (Yukawa & Masuda, 1996; Gagné, 2004). Therefore, the generic position of *O. faggalli* should be reconsidered.

Preliminary morphological study of *J. infrafoli* suggests that its generic position is also doubtful, although species of *Janetiella* are associated with various botanical families (Gagné, 2004). However, it is not treated in this paper.

In this paper, *O. faggalli* is redescribed and combined with an appropriate genus of the tribe Oligotrophini. Its distributional information and life history pattern are presented, and individuals from different galling sites, upper and under surfaces of the host leaves, were compared morphologically and at the DNA level.

Materials and Methods

Collection and preservation of specimens

Midge galls on *F. crenata* were collected by myself and other colleagues (see Acknowledgements) from various localities in Japan during the period from 1979 to 2000. Some of the galls collected were dissected under a binocular microscope. Mature larvae or pupae, if any, were picked out of the galls and divided into 2 groups. One group was stored in 75 % ethanol to make slide-mounted specimens for morphological studies and the other in 99.5 % acetone for DNA analysis. When some of the dissected galls contained healthy mature larvae or pupae, the rest of collected galls were maintained in plastic bottles (30 cm in diameter, 37 cm in depth) to rear adult midges under the laboratory conditions. Emerged adults were put into 75 % ethanol or 99.5 % acetone for the aforementioned purposes.

Morphological studies and terminology

Some of the ethanol-stored specimens were mounted on slides for microscopic study in Canada balsam using ethanol and xylene. Some larvae were mounted on slides in

Table 1. Specimens of *Hartigiola faggalli* and *Mikiola fagi* (an outgroup) used for DNA analysis.

Gall midge	Host plant	Collection site	Collection date	Stage	n	Isolation name	Accession no.
H. faggalli[2)]	*F. crenata*	Morioka, Iwate Pref.	07 Mar. 2000	Pupa	2	S7-S8	AB162829 -AB162830
	F. crenata	Morioka, Iwate Pref.	16 Mar. 2001	Pupa	8	S245-S250	AB162831 -AB162836
						S255-S256	AB162837 -AB162838
H. faggalli[3)]	*F. crenata*	Morioka, Iwate Pref.	07 Mar. 2000	Pupa	2	S1-S2	AB162839 -AB162840
	F. crenata	Morioka, Iwate Pref.	16 Mar. 2001	Pupa	7	S258-S264	AB162841 -AB162847
M. fagi	*F. sylvatica*	Babia Góra National Park, Poland	Sep. 2000	Larva	1	S151	AB162848

1) Nucleotide sequence data obtained in this study are available from DDBJ (DNA Data Bank of Japan), EMBL (European Molecular Biology Laboratory), and GenBank nucleotide sequence databases.
2) Specimens obtained from the galls induced on the upper surface of the host leaves.
3) Specimens obtained from the galls induced on the under surface of the host leaves.

lactic acid using glycerol and formalin for quick observation of lateral and ventral papillae. Drawings were made with the aid of a drawing tube.

Adult morphological terminology follows usage in Gagné (1981), except for thoracic setae and the mediobasal lobe of male genitalia, of which terminology follows usage in Yukawa & Ohsaki (1988) and Nijveldt & Yukawa (1982), respectively. Morphological terminology of larva generally follows usage in Möhn (1955; originally written in German) that was translated into English in Yukawa (1971), and the terminology of pupa follows that in Gagné (1994).

DNA extraction, amplification, and sequencing

Acetone-preserved specimens of some *Fagus* gall midges were used for DNA analysis (Table 1). *Mikiola fagi* was used as an outgroup species for the analysis. For every individual, total DNA was extracted from the whole body with the Dneasy tissue kit (Qiagen, Japan), following the manufacturer's instructions. A region of the cytochrome oxidase subunit I (COI) gene of mtDNA was amplified, purified, sequenced, and electrophoresized following the methods described by Yukawa *et al.* (2003). DNA of each specimen was analyzed using the following primers: forward; 5'-GGATCACCTGATATAGCATTCCC-3' (COIS) and reverse; 5'-CCCGGTAAAATTAAAATATAAACTTC-3' (COIA). These primers have been effectively used for many gall midges to determine intra- and inter-specific relations

(e.g., Shirota *et al.*, 1999; Yukawa *et al.*, 2003; Uechi *et al.*, 2003; Uechi *et al.*, 2004). The amplified COI gene fragment of mtDNA was 439 bp long. This region corresponded to the bases 1752-2190 of the genome of *Drosophila yakuba* Burla (Diptera: Drosophilidae) (Clary & Wolstenholme, 1985).

Molecular phylogenetic analysis

The DNA sequence data were edited using DNASIS (Hitachi Software Engineering Co.). Evolutionary distances were computed by Kimura's two-parameter method (Kimura, 1980). Phylogenetic analysis was conducted by the neighbor-joining (NJ) method (Saitou & Nei, 1987) using the software package PHYLIP ver. 3.573c. The resulting tree was evaluated by the bootstrap test (Efron, 1982; Felsenstein, 1985) based on 1,000 replications for the NJ tree.

Genus *Hartigiola* Rübsaamen

Hartigiola Rübsaamen, 1912: Type-species, *Cecidomyia annulipes* Hartig, 1839.
Phegobia Kieffer, 1913a: Type-species, *Cecidomyia tornatella* Bremi, 1847.

Adult.

Eyes joined at vertex. Palpus 3 or 4 segmented. Number of antennal flagellomeres variable with sexes and species; flagellomeres of male, except terminal one, each consisting of a cylindrical basal enlargement and a relatively long distal neck; female flagellomeres each consisting of a cylindrical basal enlargement and a very short distal neck; tarsal claw without teeth; empodium nearly as long as tarsal claws; R_5 joining costa slightly before wing apex. Male genitalia: cerci separate, large, rounded apically; hypoproct nearly as long as and distinctly narrower than cerci, the apex concave; gonostylus weakly arched, tapering distally, with a strong apical tooth; inner angle of gonocoxite ventrally developed into a setose lobe, which is rounded apically and provided with several short setae near apex; aedeagus cylindrical, tapering slightly to rounded apex. Ovipositor relatively long, soft, cerci fused, elongate.

Mature larva.

Four dorsal papillae present on all thoracic segments and 4 or 6 dorsal papillae on first to seventh abdominal segments, each with a seta; 2 dorsal papillae of eighth abdominal segment each with a seta; 2 pleural papillae on all thoracic and abdominal segments each with a seta; 3 or 4 pairs of terminal papillae present on terminal segment; sternal spatula absent; 1 of 3 pairs of lateral papillae each with a seta; sternal papillae all without seta on all thoracic segments; no anterior ventral papillae present on thoracic segments; 4 anterior ventral papillae present on first to seventh abdominal segments, all

segments; 4 anterior ventral papillae present on first to seventh abdominal segments, all without seta; 2 posterior ventral papillae present on first to seventh abdominal segments, each with a seta; 4 ventral papillae present on eighth abdominal segment; 4 anal papillae all without seta.

Pupa.

Base of antenna well sclerotized, undeveloped, tapering gradually from base to bluntly pointed apex; cephalic pair of setae elongate; frons smooth; 1 of 2 pairs of lower facial papillae each with a seta; 1 of 2 pairs of lateral facial papillae each with a seta; prothoracic spiracle relatively long, pointed apically; abdominal spiracles elongate, present on second to sixth abdominal segments; each abdominal segment, except terminal one, with many short spines dorsally and ventrally; terminal abdominal segment with many short spines only dorsally.

Remarks. The genus *Hartigiola* contained only one species, *Hartigiola annulipes* (Hartig, 1839), which induces leaf galls on *F. sylvatica*. Since *Hartigiola* was insufficiently described by Hartig (1839) and Rübsaamen (1912), it is redescribed herein based on the specimens collected by K. M. Harris in October 2001 from W. Hanger, UK. The generic diagnosis was broadened to contain the Japanese species redescribed in this paper.

The genus has been placed in the tribe Oligotrophini (Skuhravá, 1997; Gagné, 2004). *Hartigiola* is similar to *Zygiobia* Kieffer, 1913b, consisting of two galling species on *Carpinus* (Betulaceae), but differs from it by having male genitalia with slender gonostyli, larval prothorax without sternal spatula, and the gall-forming habit on *Fagus* (Möhn, 1955; Skuhravá, 1997).

***Hartigiola faggalli* (Monzen) comb. n.**

Japanese name: Buna-kaigara Tamabae

English name: *Fagus* bivalve-shaped gall midge

Oligotrophus faggalli Monzen, 1955, 47 (Japan: Honshu); Yukawa, 1971: 101; Yukawa & Masuda, 1996: 41, 172.

Male.

Eye bridge 3 to 5 facets long medially; frontoclypeal setae dense, 16 to 39 in number (Table 2). Palpus 3 segmented; first palpal segment 0.9 to 1.3 times as long as wide; second 0.8 to 1.1 times as long as first; third 1.2 to 1.8 times as long as second. Antenna: scape ventro-laterally with 23 to 44 setae; pedicel ventrally and dorsally with 4 to 12 setae; with 14 to 15 flagellomeres; basal enlargement of third flagellomere 0.7 to 1.4 times as long as wide; terminal flagellomere relatively small, rounded apically. Wing

apex. Legs densely with blackish brown hairs; claws of all legs without tooth; empodium as long as claw; length of respective segments as in Table 2; foreleg with femur slightly longer than tibia and distinctly longer than tarsomere II, tarsomere IV 1.0 to 1.5 times as long as tarsomere V; midleg with femur longer than tibia and distinctly longer than tarsomere II, tarsomere IV 1.1 to 1.5 times as long as tarsomere V; hindleg with femur slightly longer than tibia and distinctly longer than tarsomere II, tarsomere IV 1.1 to 1.9 times as long as tarsomere V. Abdominal tergites I to VII rectangular, wider than long, with caudal rows of setae and a pair of anterior trichoid sensilla, tergite VIII narrower than preceding, with sparsely scattered setae and a pair of anterior trichoid sensilla. Ab-

Table 2. *Hartigiola fagalli*: front-clypeal and mesepimeral setal counts, and measurements of wing, palpus, third flagellomere, and legs.

	♂			♀		
	n	mean±s. d.	(range)	n	mean±s. d.	(range)
Front-clypeal setae	9	23.22±7.22	(39-16)	9	16.67±2.12	(21-15)
Mesepimeral setae	8	16.50±4.00	(21-9)	9	11.67±3.04	(16-6)
Wing length (mm)	10	2.16±0.19	(2.56-1.94)	10	2.60±0.27	(2.83-1.88)
Wing width (mm)	10	1.07±0.10	(1.19-0.91)	10	1.24±0.14	(1.38-0.88)
Length/width	10	2.02±0.08	(2.16-1.89)	10	2.10±0.04	(2.18-2.04)
Palpus (μm)						
Length of 1st segment	8	26.3±4.1	(31.3-18.8)	6	17.5±3.4	(22.5-12.5)
Length of 2nd segment	10	26.6±4.3	(32.5-18.8)	8	26.1±3.7	(31.3-18.8)
Length of 3rd segment	9	37.2±5.5	(43.8-25.0)	8	33.6±4.0	(40.0-27.5)
3rd flagellomere (μm)						
Length of basal enlargement	9	65.6±10.8	(78.8-43.8)	10	58.6±4.5	(65.0-48.8)
Width of basal enlargement	9	59.0±6.3	(68.8-50.0)	10	49.5±4.9	(56.3-37.5)
Length of distal stem	9	40.0±6.5	(50.0-32.5)	10	4.3±1.1	(6.3-3.8)
Fore leg (mm) Fe	10	0.65±0.11	(0.80-0.45)	9	0.67±0.08	(0.77-0.50)
Ti	9	0.64±0.11	(0.80-0.50)	9	0.62±0.06	(0.70-0.50)
T_2	9	0.38±0.05	(0.43-0.30)	7	0.30±0.05	(0.35-0.23)
T_3	9	0.20±0.03	(0.23-0.15)	7	0.18±0.03	(0.23-0.13)
T_4	9	0.14±0.02	(0.16-0.10)	8	0.13±0.02	(0.15-0.10)
T_5	9	0.11±0.00	(0.13-0.10)	9	0.11±0.02	(0.14-0.09)
Mid leg (mm) Fe	7	0.65±0.08	(0.75-0.58)	9	0.67±0.07	(0.75-0.50)
Ti	9	0.60±0.07	(0.68-0.45)	9	0.59±0.06	(0.68-0.48)
T_2	9	0.34±0.05	(0.43-0.28)	9	0.30±0.03	(0.33-0.23)
T_3	8	0.19±0.03	(0.23-0.15)	9	0.18±0.02	(0.20-0.13)
T_4	8	0.14±0.02	(0.15-0.11)	9	0.13±0.02	(0.14-0.09)
T_5	8	0.10±0.01	(0.13-0.10)	9	0.10±0.01	(0.14-0.09)
Hind leg (mm) Fe	8	0.77±0.10	(0.90-0.60)	10	0.83±0.10	(0.93-0.60)
Ti	9	0.74±0.10	(0.87-0.63)	9	0.72±0.07	(0.83-0.55)
T_2	9	0.43±0.06	(0.54-0.33)	8	0.35±0.05	(0.40-0.25)
T_3	9	0.23±0.04	(0.28-0.18)	9	0.20±0.05	(0.23-0.13)
T_4	9	0.16±0.02	(0.20-0.13)	9	0.15±0.02	(0.18-0.11)
T_5	9	0.10±0.01	(0.13-0.08)	9	0.10±0.01	(0.11-0.09)

dominal sternites I to VII rectangular, wider than long, with caudal rows of setae, with scattered setae elsewhere, sternite VIII rectangular, narrower than preceding, the vestiture similarly arranged. Genitalia (Fig. 1) yellowish brown; cerci separate, large, rounded apically; hypoproct nearly as long as and distinctly narrower than cerci, the apex concave; gonostylus relatively short, weakly arched, tapering distally, with a strong claw apically and fine hairs uniformly; inner angle of gonocoxite ventrally developed into a setose lobe, which is provided with a few protuberances; aedeagus longer than hypoproct, cylindrical, tapering slightly to rounded apex. See Table 2 for detailed data of setal counts and measurements.

Female.

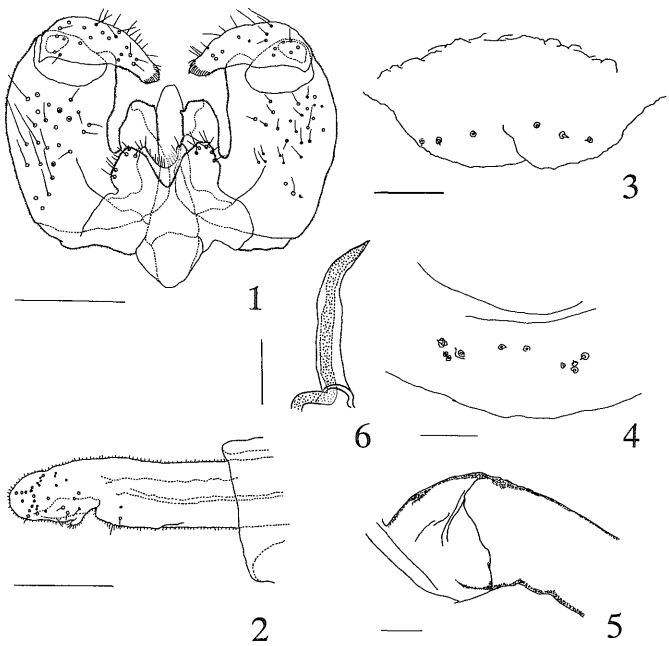
Eye bridge 3 to 4 facets long medially. Palpus 3 segmented. Antenna with 13 flagellomeres; basal enlargement of third flagellomere 1.0 to 1.3 times as long as wide. Wing 1.8 to 2.9 mm, 2.0 to 2.2 times as long as wide (Table 2). Tarsomere IV 1.0 to 1.3 times as long as tarsomere V on foreleg, 1.0 to 1.4 times as long as tarsomere V on midleg, and 1.2 to 1.6 times as long as tarsomere V on hindleg. Abdominal tergites I to VI rectangular, wider than long, with caudal rows of setae and a pair of anterior trichoid sensilla, tergite VII narrower than preceding, the vestiture similarly arranged, tergite VIII distinctly narrower than tergite VII, bare, with only a pair of anterior trichoid sensilla. Abdominal sternites I to VI rectangular, wider than long, with rows of caudal setae, with scattered setae elsewhere, sternite VII rectangular, narrower than preceding, with caudal rows of setae, sternite VIII not sclerotized, bare. Ovipositor yellowish brown, elongate, 370.0 to 520.0 μm long, not sclerotized; cerci elongated, entire (Fig. 2). See Table 2 for detailed data of setal counts and measurements.

Mature larva.

Second antennal segment about 22.0 μm long; cervical papillae without seta; 4 dorsal papillae present on all thoracic and first through seventh abdominal segments, each with a seta, 7.0 μm long; 2 dorsal papillae of eighth abdominal segment, each with a 7.0 μm long seta; and 2 pleural papillae, each with a 7.0 to 12.0 μm long seta; stigma normal in number and position; 2 of 3 pairs of terminal papillae each with a seta, which is 6.0 to 9.0 μm long (Fig. 3); sternal spatula absent; 1 of 3 pairs of lateral papillae each with a minute seta (Fig. 4); sternal papillae on all thoracic segments, each without seta; inner pleural papillae with a seta on all thoracic segments; on first to seventh abdominal segments, 4 anterior ventral papillae without seta and 2 posterior ventral papillae each with a seta; on eighth abdominal segment, 4 ventral papillae without seta; 4 anal papillae without seta.

Pupa.

Base of antenna undeveloped, gently rounded apically (Fig. 5); cephalic pair of setae

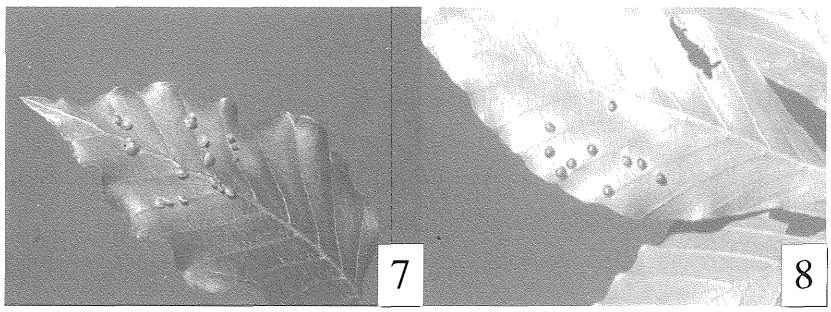


Figs. 1-6. *Hartigiola faggalli* (Monzen) comb. n. 1: male genitalia, 2: ovipositor, 3: terminal segment of mature larva (dorsal view), 4: papillae on larval prothoracic segment (ventral view), 5: basal portion of antenna in pupa, 6: prothoracic spiracle of pupa. Scale: 0.05 mm (3, 4, 5, 6); 0.1 mm (1, 2).

elongate, 15.0 to 59.0 μm in length; frons smooth; 1 of 2 pairs of lower facial papillae each with a seta, which is 4.0 to 6.0 μm long; 1 of 2 pairs of lateral facial papillae each with a 4.0 μm long seta; prothoracic spiracle 147.0 μm in length, pointed apically (Fig. 6); abdominal spiracles long, pointed apically, present on second to sixth abdominal segments; each abdominal segment, except first and terminal ones, with many short spines dorsally and ventrally; first and terminal abdominal segment with many short spines only dorsally.

Host plant. Fagus crenata Blume "Buna" in Japanese [Fagaceae].

Specimens examined. Neotype, male (on slide, Type No. 3188, Cecid. No. A8901), a gall collected from Kuriyagawa, Morioka City, Iwate Prefecture, March 7, 2000, by T. Goto, reared under the laboratory conditions, emerged on March 25, 2000. Other specimens, 5 males (on slide, Cecid. No. A8902-06), galls collected from Mt. Shibi, Miyanojo Town, Kagoshima Prefecture, December 4, 1979, by K. Tsuda; 4 males (on slide, Cecid. No. A8907-10), galls collected from Kuriyagawa, Morioka City, Iwate



Figs. 7-8. Galls of *Hartigiola faggalli* on the upper surface of the host leaf (Fig. 7), and those on the under surface (Fig. 8).

Prefecture, March 7, 2000, by T. Goto; 10 females (on slide, Cecid. No. A8911-20), galls collected from Kuriyagawa, Morioka City, Iwate Prefecture, March 7, 2000, by T. Goto; 9 mature larvae (on slide, Cecid. No. A8921-29), galls collected from Mt. Takanawa, Hojo City, Ehime Prefecture, September 26, 1999, by S. Sato; 7 pupae (on slide, Cecid. No. A8930-36), galls collected from Mt. Sobo, Ogi Town, Oita Prefecture, April 21, 1980, by K. Tsuda; 5 pupae (on slide, Cecid. No. A8937-41), galls collected from Kuriyagawa, Morioka City, Iwate Prefecture, March 7, 2000, by T. Goto.

Hartigiola faggalli was described as *Oligotrophus faggalli* without the designation of type specimens. Therefore, a series of the specimens used by Monzen (1955) could be defined as syntype, if any. However, the existence of the syntype could not be confirmed at Iwate University, Morioka, Japan, to which he belonged at that time.

The shape and structure of *H. faggalli* gall indicate similarity to the description of *O. faggalli* gall in Monzen (1955). In many cases, the appearance and structure of midge galls are specific to gall-midge species, and each gall is induced on particular plant taxa. Therefore, the galls are regarded as an extended phenotype of respective gall-midge species, except for some polyphagous species (e.g., Yukawa *et al.*, 2003; Uechi *et al.*, 2003; Uechi *et al.*, 2004). On the basis of such species specificity in the gall shape, we consider *H. faggalli* to be identical with *O. faggalli*.

In this paper, we designate the neotype for *H. faggalli* using a male specimen reared from a gall that had been collected from Morioka, Iwate Prefecture, Japan; one of the localities where the galls of *O. faggalli* were collected (Monzen, 1955). The neotype and the other specimens examined in this paper are preserved in the collection of Entomological Laboratory, Kyushu University, Fukuoka, Japan.

Gall.

Smooth, brown or blackish brown bivalve-shaped swelling on upper- or under surface of the leaf blade (Figs. 7, 8), with a height 1.4 to 2.1 mm and a maximum width 1.2 to 2.4 mm; monothalamus, containing one midge larva; galls that are produced on the upper

surface of the leaf standing mainly on the side veins or rarely on the midrib (Fig. 7), while those on the under surface lying down on the leaf blade (Fig. 8); sometimes more than 30 galls produced on a leaf. Japanese name of the gall: 'Buna-ha-kaigarafushi' (Monzen, 1929; Tsuda, 1982; Yukawa & Masuda, 1996).

Distribution. Japan (Hokkaido, Honshu, Shikoku, Kyushu).

Life history. This species is univoltine. According to field surveys in Honshu by Takizawa (1993), the emergence of adults usually started from late April to mid May and mating occurred immediately after emergence. Females located on the host leaves that were about to open and laid their eggs along the side veins of upper leaf surface or on the under leaf surface. Mean adult longevity was 2 and 3 days for males and females, respectively. Egg stage lasted for about 4 days, and first instars settled themselves at the places where they had hatched.

Galls became conspicuous in June and the midge larvae matured in October in the galls. Then galls dropped to the ground together with the leaf blade. The mature larvae overwintered in the galls on the ground, and pupated in the galls from late March to mid April. The pupal stage lasted for about 4 weeks (Takizawa, 1993).

Remarks. Monzen (1955) described this species as *Oligotrophus faggalli* Monzen, but its generic position has been doubted (Yukawa & Masuda, 1996), since the genus *Oligotrophus* has an association with plant species of the genus *Juniperus* (Cupressaceae). In the course of this study, *O. faggalli* was confirmed not to fit the generic diagnosis of *Oligotrophus* that has tarsal claws much shorter than empodia on all legs (Skuhravá, 1997) and 6 dorsal papillae on thoracic and the first through seventh abdominal tergites of mature larvae (Möhn, 1955). The Japanese species described as *O. faggalli* has tarsal claws as long as empodia and only 4 dorsal papillae, hence it does not belong to *Oligotrophus*. This species should be combined with the genus *Hartigiola* Rübsaamen, 1912 in the view of basic characters mentioned in the generic remarks. *Hartigiola faggalli* differs from *Hartigiola annulipes* (Hartig, 1839) as follows: antenna with 13 to 15 (not 17 to 19 as in *H. annulipes*) flagellomeres; palpus 3 (not 4) segmented; gonostylus uniformly pubescent (pubescent only basally in *H. annulipes*); aedeagus longer than hypoproct (not as long as hypoproct); 3 (not 4) pairs of terminal papillae each with a seta on larval terminal segment; 4 (not 6) dorsal papillae present on larval first to seventh abdominal segments; ventral papillae on larval eighth abdominal segment all without seta (2 or 3 ventral papillae each with a seta in *H. annulipes*).

Within a *H. faggalli* population, there were no clear morphological differences in adult, pupa, and mature larva between individuals obtained from galls that were induced on the upper surface of the leaf and those from the galls induced on the under surface of the leaf.

However, Takizawa (1983) considered that they might be different from each other be-

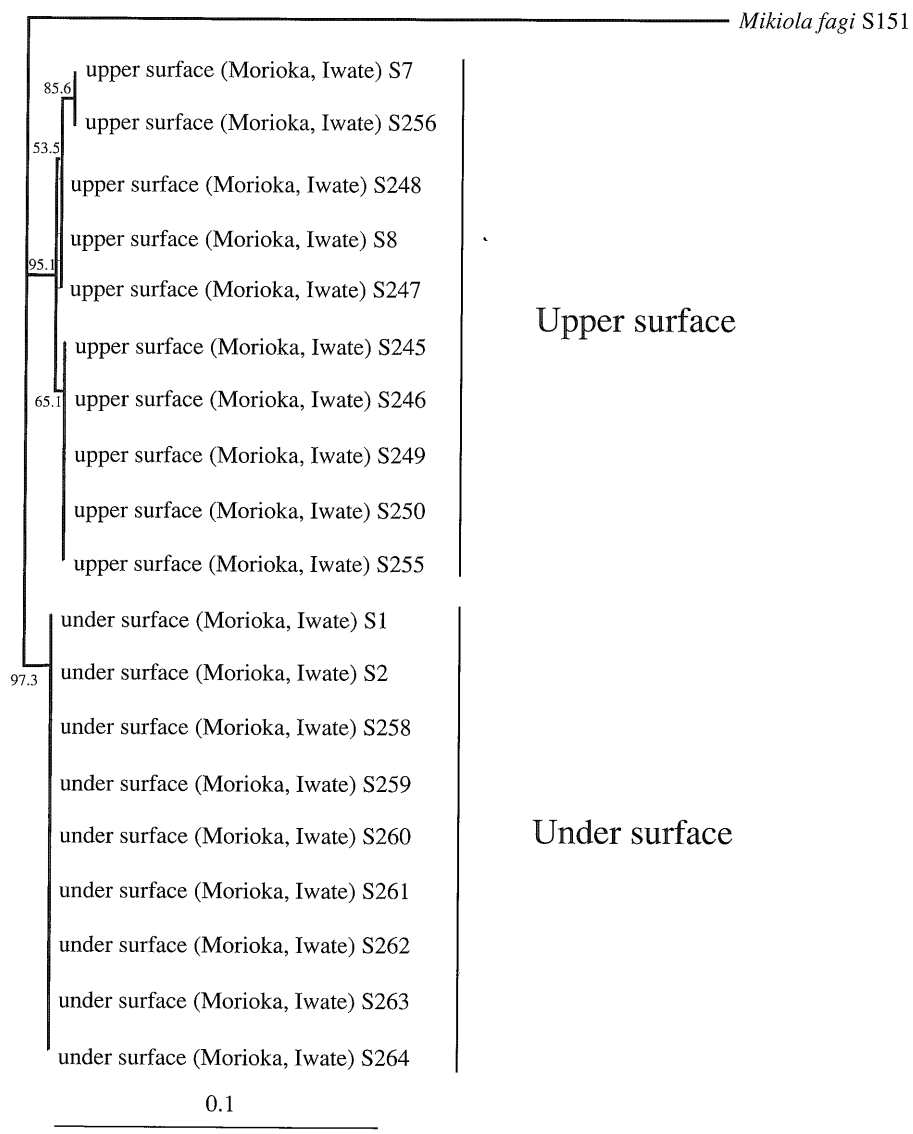


Fig. 9. NJ-tree for *Hartigiola faggalli* individuals obtained from the galls that were induced on the upper and under surfaces of *Fagus crenata* leaves. Isolation names correspond to the respective accession numbers registered in DNA database.

cause of different colors on egg surface.

DNA analysis indicated that there were 3 haplotypes within 10 individuals obtained from the galls on the upper surface of the leaf. These haplotypes were included in a clade supported by a 95.1 % bootstrap value (Fig. 9) and there were no differences among them in the 146 deduced amino acid residues.

Nine individuals from the galls on the under surface of the leaf were represented by only 1 haplotype (Fig. 9), which was 8 bp (1.82 %) to 10 bp (2.28 %) different from the haplotypes from the galls on the upper surface. In addition, there was difference in one of the 146 deduced amino acid residues between the haplotype on the under surface and each of the 3 haplotypes on the upper surface.

This means that the gall midges from upper and under surfaces are not different species because there are no morphological differences and the sequential differences are not big enough. Such a small extent in intra-specific sequential variations has been demonstrated for several gall midge species; *Aphidoletes aphidimyza* (Rondani) (Shirota *et al.*, 1999), *Asphondylia yushimai* Yukawa et Uechi (Yukawa *et al.*, 2003), *Contarinia maculipennis* Felt (Uechi *et al.*, 2003), *Asphondylia gennadii* (Marchal) (Uechi *et al.*, 2004), and *Asphondylia baca* Monzen (Uechi *et al.*, 2004). However, *H. faggalli* seems to be on the way to speciation. If so, it is an interesting phenomenon, exhibiting a possible sympatric speciation process on a single host plant species.

Acknowledgements

We wish to express our cordial thanks to Dr. K. M. Harris (Former Director of the International Institute of Entomology, UK), Mr. K. Tsuda (Kagoshima Prefecture), and Dr. T. Goto (Forestry and Forest Products Research Institute, Tohoku Branch) for their kindness in offering galls and gall-midge specimens. One of us, SS, also thanks Assoc. Prof. O. Tadauchi, Assist. Prof. S. Kamitani, and Mr. D. Yamaguchi (Entomological Laboratory, Kyushu University) for their continuous guidance and support.

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