LIFE HISTORY TRAITS, LARVAL HABITS AND LARVAL MORPHOLOGY OF A LEAFMINER, *COPTOTRICHE JAPONIELLA* (TISCHERIIDAE), ON AN EVERGREEN TREE, *EURYA JAPONICA* (THEACEAE), IN JAPAN

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ABSTRACT. *Coptotriche japoniella* (Tischeriidae) is a leafminer that is specialized on the evergreen trees, *Eurya japonica* and *E. emarginata* (Theaceae). We investigated its larval habits, seasonal development and larval morphology on *E. japonica* in a red-pine forest on Takamado Hill, Nara, Japan. *C. japoniella* has a univoltine life cycle with six larval instars and a long larval period of 9 months from August to May of the following year. The larvae possess crochets on the ventral prolegs, as do other *Coptotriche* larvae associated with Rosaceae and Fagaceae. We discuss the relationship between voltinism of tischeriid species and leaf type (i.e. deciduous or evergreen) of their host plants, and also examine crochets on the ventral prolegs as a diagnostic character among the three tischeriid genera, *Coptotriche*, *Tischeria* and *Astrotischeria*.

Additional key words: adult longevity, larval crochets, leaf selection for oviposition

The family Tischeriidae is a small group of leafmining moths, consisting of 114 described species (Puplesis & Diškus 2003). As of the early 1990s, the family was considered to be made up of only one genus (i.e., *Tischeria* Zeller, 1839). Leraut (1993) erected a new genus, *Emmetia*, and indicated that larvae of the new genus possess ventral prolegs with crochets on the 3rd to 6th abdominal segments, whereas those of *Tischeria* (*s. str.*) have ventral prolegs lacking crochets. However, he did not examine any *Tischeria* larvae that are associated with Asteraceae or Malvaceae in North America. Unfortunately, he did not notice that his newly erected genus agreed with the genus *Coptotriche* that was erected by Walsingham (1890) for a North American species; *Coptotriche* had been commonly treated as a junior synonym of *Tischeria* (Braun 1972). Puplesis & Diškus (2003) made a cladistic analysis of the family on the basis of 34 characters, thereby recognizing three main lineages of generic rank: *Tischeria* (27 described species for the world fauna), *Coptotriche* (57 species) and *Astrotischeria* Puplesis & Diškus (30 species). They treated the genus *Emmetia* as a junior synonym of *Coptotriche* and erected the new genus *Astrotischeria*, which is distributed in North and South America. In their cladogram, *Tischeria* and *Astrotischeria* form a sister group. They also presented all known host plants of tischeriids. According to them, main host-plant families are as follows: Fagaceae, Rhamnaceae and Tiliaceae in *Tischeria*, Rosaceae and Fagaceae in *Coptotriche*, and Asteraceae and

Malvaceae in *Astrotischeria*. Oddly enough, they made no reference to crochets of ventral prolegs by which Leraut (1993) characterized *Coptotriche* [=*Emmetia* in his sense] larvae. Consequently, crochets on the ventral prolegs remain unclear as a diagnostic character among the genera.

Compared with the taxonomic study of Tischeriidae, ecological study of the family has made little progress. Although voltinism of 18 species is known (Braun 1972; Opler 1974; Emmet 1976; Hisai 1979; Sato 1990; Puplesis & Diškus 2003), their life histories have not been examined in detail except for *T. ceanothi* Walsingham infesting *Ceanothus griseus* L. (Rhamnaceae) (Fasoranti 1984) and *T. ekebladella* (Bjerkander) infesting *Quercus robur* L. (Fagaceae) (Jordan 1995). Furthermore, parasitoid assemblages have been investigated in only three species, *T. ekebladella* (Jordan 1995), *T. quercifolia* Kuroko and *T. decidua* Wocke (Sato 1990).

Coptotriche japoniella Puplesis & Diškus is associated with the evergreen trees, *Eurya emarginata* (Thunb.) and *E. japonica* Thunb. (Theaceae). Before it was described as a new species by Puplesis & Diškus (2003), its life history had been investigated initially on *E. japonica* in Tokyo, Japan, by Hisai (1979). He reported that *C. japoniella* has a univoltine life cycle; the adults emerge in late May to June; the larvae hatch in late August, grow into 5th instar in late February of the following year, and pupate in mid-April to early June. Thus, *C. japoniella* has a unique life history in two

respects: (1) it is the only species to feed on plants of Theaceae among Tischeriidae, and (2) its larvae spend a long period of 9 months within the mine. In the current study, we investigated larval habits, seasonal development and leaf selection for oviposition according to leaf age in Nara, Japan. In addition, we examined larval morphology to consider whether crochets of prolegs can be used as a diagnostic character for *Coptotriche*. We have already published a paper on the parasitoid assemblage associated with *C. japoniella* (Oishi & Sato 2008).

Materials and Methods

Study site. We carried out this study in a red pine (*Pinus densiflora* Siebold & Zuccarini, Pinaceae) forest on Takamado Hill (400 m a.s.l., 34°40'N, 135°52'E) near the urban area of Nara. The understory vegetation of the forest was dominated by *E. japonica*.

Host plant. *Eurya japonica* is an evergreen tree, occurring widely in China, Taiwan, Korea and from the middle to the southern part of Japan (Kitamura & Murata 1979). New leaves break buds in late April in Nara, Japan (Oishi & Sato, unpublished), and mature leaves can remain on the tree for 3 yr or more (Nitta & Ohsawa 1997; Oishi & Sato, unpublished).

Seasonal development of *C. japoniella* larvae. We randomly sampled about 30 leaves with an intact mine of *C. japoniella* from about 10 *E. japonica* trees at intervals of 10 d from 22 August 2003 to 17 June 2004. When we found a pupal exuvium protruding from the mine or a small slit at the edge, we classified the mine as an adult emergence. In other cases, we dissected mines with forceps to check whether they contained a larva or pupa. To determine the larval instar, we measured head widths of the larvae to the nearest 1×10^{-4} mm on the basis of digital photographs taken with a microscope at 50X. We scanned 10 leaves with an image scanner (CanonScan D1250U2F, Canon Co., Japan), and measured leaf and mine areas to the nearest 1×10^{-2} mm² with free software for image analysis (LIA for Windows32, ver. 0.376β1; Yamamoto 1997).

Leaf selection by females for oviposition according to leaf age. We selected 73 trees within a quadrat of 2 m x 15 m on 5 March 2003, and tagged three to six shoots of each tree (in total, 225 shoots). For these shoots, we checked leaves on the old nodes and leaves on the current-year nodes on 17 October, and recorded the number of newly emerged mines on each leaf. We recorded the number of mines for the same shoots again on 16 November of the following year.

Larval habits and adult longevity. We sampled 199 mined leaves from nine randomly selected trees on 22 November 2004, and put them individually in a plastic case in which a sheet of moistened filter paper was laid. We kept them in an incubator at a temperature of 15°C with 10L:14D photoperiod until 26 January 2005, 17°C with 10L:14D until 10 February 2005, and thereafter 20°C with 12L:12D. We observed larval behavior during rearing. To examine adult longevity, we transferred adult moths (8 males and 6 females) that emerged from 12 to 13 May 2005, separately, into a plastic case in which two absorbent cotton pieces with distilled water and 10% honey, respectively, were laid. Afterward, we counted dead adults every 24 hr until all the individuals died.

Results and Discussion

Leaf selection for oviposition according to leaf age. The eggs of *C. japoniella* are cemented to the lower surface of the leaf. They are elliptic, 0.9 mm in length, 0.5 mm in width, with an iridescent semitransparent surface (Fig. 1A). Very few or no eggs were found on current-year leaves, but almost all eggs were laid on leaves aged 1 yr old or more (Fig. 2). Current-year leaves expanded during May, while adult females oviposited after June (see below). Thus, females exclusively select old leaves for oviposition.

Larval habits. The larva chews through the bottom of the egg, and directly enters the leaf tissue (Fig. 1A). It consumes the palisade tissue layer and the upper half of the spongy tissue layer (Fig. 1E), forming an irregular blotch mine with a yellowish green surface (Fig. 1B). It ejects frass through several arch-shaped slits on the lower surface of the mine. It feeds on the entire exuvium and leaves only the head capsule within the mine. When two mines on a leaf connect and the larvae encounter each other within the mine, cannibalism often occurs (Fig. 1D). The mature larva firmly backs the mine with silk, so that the upper surface of the mine is lined with a few folds (Fig. 1C). It also makes a crescent-shaped slit at the edge of the upper surface, and afterward closes it with silk. It pupates near the center of the mine. The pupa moves to the edge, and protrudes the upper part of its body from the slit at adult emergence.

Seasonal development. We estimated six larval instars from six peaks in the frequency distribution of larval head width (Fig. 3). Although the boundary between 1st and 2nd instars appeared to be obscure, it was confirmed by the existence of two head capsules remaining within the mine. However, Hisai (1979) mentioned that *C. japoniella* has five larval instars. He most likely missed the 3rd instar larvae because they made no field sampling in mid- to late September. Although Fasoranti (1984) and Jordan (1995) considered *T. ceanothi* and *T. ekebladella* to have four

larval instars, they did not seem to distinguish head capsules of 1st and 2nd instars, judging from the fact that the head-capsule width that they defined as the 1st instar's has a much larger coefficient of variance than those of other instars. Thus, *T. ceanothi* and *T. ekebladella* probably have five larval instars.

The 1st instar larvae hatched in mid- to late August, feeding on leaf tissue within the mine through the summer and early winter (Fig. 4B). They overwintered as 5th instars. Cessation of mine enlargement from December to March (Fig. 4A) indicates that 5th instar larvae scarcely consumed leaf tissue during the winter. Overwintered larvae resumed growing in early April, and pupated in mid-May to early June. The mean area of fully extended mines was 272.2 ± 31.8 mm² (mean ± SDs, n=10). Consequently, the larvae spent over 9 months within the mine.

Adult longevity and oviposition period. The adults emerged in mid-June (Fig. 2B). The mean longevity of adults under rearing conditions was 12.1 ± 2.03 days (mean ± SDs) for males and 14.7±1.21 days for females. Females already had mature ovaries 1 d after their emergence. These results suggest that oviposition of *C. japoniella* continues for 2 wk at most after adult emergence. Thus, it takes 2 months for the eggs to hatch in August, suggesting that eggs are in the state of aestivation after they are laid.

Life history traits. As mentioned above, *C. japoniella* is a univoltine leafminer with a long larval period of 9 months. As far as we know, *C. discreta* (Braun), which is distributed in North America and associated with evergreen oaks (*Quercus* spp.), is the
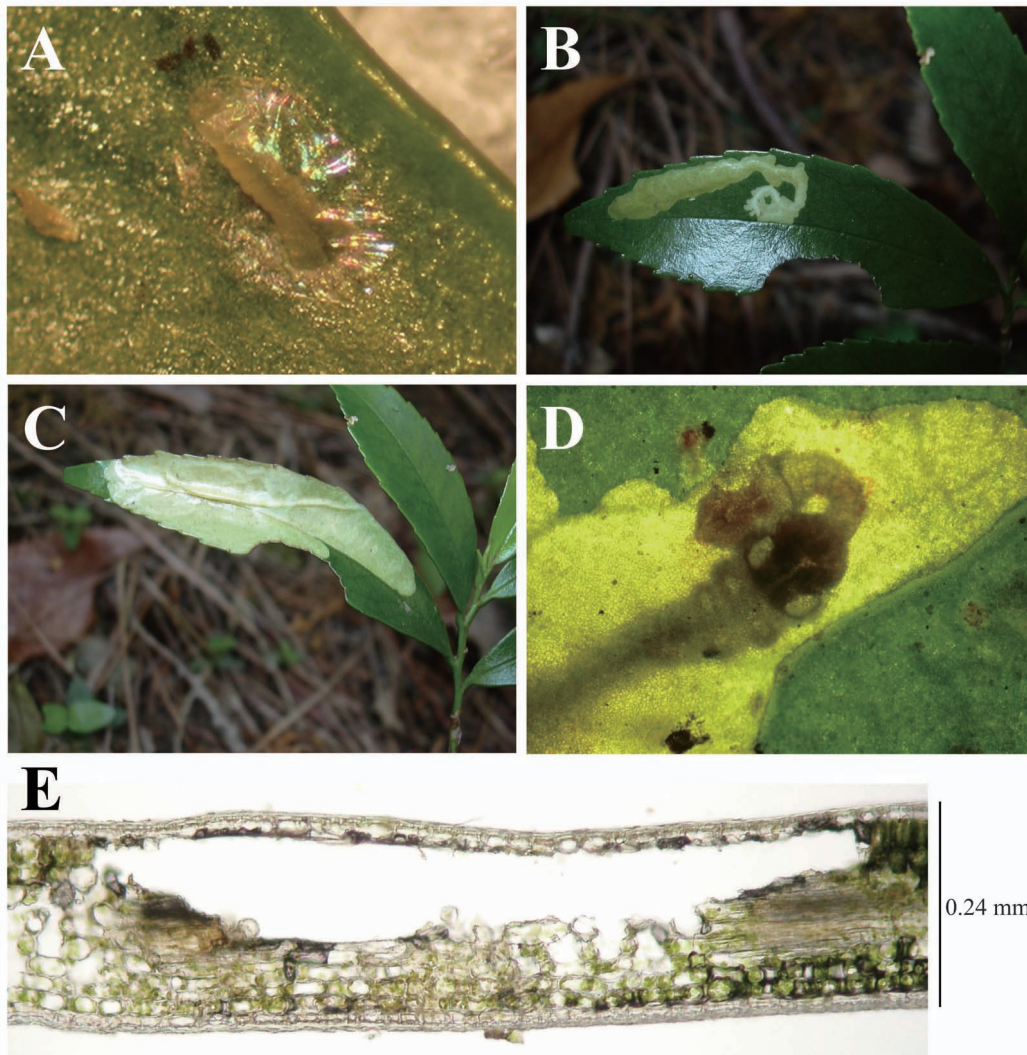

FIG.1. Immature stages of *Coptotriche japoniella*. **A,** egg shell and newly hatched larva directly mining into mesophyll under the egg; **B,** irregular blotch mine of 5th instar larva; **C,** fully expanded mine lined with a few folds; **D,** larval cannibalism within a mine which was formed by two mines connected; **E,** transverse section of mine of 3rd instar larva.
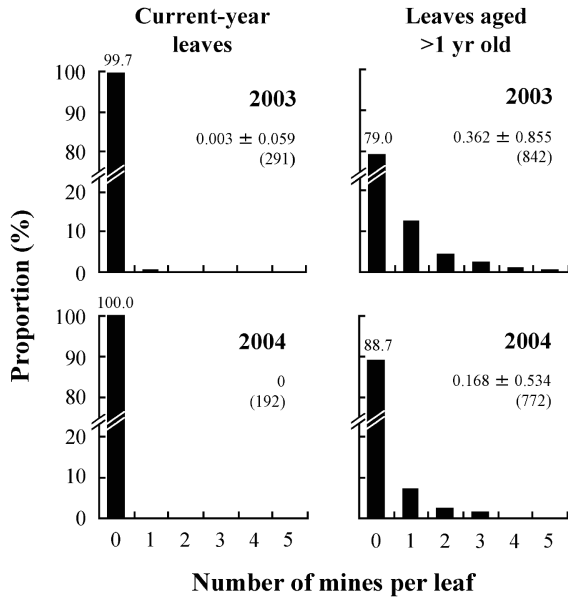
FIG. 2. Frequency distribution of *Coptotriche japoniella* mines on leaves newly expanded in the year and leaves aged 1 yr old or more in 2003 and 2004. Mean density per leaf is given with S.D. and sample size.
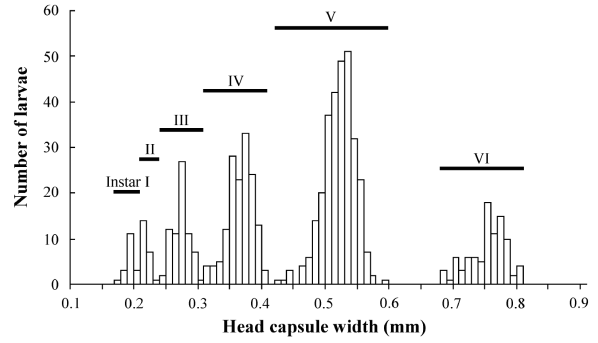
FIG. 3. Frequency distribution of head capsule width of *Coptotriche japoniella* larvae.
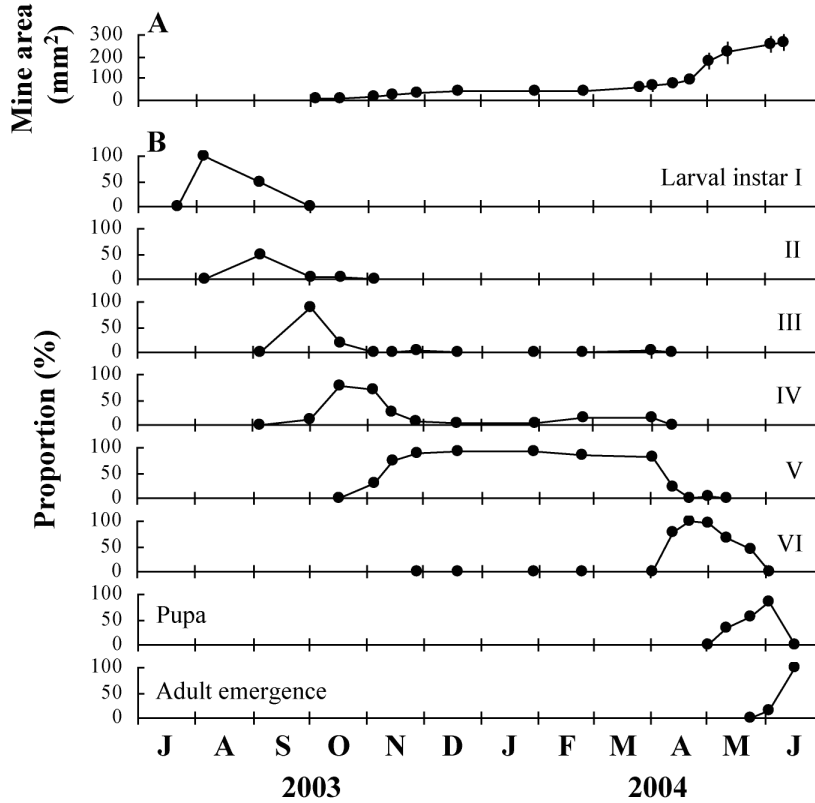
FIG. 4. Seasonal development of mines and immatures of *Coptotriche japoniella*. A, mean area of mines (± SD); B, frequencies of individuals in eight developmental stages.
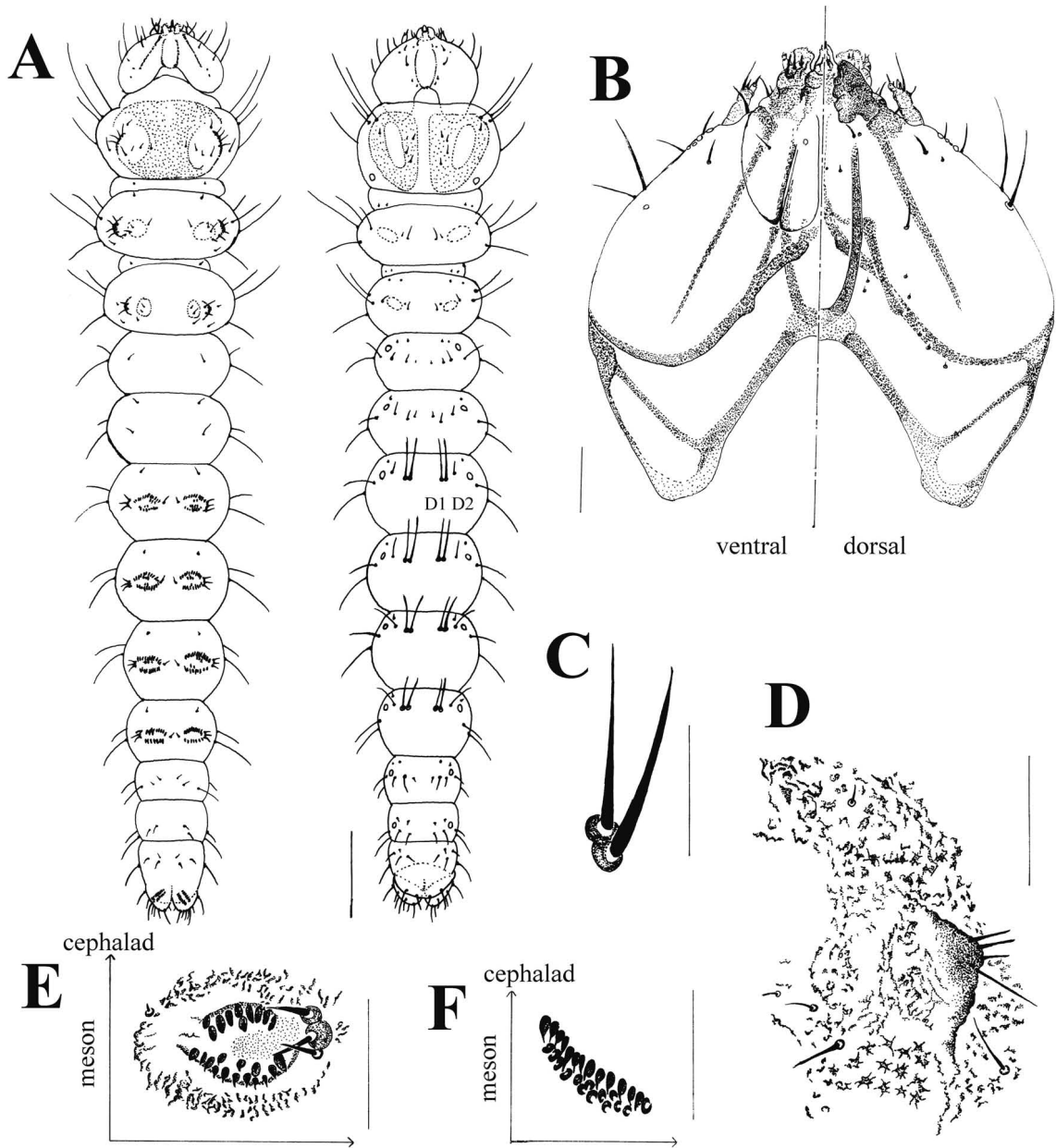
FIG. 5. Larval morphology of *C. japoniella*. **A,** ventral (left) and dorsal (right); **B,** head capsule (left: ventral, right: dorsal); **C,** D1 and D2 setae on 3rd abdominal segment; **D,** rudimentary leg on 1st thoracic segment; **E,** abdominal proleg with crochets on 5th abdominal segment; **F,** anal proleg with crochets. Bars indicate 0.1 mm.

only tischeriid besides *C. japoniella* to have such life history traits (Opler 1979).

Until now, 12 *Coptotriche* and 6 *Tischeria* species out of 114 tischeriids have been examined for both voltinism and host plants (Table 2). Three species with a facultatively bivoltine life cycle occur in Europe, showing univoltinism in the northern region and bivoltinism in the middle to southern region. The

geographic variation in voltinism of these species is probably due to temperature conditions. Although *C. gaunacella* (Duponchel), which also occurs in northern to southern Europe, has been regarded as univoltine (Emmet 1976), it may be bivoltine in the southern region. Only *C. japoniella* and *C. discreta* are tischeriids that have a univoltine life cycle and are associated exclusively with evergreen plants in temperate regions.

TABLE 1. Voltinism and leaf type (i.e. evergreen and deciduous) of host plants of 12 *Coptotriche* and 6 *Tischeria* species.

Species	Voltinism	Host plants		References
		Evergreen	Deciduous	
Coptotriche discreta (Braun)	Univoltine	+		Opler (1979)
Coptotriche japoniella Puplesis & Diskus	Univoltine	+		Present study
Coptotriche gaunacella (Duponchel)	Univoltine		+	Emmet (1983)
Coptotriche angusticollella (Duponchel)	Facultatively bivoltine		+	Kuroko (1982), Emmet (1983)
Tischeria ekebladella (Bjerkander)	Facultatively bivoltine		+	Jordan (1995)
Tischeria dodonaea Stainton	Facultatively bivoltine		+	Emmet (1983), De Prins & Steeman (2007)
Coptotriche heinemanni (Wocke)	Bivoltine		+	Kuroko (1982)
Coptotriche marginea (Haworth)	Bivoltine		+	Emmet (1983)
Coptotriche malifoliella (Clemens)	Bivoltine		+	Braun (1972)
Coptotriche citrinipennella (Clemens)	Multivoltine		+	Braun (1972)
Coptotriche badiiella (Chambers)	Multivoltine		+	Braun (1972)
Coptotriche purinosella (Chambers)	Multivoltine		+	Braun (1972)
Coptotriche crataegifoliae (Braun)	Bivoltine		+	Braun (1972)
Tischeria decidua Wocke	Bivoltine		+	Teramoto (1996)
Tischeria quercifolia Kuroko	Bivoltine		+	Kuroko (1982)
Tischeria naraensis Sato	Bivoltine		+	Sato (unpublished)
Coptotriche consanguinea (Braun)	Multivoltine	+	+	Braun (1972)
Tischeria ceanothi Walsingham	Bivoltine	+	+	Fasoranti (1984)

On the whole, therefore, tischeriids depending on deciduous plants tend to be bi- or multivoltine, while those depending on evergreen plants tend to be univoltine.

The voltinism in tischeriids can relate to nutritional differences in foliage between deciduous and evergreen plants. Reich *et al*. (1998) reviewed published data, and demonstrated that leaves aged 1 yr or more have a smaller amount of nitrogen (15.0 ± 6.1 mg g^{-1} (mean ± SDs) for 27 species) than leaves of deciduous trees or current-year leaves of evergreen trees (22.2 ± 6.5 mg g^{-1}, for 91 species). Actually, 1 year old leaves of *E. japonica* contain as large an amount of nitrogen (15.2 ± 0.9 mg g^{-1}, n=10) as those of other evergreen trees (Oishi & Sato, unpublished data). In general, nitrogen contents greatly influence the growth performance of folivorous insect larvae (Schoonhoven *et al*. 1998; Chown & Nicolson 2004; Oishi & Sato 2006). If this is true for tischeriids, a smaller amount of nitrogen contents in evergreen leaves would have delayed larval development of the leafminers, resulting in the evolution of univoltine life cycles.

Larval morphology. Davis (1987) presented the following diagnostic characters of tischeriid larvae: a strongly depressed head, deep epicranial notch, four to six stemmata arranged in a horizontal line along the lateral edge of the head, setae D1 and D2 arising extremely close together on the 3rd to 6th abdominal segments, the absence of thoracic legs, and poorly developed ventral prolegs. As Fig. 5 shows, we recognized these characters except for stemmata in *C. japoniella*; the number of stemmata was four on each side of the head. In addition, we present rudimentary legs on the three thoracic segments as a diagnostic character (Fig. 5A, D); this character has been found in other tischeriid species (Braun 1972; Davis 1987).

Davis (1987) also mentioned that crochets are usually present on the ventral prolegs, and arranged in multiserial bands or incomplete ellipses. When Leraut (1993) erected a new genus *Emmetia* for species that were associated with Rosaseae, he indicated that larvae of the new genus possess crochets on the ventral prolegs, whereas those of *Tischeria* (in his sense) that are associated with Fagaceae lack them. However, he examined no tischeriids that are associated with Asteraceae or Malvaceae. Later Puplesis & Diškus (2003) classified those tischeriids into a new genus, *Astrotischeria*, and also synonymized *Emmetia* with *Coptotriche*, without referring to the crochets of the ventral prolegs. We confirmed the presence of crochets

on the ventral prolegs in a *Coptotriche* sp. that is associated with evergreen oaks (*Quercus* spp.) besides *C. japoniella* in Japan (Sato, unpublished), thus suggesting that the presence of crochets on the ventral prolegs is common to *Coptotriche*. In contrast, it seems that *Astrotischeria* larvae do not always possess crochets on the ventral prolegs. Braun (1972: fig. 22) clearly drew crochets on the ventral prolegs in the larva of *A. heliopsisella* (Chambers), whereas we found them in *Astrotischeria* sp. that were collected from *Montanoa hibiscifolia* (Benth.) (Asteraceae) in Costa Rica (Sato, unpublished). Taking it into account that crochets are often used as a diagnostic character for families and genera of Lepidoptera (Stehr 1987), the genus *Astrotischeria* might be possibly divided into two or more genera. Further studies of larval crochets, especially in *Astrotischeria*, should be undertaken.

ACKNOWLEDGEMENTS

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