Intense inhabitation and relaxed host-leaf preference of aquatic chironomid leaf-miners in headwater streams in Asian lucidophyllous forests

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Submerged leaf litter in headwater streams covered by Asian lucidophyllous (glossy broad-leaved evergreen) forests was found to be frequently processed by leaf-mining larvae of a chironomid, *Stenochironomus okialbus*. The larva gnaws plant tissue of the mine wall while swimming and undulating its body in the water-filled mine. To detect the level of the inhabitation and host-preference of the aquatic leaf-miner, extensive samplings of mined leaves were made in various headwater streams covered by lucidophyllous forests in the Japanese Island Arc. The chironomid showed significant preference for leaf texture (evergreen to deciduous leaves) and for specific taxonomic groups of the host plants (Fagaceae and Lauraceae), whereas diverse taxa of leaves were utilised. The relaxed host-leaf preference unique among herbivorous insects is suggested to be caused by the larval water-circulating behaviour in their porous mines and by leaching and microbial decomposition of plants’ secondary metabolites in submerged leaves.

**Keywords:** host-leaf preference; aquatic leaf-miner; lucidophyllous forest; detritus passway; chironomid midge

Introduction

In forested headwater stream ecosystems, leaf litter of vascular plants is the most important component in the organic matter budgets, and the plant breakdown proceeds in leaching, microbial decomposition and conditioning, and fragmentation by invertebrate shredders (Kaushik and Hynes 1968; Webster and Benfield 1986; Wallace and Webster 1996; Gessner et al. 1999). The relative importance of microbial vs macroinvertebrate processing changes on a latitudinal gradient with macroinvertebrate shredders more abundant and diverse in temperate than in tropical streams (Boyero et al. 2011, 2012), and among tropical streams macroinvertebrate shredders are more important at high altitudes (Irons et al. 1994; Yule et al. 2009).

Between the microbial and the invertebrate processings, however, a unique process – that is, leaf-mining by *Stenochironomus* larvae (Insecta: Diptera: Chironomidae) – occurs in some restricted ecosystems such as clear lentic or lotic water in the holarctic region (Borkent 1984) and in the Neotropics (Wantzen and Wagner 2006). Although there are several genera of leaf-mining chironomids which live by filter-feeding, *Stenochironomus* larvae feed on tissue of submerged leaves (Borkent 1984). In terrestrial ecosystems, leaf-mining habits are ubiquitous among diverse phytophagous insect lineages, and host-specific leaf-miners are quite common.

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on diverse plant species (Hering 1951). In contrast with the great diversity and abundance of these terrestrial leaf-miners, aquatic leaf-miners of submerged leaf litter are generally less common and have attracted little ecological attention.

Through my ecological survey of submerged leaf litter in headwater streams in the Japanese Arc, a great abundance of Stenochironomus leaf-mines has been found in lotic water running in lucidophyllous (evergreen broad-leaved) forests, the dominant trees of which have thick, glossy, long-lived leaves with developed cuticle (Ohsawa 2009). The textural and ecological characteristics of the leaves are considered to be adaptations to various environmental and biological stresses such as much rainfall caused by monsoon during the summer season, cool dry weather in winter, soil nutrient deficiency and intense herbivory (Chabot and Hicks 1982; Prentice et al. 1992; Onoda et al. 2011). The leaves fallen into streams are transported by currents and gathered into brinks, pools or interstitials among gravels and boulders. The thick, glossy, long-lived leaves of lucidophyllous trees are more water-persistent than thin, unglossy, deciduous leaves, whereas both types of leaves are conditioned by aquatic fungi and microorganisms. The conditioned and softened leaves are often mined by the chironomid leaf-miners, before being consumed by shredders such as trichopteran, ephemeropteran and plecopteran insects, shrimps, crabs and tadpoles.

Litter fall is provided by diverse tree species in lucidophyllous forests as in tropical forests (Covich 1988). The physical and chemical attributes of leaves vary among different tree species, and are known to influence leaf litter utilisation by shredders (Anderson and Sedell 1979; Webster and Benfield 1986; Bastian et al. 2007). Aquatic leaf-miners might be more directly influenced by the chemical defence substances than shredders, because they are living in leaf tissue. However, almost no analyses on leaf utilisation pattern of these aquatic leaf-miners have been conducted.

In order to clarify the abundance, biology and ecological role of the Stenochironomus species in Asian lucidophyllous forests, I conducted extensive sampling of submerged leaf litter in various streams in the Japanese Arc, and examined density and host-plant utilisation patterns of the chironomid larvae. In this paper, I report the natural history, distribution pattern and relaxed host-leaf preference of the aquatic leaf-miner, which contrasts with the high host-plant specificity of terrestrial leaf-miners. In compiling these data, I discuss an ecological role of the Stenochironomus species in the decomposition chain in streams in lucidophyllous forests.

Materials and methods

After a preliminary search for aquatic leaf-miners, extensive samplings of submerged leaf litter in streams were made throughout the Japanese island arc from 1999 to 2010. A scoop of submerged leaves was collected from bathing brinks or pools in streams with a shafted hand net (diameter = 40 cm). Among 48 sampling sites (Figure 1), mined leaves were found at 11 sites (S1–S11) at altitudes from 5 to 250 m and latitudes from 24 to 38°N (Table 1). S1–S7 are located in the Japanese Archipelago, and S8–S11 in the Ryukyu Archipelago. Samples which were collected more than one time at the same site were merged. No specific permissions were required for these locations/activities, and the field studies did not involve endangered
or protected species. Although periodic samplings were not made at each site, the sampling dates collectively totally cover all seasons.

For the samples at S1–S11, all leaves were identified and examined for presence/absence of leaf-mines. The common plant species, for which more than nine leaves were collected, are listed in Table 2. For three samples at S8–S10 (S8, 27 August 2006; S9, 30 April 2006; S10, 9 May 2002), frequency distribution of leaf-mines per leaf was investigated. Mining larvae were reared in aquaria whose water was changed every day, and their behaviour and morphology were observed under a microscope.
| Site | Locality | Latitude, longitude | Altitude (m) | Sampling date | Dominant tree species of the surrounding forest | No. sampled leaves |
|------|----------|---------------------|--------------|--------------|-----------------------------------------------|-------------------|
| S1   | Tsuzura-buchi, Shirahama, Wakayama Pref. | 33°38’45”N, 135°25’26”E | 30 | 3 Aug., 2 October 2004 | Quercus glauca, Castanopsis sieboldii, Myrica rubra | 189 |
| S2   | Kotonotaki, Susami, Wakayama Pref. | 33°33’37”N, 135°32’26”E | 140 | 2 September 2002, 2 August 2004 | Castanopsis sieboldii, Quercus glauca, Quercus phillyraeoides | 203 |
| S3   | Takase, Koza-gawa, Wakayama Pref. | 33°32’20”N, 135°45’53”E | 5 | 11 November 2005, 5 May 2008 | Castanopsis sieboldii, Machilus japonica, Alnus serrulatoides | 333 |
| S4   | Takinohai, Kozagawa, Wakayama Pref. | 33°36’22”N, 135°45’54”E | 70 | 19 November 2005 | Quercus glauca, Zelkova serrata, Quercus phillyraeoides | 343 |
| S5   | Nachi, Nachi-Katsuura, Wakayama Pref. | 33°40’38”N, 135°53’57”E | 180 | 28 Auf. 2003 | Quercus glauca, Castanopsis sieboldii, Quercus salicina | 936 |
| S6   | Nakatani, Sakamoto, Kumamoto Pref. | 32°26’47”N, 130°39’47”E | 30 | 11 December 2005 | Quercus glauca, Celtis sinensis, Wisteria floribunda | 635 |
| S7   | Tashiro, Kinkou, Kagoshima Pref. | 31°9’38”N, 130°52’31”E | 250 | 11 December 2005 | Castanopsis sieboldii, Distylium racemosum, Ligustrum japonicum | 203 |
| S8   | Akina, Amami Is., Kagoshima Pref. | 28°25’6”N, 129°33’26”E | 60 | 4 July 1999, 8 June 2002, 19 December 2005, 27 April 2006, 5 December 2009 | Castanopsis sieboldii, Quercus glauca, Machilus japonica | 1764 |
| S9   | Yakukachi, Amami Is., Kagoshima Pref. | 28°13’34”N, 129°21’20”E | 25 | 18 December 2005, 30 April 2006, 27 April 2007 | Castanopsis sieboldii, Styrax japonica, Machilus japonica | 1928 |
| S10  | Yona, Okinawa Is., Okinawa Pref. | 26°45’13”N, 128°13’12”E | 80 | 2 May 2002 | Castanopsis sieboldii, Alnus formosana, Machilus japonica | 735 |
| S11  | Arakawa, Ishigaki Is., Okinawa Pref. | 24°26’49”N, 124°10’46”E | 30 | 16 October 2002, 23 January 2011 | Castanopsis sieboldii, Calophyllum inophyllum, Schefflera octophylla | 252 |
Table 2. A list of species of submerged fallen leaves recorded from headwater streams at 11 sites, with their habit, desiduosity, leaf texture and numbers of unmined/mined leaves.

| Family              | Species                     | H | D | LT | Site | Total |
|---------------------|-----------------------------|---|---|----|------|-------|
|                     |                             | S1 | S2 | S3 | S4  | S5   | S6   | S7   | S8  | S9  | S10 | S11 |
| Aceraceae           | *Acer palmatum*             | T  | D  | C  | 0/0 | 0/0  | 0/0  | 66/0 | 0/0 | 0/0 | 0/0 | 0/0 | 0/0 | 66/0 |
| Anacardiaceae       | *Rhus succedanea*           | T  | D  | C  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 15/0 | 5/0 | 0/0 | 0/0 | 20/0 |
| Apocynaceae         | *Trachelospermum asiaticum* | L  | E  | G  | 0/0 | 0/0  | 2/0  | 0/0  | 0/0  | 9/1 | 0/0 | 1/0 | 0/0 | 0/0 | 12/1 |
| Aquifoliaceae       | *Ilex integra*              | T  | E  | G  | 0/0 | 0/0  | 12/0 | 2/0  | 1/0  | 0/0  | 0/0  | 0/0 | 0/0 | 0/0 | 15/0 |
| Araliaceae          | *Schefflera octophylla*     | T  | E  | G  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 48/1 | 27/0 | 2/0 | 11/3 | 88/4 |
| Betulaceae          | *Alnus formosana*           | T  | D  | C  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 1/0 | 122/5 | 0/0 | 123/5 |
| Betulaceae          | *Alnus serrulatoides*       | T  | D  | C  | 0/0 | 0/0  | 38/0 | 3/1  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 41/1 |
| Caprifoliaceae      | *Viburnum sieboldii*        | S  | D  | C  | 0/0 | 0/0  | 15/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 15/0 |
| Clusiaceae          | *Calophyllum inophyllum*    | T  | E  | G  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 12/2 |
| Elaeocarpaceae      | *Elaeocarpus sylvestris*    | T  | E  | G  | 0/0 | 0/0  | 9/0  | 0/0  | 0/0  | 5/0 | 6/0 | 0/0  | 0/0  | 0/0  | 21/0 |
| Euphorbiaceae       | *Mallotus japonicus*        | T  | D  | C  | 0/0 | 0/0  | 4/0  | 3/0  | 15/0  | 0/0  | 11/0 | 0/0  | 0/0  | 0/0  | 33/0 |
| Fabaceae            | *Wisteria brachybotrys*     | L  | D  | C  | 0/0 | 0/0  | 0/0  | 0/0  | 120/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 120/0 |
| Fabaceae            | *Wisteria floribunda*       | L  | D  | C  | 7/0 | 0/0  | 0/0  | 25/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 32/0 |
| Fagaceae            | *Castanopsis sieboldii*     | T  | E  | G  | 12/3 | 100/4 | 91/4 | 15/2 | 143/20  | 0/0 | 30/22 | 481/42 | 566/39 | 192/133 | 105/18 | 1743/99 |
| Fagaceae            | *Quercus gigia*             | T  | E  | G  | 61/7 | 40/0 | 39/1 | 120/52 | 394/5 | 130/9 | 8/0 | 234/81 | 18/6 | 0/0  | 0/0  | 1044/161 |
| Fagaceae            | *Quercus glauca*            | T  | E  | G  | 0/0 | 0/0  | 0/0  | 5/0  | 48/6 | 0/0  | 0/0  | 0/0  | 0/0  | 71/12 | 19/0  | 20/92 |
| Fagaceae            | *Quercus myrsinfolia*       | T  | E  | G  | 0/0 | 0/0  | 0/0  | 5/0  | 48/6 | 0/0  | 0/0  | 0/0  | 0/0  | 71/12 | 19/0  | 20/92 |
| Fagaceae            | *Quercus phillyraeoides*    | T  | E  | G  | 4/2 | 2/1  | 0/0  | 28/0 | 12/0 | 0/0  | 0/0  | 0/0  | 0/0  | 71/12 | 19/0  | 20/92 |
| Fagaceae            | *Quercus salicina*          | T  | E  | G  | 0/0 | 6/0  | 0/0  | 0/0  | 70/5 | 0/0  | 0/0  | 0/0  | 0/0  | 70/5 | 0/0  | 76/5 |
| Fagaceae            | *Quercus sessilifolia*      | T  | E  | G  | 0/0 | 0/0  | 0/0  | 8/0  | 15/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 23/0 |
| Hamamelidaceae      | *Distylium racemosum*       | T  | E  | G  | 0/0 | 0/0  | 0/0  | 0/0  | 32/0 | 0/0  | 1/0 | 0/0  | 0/0  | 0/0  | 0/0  | 33/0 |
| Lamiaceae           | *Premna corymbosa*          | S  | E  | G  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 15/0 | 15/0 | 15/0 |
| Lardizabalaceae     | *Akebia trifoliata*         | L  | E  | G  | 0/0 | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 0/0  | 21/0 | 0/0  | 21/0 |
| Lauraceae           | *Cinnamomum tenuifolium*    | T  | E  | G  | 1/0 | 5/0  | 0/0  | 0/0  | 26/0 | 8/0  | 0/0  | 9/0  | 0/0  | 46/8 | 15/0 | 110/8 |

(Continued)
| Family      | Species            | H  | D  | LT | Site      | Total |
|-------------|--------------------|----|----|----|-----------|-------|
| Lauraceae   | Machilus japonica | T  | E  | G  | 4/2 3/0 114/0 0/0 11/1 0/0 0/0 43/6 | 320/27 |
| Lauraceae   | Machilus thunbergii | T  | E  | G  | 0/0 4/0 0/0 0/0 0/0 2/0 12/0 13/4 70/5 9/3 7/0 | 117/12 |
| Moraceae    | Ficus ampelopsis   | S  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 18/0 0/0 3/0 5/0 | 26/0   |
| Moraceae    | Ficus caulocarpa   | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 11/0 11/0 |
| Moraceae    | Ficus erecta       | S  | D  | C  | 0/0 0/0 3/0 0/0 14/0 4/0 0/0 0/0 5/0 0/0 0/0 0/0 26/0 |
| Moraceae    | Morus australis    | T  | D  | C  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 33/0 0/0 0/0 0/0 33/0 |
| Moraceae    | Myrica rubra       | T  | E  | G  | 22/10 4/0 0/0 0/0 1/0 0/0 12/0 5/0 30/20 2/1 0/0 76/31 |
| Myricaceae  | Ardisia sieboldii  | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 16/0 30/0 0/0 3/0 49/0 |
| Myrsinaceae | Myrsine seguini     | T  | E  | G  | 10/0 10/0 0/0 0/0 4/0 0/0 0/0 2/0 15/0 0/0 1/0 42/0 |
| Oleaceae    | Ligustrum japonicum | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 42/0 0/0 0/0 0/0 42/0 |
| Phyllanthaceae | Glochidion lanceolatum | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 10/0 10/0 |
| Poaceae     | Pleioblastus linearis | S  | S  | C  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 11/0 0/0 0/0 0/0 11/0 |
| Rhamnaceae  | Berchemia magna    | S  | D  | C  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 11/0 0/0 0/0 0/0 11/0 |
| Rosaceae    | Photinia glabra     | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 21/0 0/0 0/0 0/0 21/0 |
| Rosaceae    | Rhaphiolepis indica | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 1/0 1/0 9/0 0/0 11/0 |
| Rubiaceae   | Psychotria serpens | L  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 15/0 0/0 0/0 0/0 15/0 |
| Rubiaceae   | Wendlandia formosana | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 3/0 8/0 1/0 0/0 12/0 |
| Sabiaceae   | Meliosma rigidia   | T  | E  | G  | 2/1 4/0 0/0 0/0 1/0 0/0 0/0 4/0 12/0 9/0 0/0 32/1 |
| Salicaceae  | Ilesia polyacarpa  | T  | S  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 141/0 0/0 0/0 0/0 141/0 |
| Simaroubaceae | Turpinia ternata  | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 38/0 6/0 0/0 0/0 44/0 |
| Simplocaceae | Simplocos glauca  | T  | S  | C  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 25/0 7/0 236/0 0/0 0/0 268/0 |
| Styracaceae | Styrax japonica    | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 10/0 |
| Theaceae    | Camellia japonica  | T  | E  | G  | 0/0 5/0 0/0 0/0 3/0 0/0 3/0 0/0 0/0 0/0 0/0 0/0 11/0 |
| Theaceae    | Cleayera japonica  | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 5/0 0/0 0/0 0/0 0/0 0/0 0/0 14/1 |
| Theaceae    | Eurya japonica     | T  | E  | G  | 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 12/0 |

(Continued)
Table 2. (Continued).

| Family  | Species             | Habit | Deciduosity | Leaf Texture | Unmined Leaves | Mined Leaves |
|---------|---------------------|-------|-------------|--------------|----------------|--------------|
| Theaceae| Schima liukiensis   | T E G |              |              | 4/0 57/0 65/4 126/0 |              |
| Ulmaceae| Celtis sinensis     | T D C |              |              | 0/0 0/0 0/0 220/0 |              |
| Ulmaceae| Trema orientalis    | T D C |              |              | 0/0 0/0 0/0 164/0 |              |
| Ulmaceae| Ulmus parvifolia    | T D C |              |              | 0/0 0/0 0/0 0/0  |              |
| Ulmaceae| Zelkova serrata     | T D C |              |              | 2/0 0/0 0/0 89/0  |              |
| Urticaceae| Boehmeria nivea  | P D C |              |              | 0/0 0/0 0/0 45/0  |              |
| Vitaceae| Ampelopsis glandulosa| L D C |              |              | 0/0 0/0 0/0 0/0  |              |
| Others  |                     |       |              |              | 10/0 12/0 7/1 34/1 187/12 |              |

Total number of unmined leaves: 156 198 327 288 899 1249 1509
Total number of mined leaves: 33 5 6 55 37 9 22 419 165 24

Note: 
- Habit: E, epiphyte; L, liana; P, perennial; S, shrub; T, tree.
- Deciduosity: D, deciduous; E, evergreen; S, semievergreen.
- Leaf texture: C, charthaceous; G, glossy coriaceous.
To examine host-leaf preference by the chironomid, plant species were sorted by habit, deciduosity, leaf texture and family (Table 2). The data set consists of numbers of unmined and mined leaves. To test the hypotheses that the proportion of mined leaves differs between tree and non-tree leaves, between evergreen and deciduous leaves and among plant taxa, likelihood rate $\chi^2$ tests were conducted.

**Results**

Leaf-mines of submerged leaves were found in clear headwater streams covered by natural lucidophyllous forests at S1–S11 in southwestern Japan, ranging from Kii Peninsula (S1–S5) of Honshu to the Ryukyu Archipelago (Figure 1; Table 1), although the distribution of lucidophyllous forests goes up beyond central Honshu. Adults that emerged from the mined leaves were proved to be *Stenochironomus okialbus* Sasa, 1990 (Figure 2J, K), whose larval biology was not previously known.

The habitats of the chironomid were bathing brinks and pools and interstitials of gravels or boulders in streams, where many fallen leaves are accumulated (Figure 2A, B). The water of all the streams where mined leaves were observed was clean and clear, and little fine sediment was deposited on submerged leaves (Figure 2C). The leaves covered by fine sediment in stagnant pools were not mined. The streams are usually inhabited by crabs (*Eriocheir japonica*), shrimps (*Cardina* spp. and *Atyopsis* spp.) and gobies (*Rhinogobius* spp.), and diverse aquatic insects, and ellipsoid leaf cases were sometimes cut out from submerged leaves by the caddisfly *Anisocentropus immunis*. The plant species of submerged leaf litter comprised 129 species, 70.0% of which were lucidophyllous trees of the genera *Castanopsis*, *Quercus* (Fagaceae), *Machilus*, *Cinamomum* (Lauraceae), *Myrica* (Myricaceae), *Schima* (Theaceae), etc. (Table 2; Figure 2C–F). The aquatic leaf-miner was found almost exclusively on submerged leaves of these lucidophyllous tree species.

Chironomid larvae mine submerged, conditioned leaves, the colour of which is black or dark brown. The larvae never mine just-fallen green leaves. The mine is linear at first, and then expands indeterminately (Figure 2D–F). The flat, elongated larva mines and feeds on conditioned tissue of the mine wall without damaging the epidermis, and builds two elongate, parallel, frass walls while undulating its elongated body (Figure 2G). The frass wall is composed of undigested plant debris. When feeding, the larva comes out from the resting site between the frass walls and infests the conditioned tissue using its mandibles (Figure 2I). A larva could complete its larval life by infesting only a part of a conditioned leaf. The larva pupates between the frass walls (Figure 2H), and an adult emerges by dashing out from the pupa in the mined leaf.

A leaf was sometimes mined by several larvae (Figure 2E, F). For samples at S8–S10, the mean number of chironomid larvae per leaf varied from 1.1 (S9) to 3.0 (S8). The proportion of mined leaves ranged from 0 to 79% at S1–S11, and peaked in Amami Islands (S8 and S9). High proportions of mined leaves were observed from April to May. Mining larvae were found throughout the year in the Ryukyu Archipelago, whereas no mining larvae were found in observations in winter at northern sites (S1–S4).

The chironomid larvae mined various species of leaves, and the plant species recorded as hosts of the chironomid reached 24 species, 15 genera and 11 families (Table 2). Among 51 plant families constituting the submerged litter, Fagaceae and
Figure 2. Habitats and leaf-mining habits of Stenochironomus okialbus. (A–B) headwater streams at S4 and S9; (C) submerged litter at S9; (D–F) leaf-mines; (G) a larva undulating in a mine; (H) a pupa; (I) head of a mining larva; (J–K) a female and a male adult midge. Plant species of the leaves: C, D, G, I: Castanopsis sieboldii; E, H: Dendropanax trifidus; E: Myrica rubra.
Lauraceae were most dominant, comprising 73.6 and 8.0% of total leaves, respectively. The most frequently mined leaves were *Castanopsis sieboldii*, *Quercus* spp. (Fagaceae), *Machilus* spp. (Lauraceae) and *Myrica rubra* (Myricaceae), all of which were lucidophyllous tree species (Figure 3). To detect host-leaf preference of the chironomid in detail, the following three hypotheses were tested: (1) tree leaves are preferred to non-tree (shrub, perennial and liana) leaves; (2) evergreen leaves are preferred to non evergreen (deciduous and semi-deciduous) leaves; (3) Fagaceae leaves are preferred to Lauraceae leaves. The proportions of mined leaves were compared between the above-mentioned paired categories, and the differences were tested by likelihood rate $\chi^2$ test. These three hypotheses were confirmed at eight, nine and five sites, respectively (Table 3). Deciduousity coincided with leaf texture, evergreen leaves were glossy coriaceous and non-evergreen leaves were charthaceous. The chironomid also showed a preference for specific taxa, e.g., Fagaceae and Lauraceae, whereas diverse taxa of leaves were utilised as hosts.

**Discussion**

In this study, a high abundance of aquatic leaf-miners (up to 79% of submerged leaves) was detected in forested headwater streams in Asian lucidophyllous forests. This finding suggests that leaf-mining by chironomid larvae is another important...
Table 3. Results of likelihood rate $\chi^2$ tests for the difference of proportions of mined leaves between categories as to plant habit (tree vs non-tree), deciduosity (evergreen vs non-evergreen) and plant families (Fagaceae vs Lauraceae).

| Statistics | Sites |  |  |  |  |  |  |  |  |  |  |
|------------|------|---|---|---|---|---|---|---|---|---|---|
|            | S1   | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10| S11|
| **Plant habit** |      |    |    |    |    |    |    |    |    |    |    |
| % mined (tree) | 19.2 | 2.5 | 1.6 | 18.3 | 4.1 | 2.2 | 11.3 | 30.4 | 22.5 | 25.1 | 10.7 |
| % mined (non-tree) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $\chi^2$ | 8.9 | 2.2 | 0.6 | 18.2 | 4.6 | 6.8 | 4.6 | 44.1 | 3.5 | 7.2 | 7.7 |
| $p$ | 0.003 | 0.138 | 0.132 | < 0.001 | 0.032 | 0.009 | 0.033 | < 0.001 | 0.063 | 0.008 | 0.005 |
| **Deciduosity** |      |    |    |    |    |    |    |    |    |    |    |
| % mined (evergreen) | 19.3 | 2.5 | 1.8 | 22.7 | 4.6 | 6.0 | 12.9 | 34.0 | 28.2 | 29.3 | 9.6 |
| % mined (non-evergreen) | 0 | 0 | 1.7 | 1.0 | 0 | 0 | 0 | 0 | 0 | 3.8 | 0 |
| $\chi^2$ | 7.7 | 2.2 | 0.0 | 35.8 | 514.3 | 25.4 | 4.9 | 177.2 | 254.2 | 49.0 | 12.7 |
| $p$ | 0.005 | 0.138 | 0.945 | < 0.001 | < 0.001 | < 0.001 | 0.026 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| **Plant family** |      |    |    |    |    |    |    |    |    |    |    |
| % mined (Fagaceae) | 17.1 | 3.2 | 3.7 | – | 5.0 | 6.5 | 36.7 | 41.2 | 36.5 | 38.7 | 14.4 |
| % mined (Lauraceae) | 28.6 | 0 | 0 | – | 2.2 | 0 | 0 | 13.3 | 9.9 | 12.9 | 3.8 |
| $\chi^2$ | 0.5 | 2.9 | 8.2 | – | 0.9 | 3.3 | 11.8 | 26.5 | 59.4 | 31.2 | 2.8 |
| $p$ | 0.442 | 0.090 | 0.004 | – | 0.334 | 0.070 | 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.097 |
option of detritus processing, in addition to microbial decomposition and fragmentation by shredders, in the stream ecosystem where litter of glossy evergreen broad-leaved leaves is amply supplied. Because mined leaves become soft and susceptible to fragmentation by shredders, the leaf-miner–shredder facilitation of leaf litter decomposition may be present as observed between shredders and tadpoles (Iwai et al. 2009). The headwater streams in lucidophyllous forests are comparable to the streams in the Neotropics (Wantzen and Wagner 2006) because aquatic leaf-miners contribute substantially to detritus processing of allochthonous organic materials.

The aquatic leaf-miner preferred tree leaves to non-tree leaves, and lucidophyllous to deciduous leaves, suggesting that glossy, thick, water-persistent textures of leaf litter are preferred. For the aquatic leaf-miner, a tough texture of the leaf litter is necessary because the mined leaves need to endure physical disturbance and fragmentation by shredders at least during their immature period.

In addition to leaf texture, the leaf-miner showed preference for particular plant taxa, for example, Fagaceae and Lauraceae, whereas both plant families were utilised as host plants. Because secondary metabolites of these two families are profoundly dissimilar, very few herbivorous insects in terrestrial ecosystems utilise both plant families. Thus, it is rather surprising that the chironomids can utilise the diverse taxa of leaves, when secondary metabolites are involved in this preference. The relaxed host-plant preference of the aquatic leaf-miner contrasts with the high host-plant specificity of terrestrial leaf-miners, each of which confronts specific chemical defences of each host plant species. The weakness of host-plant preference in the aquatic leaf-miner is considered to be caused by leaching and microbial decomposition of secondary metabolites, as observed in stream shredders (Cummins et al. 1989). This result is comparable to the less-specific plant–decomposer interactions, where well-defended leaves are not necessarily less decomposable litter in Asian tropical forest ecosystems (Kurokawa and Nakashizuka 2008).

The evergreen leaves of lucidophyllous trees are long-lived (maximum leaf age 2–5 years; Nitta and Ohsawa 1997), and characterised by a glossy, thick texture. The developed cuticle of the leaf epidermis is an adaptation to various stresses such as cool dry weather in winter, and the water persistence of the leaves caused by the glossy epidermis indirectly contributes to foster the aquatic leaf-miner. A high density of the leaf-miner was observed from April to May, when leaf flushing and leaf fall of the dominant tree Castanopsis sieboldii occur in Asian lucidophyllous forests (Kurokawa and Nakashizuka 2008). The abundance of the aquatic leaf-miner is threatened especially in the Ryukyu Archipelago by dam construction which destroys stream habitats, and by deforestation, which reduces input of leaf litter of lucidophyllous trees, expunges the shady habitats where chironomid adults mate and oviposit, and causes sedimentation on submerged leaves through increased soil runoff, as observed in amphibians and reptiles living in riparian habitats (Ota 2000).

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