

Patterns of hind-wing degeneration in Japanese riffle beetles (Coleoptera: Elmidae)

MASAKAZU HAYASHI¹, SIMON D. SONG² and TEIJI SOTA^{2*}

¹Hoshizaki Green Foundation, Izumo, Shimane, 691-0076, Japan; e-mail: hgf-haya@green-f.or.jp

²Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto, 606-8502, Japan;
e-mail: sota@terra.zool.kyoto-u.ac.jp

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Abstract. We conducted a molecular phylogenetic analysis based on mitochondrial cytochrome oxidase subunit I and nuclear 28S rRNA gene sequences of species of Japanese elmids (23 species from 12 genera) and examined the hind-wings of 24 species in order to determine the incidence of hind-wing degeneration among species and the presence of dimorphic species with respect to hind-wing degeneration. Based on the molecular phylogenetic analysis, we determined that the previously separated winged and wingless species, *Stenelmis vulgaris* and *S. miyamotoi*, and *Leptelmis gracilis* and *L. parallela*, are two forms of the same species. Of the 24 species whose hind wings were studied, we found apterous (3 species of *Zaitzeviaria*), brachypterous (2 species of each of *Optioservus* and *Paramacronychus*) and dimorphic species (2 species as above) in separate clades of the phylogeny. These were the smallest or medium-sized species. Dimorphic species occurred in mid- to downstream areas and used reeds and wood as substrates. The percentage of species with hind-wing degeneration (wingless or dimorphic) was high among the species (29%) studied compared to the perceived percentage for temperate beetles (<10%). Thus, we found that the degeneration of hind wings has occurred repeatedly in these elmids. However, we identified only ambiguous habitat and life history correlates of hind-wing degeneration, and the adaptive significance of hind-wing degeneration in these species of elmids remains unclear.

INTRODUCTION

Hind-wing degeneration is known to occur in many insect groups, including Coleoptera (Roff, 1990). Although flight is advantageous when searching for food, mates and a suitable habitat, the production and use of the flight apparatus is costly, and degeneration of flight muscles and wings occurs based on the trade-off between flight capability and reproduction (Roff, 1990; Roff & Fairbairn, 1991; Wagner & Liebherr, 1992). A recent study suggested that the loss of flight ability promotes allopatric differentiation and consequently contributes to the species richness of beetles (Ikeda et al., 2012).

The loss of flight is rare in freshwater insects that have aquatic larvae but rely on dispersal by flight. Among Japanese Coleoptera whose larvae and adults can live under water, wingless species are limited to species in the family Elmidae (Kawai & Tanida, 2005). Riffle beetles of the family Elmidae are small-sized aquatic insects that live in running water from their immature through adult stages, except for some periods of the adult stage when they fly outside the water (Brown, 1987; Elliott, 2008). Elmid beetles cannot swim and are subject to downstream drift. Therefore, even though streams are regarded as relatively permanent freshwater habitats where flightless species occur relatively frequently (Southwood, 1962), the ability to fly would be advantageous for locating more suitable habitats. Apterous species or dimorphic species with respect to hind wings are known to occur in Elmidae (Brown, 1987; Elliott, 2008), although the proportion of

wingless and dimorphic species and the factors that affect the evolution of winglessness have not yet been elucidated. Among the Japanese species in this family, some are wingless and a few dimorphic (i.e., species with winged and wingless forms) (Ogata & Nakajima, 2006; Kamite, 2009). Moreover, Inoue & Nakajima (2009) note the possibility of two additional dimorphic species in which the winged and wingless forms are currently treated as different species (*Stenelmis vulgaris* and *S. miyamotoi*; and *Leptelmis parallela* and *L. gracilis*). Hayashi & Sota (2010) also refer to this possibility based on the fact the two species in each pair are indistinguishable based on their mitochondrial *COI* gene sequences. These pairs of winged and wingless species differ slightly in the shapes of their bodies, which are likely to be related to the presence or absence of hind wings and flight muscles, which may represent a confusing pattern of dimorphism, as is known for species of *Zaitzeviaria* (Ogata & Nakajima, 2006) and *Heterlimnius* (Kamite, 2009). In general, wingless species of beetles have narrower basal elytra than winged species, but this structural difference might be expected to be rare within species. In Japan, there are approximately 50 species of elmid beetles, classified into 17 genera (Satô, 1985, 1999; Jeng & Yang, 1998; Yoshitomi & Satô, 2005; Kamite et al., 2006; Yoshitomi & Nakajima, 2007; Kamite, 2009). Their phylogenetic relationships and hind-wing states are not well studied.

In this study, we aimed to elucidate patterns in hind-wing degeneration in Japanese elmids in relation to phy-

* Corresponding author.

logeny, habitat and life history (body size). We first analyzed phylogenetic relationships among species of Japanese Elmidae to determine whether two pairs of macropterous and apterous species are in fact two species that have macropterous and apterous forms. We used a molecular phylogeny to address these taxonomic and phylogenetic questions because of the difficulties inherent in interpreting such patterns from morphological data alone. We then describe the hind-wing condition of each of the elmid species studied and discuss the occurrence of different hind-wing forms in relation to phylogeny, habitat and body size.

MATERIAL AND METHODS

Sampling

We collected adult specimens of 23 species of Elmidae in Japan for DNA extraction (Table 1). We used Satô (1985) to assist with the identification of the species. Individuals collected for DNA extraction were preserved in 99% ethanol. Adult beetles that were collected for the morphological analysis were preserved as dry specimens. Data for five species in the genus *Eubrianax* (Psephenidae) studied by Hayashi et al. (2012) were included as out-group taxa based on their placement as near relatives of Elmidae in Hunt et al. (2007). For the morphological analysis, we used a total of 481 specimens representing 24 species, including the 23 species from which DNA was extracted and one additional species (*Dryopomorphus nakanei*) [Supplementary Online Information (SOI) Tables S1–2]. All specimens used in this study are kept by M.H. at Hoshizaki Green Foundation, Izumo, Japan.

DNA extraction and sequencing

Total genomic DNA was extracted from the tissues of adults or larvae that were preserved in 99% ethanol using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) or a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). We used partial sequences of the mitochondrial cytochrome oxidase subunit I (*COI*) gene and the nuclear 28S rRNA (*28S*) gene for the phylogenetic analysis. The *COI* gene region was PCR-amplified using the primers C1-J-2195 (5'-TTG ATT TTT TGG TCA TCC AGA AGT-3') and TL2-N-3014 (5'-TCC AAT GCA CTA ATC TGC CAT ATT A-3') (Simon et al., 1994). The nuclear 28S rDNA gene region was PCR-amplified using primers 28S-01 (5'-GAC TAC CCC CTG AAT TTA AGC AT-3') and 28S-R01 (5'-GAC TCC TTG GTC CGT GTT TCA AG-3') (Kim et al., 2000). Purified PCR products were used in dye terminator cycle-sequencing reactions using an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit, and the products were sequenced using an ABI3130XL sequencer (Applied Biosystems, Foster City, CA). Sequence data obtained in this study were deposited in DDBJ/GenBank (accession numbers AB764139–AB764228).

Phylogenetic analyses

In addition to newly obtained sequences, we used previously obtained *COI* gene sequences for elmid species (Hayashi & Sota, 2010). Sequences obtained from larval specimens in the previous study were included only when the sequences were unique compared to those obtained from adult specimens. The larval specimens were identified to genus and species (whenever possible), based on morphology using a key by Hayashi & Sota (2010) or based on monophyletic grouping with sequences from adult specimens of a single species in the *COI* gene tree. Note that we included sequences from larvae for thoroughness but did not use them for consideration of dimorphic or cryptic species.

The GenBank/DDBJ accession numbers for sequences for individual specimens are given in Table 1. The alignment of *COI* sequences was done manually. The *28S* sequences were aligned using Clustal X version 1.83 (Chenna et al., 2003) with default parameter settings. The *COI* sequence (814 bp) corresponded to positions 2196–3009 of the *Drosophila yakuba COI* sequence (Clary & Wolstenholme, 1985), and the data set was aligned manually. Bayesian analyses and maximum-likelihood (ML) analyses were performed using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist, 2001) and Treefinder ver. March 2011 (Jobb et al., 2004), respectively, for the *COI* and *28S* data separately and for a combined analysis using both genes. Substitution models for the Bayesian and ML analyses and the optimal partitioning schemes were selected using KAKUSAN ver. 4 (Tanabe, 2007). Different codon positions for *COI* were treated as different partitions, while *28S* was treated as a single partition. Selected models were SYM+G for *28S* and GTR+G for each *COI* partition. In the simultaneous analyses with two genes, these same substitution models were applied to each of the four partitions. In the Bayesian analyses with MrBayes, we ran 2 million generations of Markov chain Monte Carlo with sampling at every 200 generations and obtained 50% majority rule consensus trees after removing the initial 1000 trees as burn-in. In the ML analyses, we performed bootstrap analyses with 100 replications in order to assess node credibility.

Morphology and ecology

We obtained data on body dimensions and ecological characters for 24 species (including *D. nakanei*, which was not used in the analysis of DNA). For each species, we observed the condition of the hind wings for 10–353 specimens and classified them as macropterous, brachypterous or apterous (see Table S1 for collection records). We also measured body length, maximum thoracic width and maximum elytron width for 8–45 specimens per species (see Table S2 for details). Body length was measured as the distance between the vertex of the head and the tip of an elytron in lateral view. The riverine habitat of each species was classified as headstream, upper stream, midstream or downstream. Ponds and lakes were described as lentic (L). The substrates to which larvae or adults were attached were classified as wood, gravel, sand, reed or moss. Each species was also checked for the occurrence of flight toward light as evidence of actual flight behaviour.

Statistical analysis

We conducted Fisher's exact test to determine the occurrence of different wing forms across different habitat and substrate types using R version 2.15.2 (R Foundation for Statistical Computing, Vienna). Differences in body size of the different wing forms in dimorphic species were examined using *t*-tests. An analysis of covariance was used to determine the effects of wing form and body length on thoracic and elytron widths in dimorphic species. Statistical analyses (except for Fischer's exact test) were carried out using the software JMP version 10 (SAS Institute, Carry, NC).

RESULTS

Molecular phylogeny and recognition of dimorphic species

The Bayesian inference and maximum-likelihood analysis produced similar phylogenies; Fig. 1 and SOI Figs S1–2 show the Bayesian consensus topologies. The *COI* gene tree (Fig. S1) has a star-like topology due to low resolution of the deep splits. There were no nucleotide differences in the *COI* gene sequences of *Stenelmis*

TABLE 1. Specimen number, locality and DDBJ/GenBank accession numbers for their *COI* and *28S* gene sequences for species studied.

Ind. No.	Species	Locality	COI (access. no.)	28S (access. no.)
HD111	<i>Dryopomorphus extraneus</i> Hinton	Chihaya, Chihayaakasaka, Osaka	AB764139	AB764139
HD112	<i>Dryopomorphus extraneus</i> Hinton	R. Yoshidagawa, Sugito, Yoshida, Shimane	AB764140	AB764140
HD037	<i>Graphelmis shirahatai</i> (Nomura)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816111	AB764159
HD038	<i>Graphelmis shirahatai</i> (Nomura)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816112	AB764160
HD039	<i>Graphelmis shirahatai</i> (Nomura)	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816112	–
HD055 (L)	<i>Grouvellinus marginatus</i> (Kono)	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	=GU816130	–
HD049	<i>Grouvellinus marginatus</i> (Kono)	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	GU816127	AB764161
HD050	<i>Grouvellinus marginatus</i> (Kono)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816128	AB764162
HD051	<i>Grouvellinus marginatus</i> (Kono)	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816127	AB764163
HD052	<i>Grouvellinus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816133	AB764164
HD053	<i>Grouvellinus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816134	AB764165
HD054	<i>Grouvellinus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816135	–
HD040	<i>Leptelmis gracilis</i> Sharp	R. Hiikawa, Shimamura, Izumo, Shimane	GU816126	AB764166
HD117	<i>Leptelmis gracilis</i> Sharp	R. Hiikawa, Shimamura, Izumo, Shimane	AB764142	AB764167
HD118	<i>Leptelmis gracilis</i> Sharp	R. Hiikawa, Shimamura, Izumo, Shimane	AB764143	AB764168
HD119	<i>Leptelmis gracilis</i> Sharp	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816126	–
HD120	<i>Leptelmis gracilis</i> Sharp	R. Hossyogigawa R, Yonago, Tottori	AB764141	AB764169
HD126	<i>Leptelmis gracilis</i> Sharp	R. Tagigawa, Taki-cho, Izumo, Shimane	=AB764142	–
HD121	<i>Leptelmis parallela</i> Nomura	R. Hiikawa, Shimamura, Izumo, Shimane	=AB764141	AB764170
HD122	<i>Leptelmis parallela</i> Nomura	R. Hiikawa, Shimamura, Izumo, Shimane	=AB764142	AB764171
HD123	<i>Leptelmis parallela</i> Nomura	R. Hiikawa, Shimamura, Izumo, Shimane	=AB764143	–
HD124	<i>Leptelmis parallela</i> Nomura	R. Hiikawa, Shimamura, Izumo, Shimane	=AB764143	–
HD110	<i>Neoriohelmis kurosawai</i> Nomura	R. Yoshidagawa, Sugito, Yoshida, Shimane	AB764144	AB764172
HD128	<i>Neoriohelmis kurosawai</i> Nomura	R. Kinoe-awa, Daisen-cho, Tottori	AB764145	AB764173
HD129	<i>Neoriohelmis kurosawai</i> Nomura	R. Kinoe-awa, Daisen-cho, Tottori	=AB764145	AB764174
HD130	<i>Neoriohelmis kurosawai</i> Nomura	R. Kinoe-awa, Daisen-cho, Tottori	=AB764145	–
HD131	<i>Neoriohelmis kurosawai</i> Nomura	R. Kinoe-awa, Daisen-cho, Tottori	=AB764145	–
HD134	<i>Neoriohelmis kurosawai</i> Nomura	Inabu-cho, Toyota, Aichi	AB764146	AB764175
HD135	<i>Neoriohelmis kurosawai</i> Nomura	Inabu-cho, Toyota, Aichi	AB764147	AB764176
HD066 (L)	<i>Optioservus</i> sp.	Sugo, Kofu-cho, Tottori	GU816140	–
HD067 (L)	<i>Optioservus</i> sp.	Sugo, Kofu-cho, Tottori	GU816141	–
HD068 (L)	<i>Optioservus</i> sp.	Sugo, Kofu-cho, Tottori	GU816142	–
HD076	<i>Optioservus hagai</i> Nomura	R. Amidagawa, Boryo, Daisen-cho, Tottori	GU816146	AB764177
HD077	<i>Optioservus hagai</i> Nomura	R. Amidagawa, Boryo, Daisen-cho, Tottori	GU816147	AB764178
HD078	<i>Optioservus hagai</i> Nomura	R. Amidagawa, Boryo, Daisen-cho, Tottori	AB764148	–
HD061	<i>Optioservus maculatus</i> Nomura	Sugo, Kofu-cho, Tottori	GU816137	AB764179
HD063	<i>Optioservus maculatus</i> Nomura	Sugo, Kofu-cho, Tottori	GU816138	AB764180
HD064	<i>Optioservus maculatus</i> Nomura	Sugo, Kofu-cho, Tottori	GU816139	AB764181
HD069	<i>Optioservus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816151	AB764182
HD070	<i>Optioservus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816149	–
HD072 (L)	<i>Optioservus nitidus</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	GU816150	–
HD073	<i>Optioservus</i> sp.	Onarubara, Kofu-cho, Tottori	GU816143	AB764183
HD074	<i>Optioservus</i> sp.	Onarubara, Kofu-cho, Tottori	=GU816143	AB764184
HD075	<i>Optioservus</i> sp.	Onarubara, Kofu-cho, Tottori	GU816145	–
HD012	<i>Ordobrevia foveicollis</i> (Schonfeldt)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816107	AB764185
HD013	<i>Ordobrevia foveicollis</i> (Schonfeldt)	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816107	AB764186
HD014 (L)	<i>Ordobrevia foveicollis</i> (Schonfeldt)	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	GU816109	–
HD015 (L)	<i>Ordobrevia foveicollis</i> (Schonfeldt)	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	=GU816107	–
HD001	<i>Ordobrevia gotoi</i> Nomura	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	GU816105	AB764187
HD002	<i>Ordobrevia gotoi</i> Nomura	R. Syojingawa, Ichinotani, Daisen-cho, Tottori	=GU816105	AB764188
HD006 (L)	<i>Ordobrevia gotoi</i> Nomura	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	=GU816105	–
HD007 (L)	<i>Ordobrevia gotoi</i> Nomura	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	=GU816105	–
HD008 (L)	<i>Ordobrevia gotoi</i> Nomura	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	=GU816105	–
HD011 (L)	<i>Ordobrevia gotoi</i> Nomura	Idotani, Nishihayashigi, Izumo, Shimane	=GU816105	–
HD003	<i>Ordobrevia maculata</i> (Nomura)	Idotani, Nishihayashigi, Izumo, Shimane	GU816106	AB764189
HD004	<i>Ordobrevia maculata</i> (Nomura)	Idotani, Nishihayashigi, Izumo, Shimane	=GU816106	AB764190
HD005	<i>Ordobrevia maculata</i> (Nomura)	R. Miyamoto-gawa, Okutagi, Taki-cho, Izumo, Shimane	=GU816106	AB764191
HD145	<i>Ordobrevia maculata</i> (Nomura)	Ganya, Dodo, Oki Is., Shimane	=GU816106	AB764192
HD146	<i>Ordobrevia maculata</i> (Nomura)	Ganya, Dodo, Oki Is., Shimane	=GU816106	–
HD107	<i>Paramacronychus granulatus</i> Nomura	Chihaya, Chihayaakasaka, Osaka	GU816169	AB764193

TABLE 1 (continued).

Ind. No.	Species	Locality	COI (access. no.)	28S (access. no.)
HD108	<i>Paramacronychus granulatus</i> Nomura	Chihaya, Chihayaakasaka, Osaka	GU816170	AB764194
HD109 (L)	<i>Paramacronychus granulatus</i> Nomura	Chihaya, Chihayaakasaka, Osaka	GU816171	–
HD041	<i>Pseudamophilus japonicus</i> Nomura	R. Hiikawa, Yokota, Okuziumo, Shimane	GU816110	AB764195
HD042	<i>Pseudamophilus japonicus</i> Nomura	R. Hiikawa, Yokota, Okuziumo, Shimane	=GU816110	AB764196
HD043	<i>Pseudamophilus japonicus</i> Nomura	R. Hiikawa, Yokota, Okuziumo, Shimane	=GU816110	–
HD026 (L)	<i>Stenelmis</i> sp.	R. Hiikawa, Izumo, Shimane	GU816121	–
HD027 (L)	<i>Stenelmis</i> sp.	Hiikawa, Hinobori, Kisuki, Unnan, Shimane	GU816122	–
HD017	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hiikawa, Shimamura, Izumo, Shimane	GU816113	AB764197
HD018	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hiikawa, Shimamura, Izumo, Shimane	GU816114	AB764198
HD019	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hiikawa, Shimamura, Izumo, Shimane	GU816115	–
HD020	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816114	–
HD137	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hossyojigawa R, Yonago, Tottori	AB764149	AB764199
HD138	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hossyojigawa R, Yonago, Tottori	=GU816113	AB764200
HD139	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Hossyojigawa R, Yonago, Tottori	AB764150	–
HD148	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Mukogawa, Sumada, Sanda, Hyogo	AB764151	AB764201
HD149	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Mukogawa, Sumada, Sanda, Hyogo	=GU816113	AB764202
HD150	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Mukogawa, Sumada, Sanda, Hyogo	=GU816113	–
HD151	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Mukogawa, Sumada, Sanda, Hyogo	=GU816113	–
HD152	<i>Stenelmis miyamotoi</i> Nomura et Nakane	R. Mukogawa, Sumada, Sanda, Hyogo	=GU816113	–
HD016 (L)	<i>Stenelmis nipponica</i> Nomura	R. Hatagawa, Tanbe, Sada-cho, Izumo, Shimane	GU816125	–
HD028	<i>Stenelmis nipponica</i> Nomura	Shakunouchi, Kisuki, Unnan, Shimane	GU816123	AB764203
HD029	<i>Stenelmis nipponica</i> Nomura	Shakunouchi, Kisuki, Unnan, Shimane	GU816124	AB764204
HD021	<i>Stenelmis vulgaris</i> Nomura	R. Hiikawa, Shimamura, Izumo, Shimane	=GU816113	AB764205
HD022	<i>Stenelmis vulgaris</i> Nomura	Hiikawa, Shimamura, Izumo, Shimane	GU816118	AB764206
HD023	<i>Stenelmis vulgaris</i> Nomura	Hiikawa, Shimamura, Izumo, Shimane	GU816119	–
HD136	<i>Stenelmis vulgaris</i> Nomura	R. Hossyojigawa R, Yonago, Tottori	AB764152	AB764207
HD147	<i>Stenelmis vulgaris</i> Nomura	R. Mukogawa, Sumada, Sanda, Hyogo	AB764153	AB764208
HD101 (L)	<i>Zaitzevia</i> sp.	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	GU816165	–
HD102 (L)	<i>Zaitzevia</i> sp.	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	GU816166	–
HD103 (L)	<i>Zaitzevia</i> sp.	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	AB764154	–
HD096	<i>Zaitzevia awana</i> (Kono)	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	GU816163	AB764209
HD097	<i>Zaitzevia awana</i> (Kono)	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	GU816164	AB764210
HD098	<i>Zaitzevia awana</i> (Kono)	R. Hiikawa, Hinobori, Kisuki, Unnan, Shimane	=GU816164	–
HD099	<i>Zaitzevia nitida</i> Nomura	R. Kasetsugawa, Kotoura-cho, Tottori	=GU816164	AB764211
HD100	<i>Zaitzevia nitida</i> Nomura	R. Kasetsugawa, Kotoura-cho, Tottori	GU816168	AB764212
HD093	<i>Zaitzevia rivalis</i> Nomura	Gakuenji, Izumo, Shimane	GU816161	AB764213
HD094	<i>Zaitzevia rivalis</i> Nomura	Gakuenji, Izumo, Shimane	=GU816161	AB764214
HD095	<i>Zaitzevia rivalis</i> Nomura	Gakuenji, Izumo, Shimane	GU816162	AB764215
HD079	<i>Zaitzeviaria brevis</i> (Nomura)	R. Syojigawa, Ichinotani, Daisen-cho, Tottori	GU816151	AB764216
HD080	<i>Zaitzeviaria brevis</i> (Nomura)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816152	AB764217
HD081	<i>Zaitzeviaria brevis</i> (Nomura)	R. Hiikawa, Shimamura, Izumo, Shimane	GU816153	AB764218
HD090	<i>Zaitzeviaria gotoi</i> (Nomura)	Gakuenji, Izumo, Shimane	GU816160	AB764219
HD091	<i>Zaitzeviaria gotoi</i> (Nomura)	Gakuenji, Izumo, Shimane	=GU816160	AB764220
HD140	<i>Zaitzeviaria gotoi</i> (Nomura)	R. Choshi-gawa, Dogo, Oki Is., Shimane	AB764155	AB764221
HD141	<i>Zaitzeviaria gotoi</i> (Nomura)	R. Choshi-gawa, Dogo, Oki Is., Shimane	=AB764155	AB764222
HD142	<i>Zaitzeviaria gotoi</i> (Nomura)	R. Choshi-gawa, Dogo, Oki Is., Shimane	=AB764155	–
HD082	<i>Zaitzeviaria ovata</i> (Nomura)	Idotani, Nishihayashigi, Izumo, Shimane	GU816154	AB764223
HD083	<i>Zaitzeviaria ovata</i> (Nomura)	Idotani, Nishihayashigi, Izumo, Shimane	GU816155	AB764224
HD084	<i>Zaitzeviaria ovata</i> (Nomura)	Idotani, Nishihayashigi, Izumo, Shimane	GU816156	–
HD085	<i>Zaitzeviaria ovata</i> (Nomura)	R. Miyamagawa, Manda-cho, Izumo, Shimane	=GU816156	AB764225
HD086	<i>Zaitzeviaria ovata</i> (Nomura)	R. Miyamagawa, Manda-cho, Izumo, Shimane	=GU816156	AB764226
HD143	<i>Zaitzeviaria ovata</i> (Nomura)	Nibu, Chibu-mura, Oki Is., Shimane	AB764156	AB764227
HD144	<i>Zaitzeviaria ovata</i> (Nomura)	Nibu, Chibu-mura, Oki Is., Shimane	=AB764156	AB764228
Outgroup				
DR010	<i>Eubrianax ramicornis</i> Kiesenwetter	Kisuki, Unnan, Shimane	EU287832	AB675827
DR025	<i>Eubrianax granicollis</i> Lewis	Kisuki, Unnan, Shimane	EU287819	AB675797
DR093	<i>Eubrianax amamiensis</i> Sato	Kinsakubaru, Amami-oshima I., Kagoshima	AB675746	AB675792
DR105	<i>Eubrianax pellucidus</i> Lewis	Takatani, Tsuruoka, Yamagata	AB675744	AB675790
DR141	<i>Eubrianax loochoensis</i> Nakane	Nago, Okinawa I.	AB675763	AB675812

L – larval specimen; the others are adults. For *Elmidae*, DDBJ/GenBank accession numbers followed by GU and AB were published by Hayashi & Sota (2010) and the present study, respectively. For *Eubrianax* (outgroup), the accession numbers followed by EU and AB were published by Hayashi & Sota (2008) and Hayashi et al. (2012), respectively.

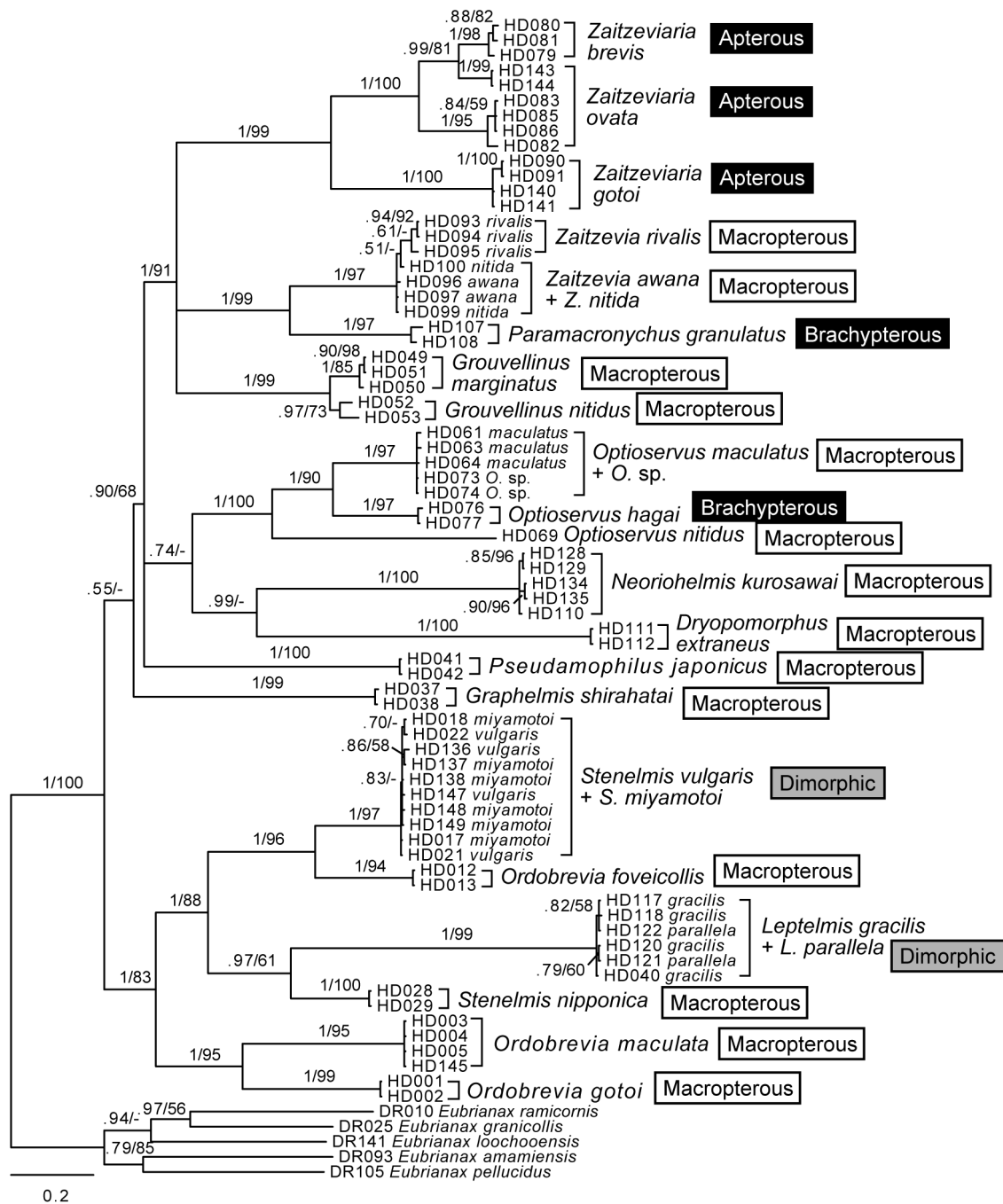


Fig. 1. The 50% majority rule consensus tree that resulted from the Bayesian phylogenetic analysis of the combined *COI* and *28S* gene sequences. Nodal support is shown in the form of Bayesian posterior probabilities and maximum-likelihood bootstrap percentages (“-” indicates below 50%).

miyamotoi and *S. vulgaris*, *Leptelmis gracilis* and *L. parallela*, *Optioservus maculatus* and *Optioservus* sp. or *Zaitzeviaria awana* and *Z. nitida*. In the *28S* gene tree (Fig. S2), species were also not distinguished within these four pairs. Thus, the results based on mitochondrial and nuclear genes were congruent. In addition, *Zaitzeviaria rivalis* shared the same *28S* sequence with *Z. awana* and *Z. nitida*. Furthermore, four species were paraphyletic within the *28S* tree (*Zaitzeviaria ovata*, *Grouvellinus marginatus*, *Ordobrevia foveicollis* and *O. maculata*). In the simultaneous analysis tree (Fig. 1), three species pairs, *S. miyamotoi* and *S. vulgaris*, *L. gracilis* and *L. parallela*,

and *O. maculatus* and *Optioservus* sp., were monophyletic, and *Z. awana* and *Z. nitida* together formed a basal part of a paraphyletic group. At the genus level, polyphyly of *Stenelmis* and *Ordobrevia* was strongly supported. These genera together with *Leptelmis* formed a monophyletic group.

Of the four pairs of species that were indistinguishable using molecular markers, *Stenelmis vulgaris* and *S. miyamotoi*, and *Leptelmis gracilis* and *L. parallela* were also indistinguishable in terms of their genital morphology (Fig. 2). We hereafter treat each pair of these species as two forms of the same species (i.e., dimorphic species).

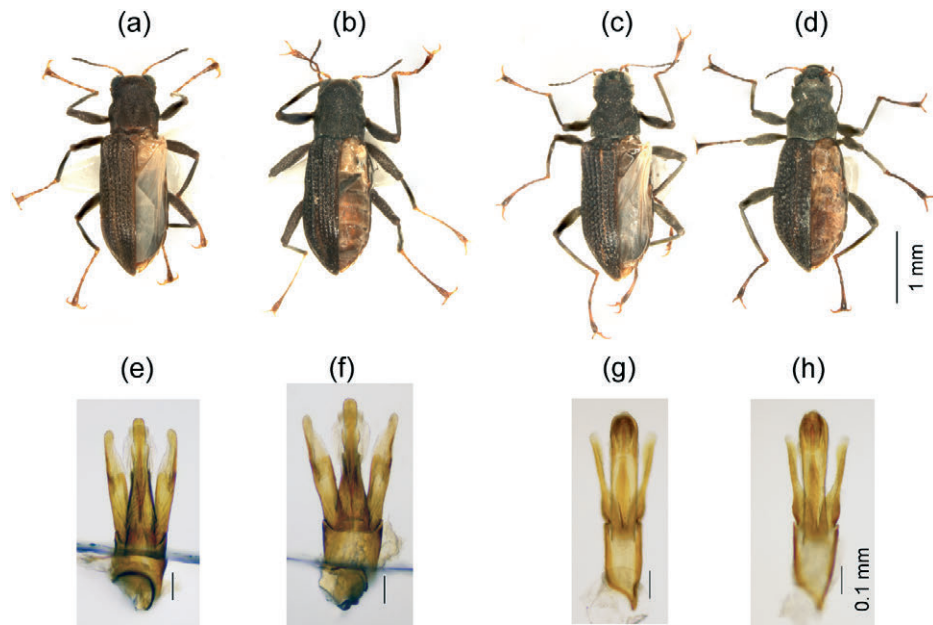


Fig. 2. Adult beetles of (a) *Stenelmis vulgaris*, (b) *S. miyamotoi*, (c) *Leptelmis parallela* and (d) *L. gracilis*. Right elytra are removed to show the condition of the hind wings. Male genitalia of (e) *Stenelmis vulgaris*, (f) *S. miyamotoi*, (g) *Leptelmis parallela* and (h) *L. gracilis*.

TABLE 2. The percentage of macropterous individuals recorded, wing type, habitat, substrate of microhabitat and tendency of each species to fly to light.

Species	% macropterous (n)	Wing type	Habitat	Substrate	Tendency to fly to light	Body length, mm (mean ± SD)	Thoracic width, mm (mean ± SD)	Elytron width, mm (mean ± SD)
<i>Dryopomorphus extraneus</i>	100.0 (15)	M	H	W	–	4.21 ± 0.13 (22)	1.78 ± 0.07 (22)	1.97 ± 0.07 (22)
<i>Dryopomorphus nakanei</i>	100.0 (10)	M	HUMD	W	–	3.18 ± 0.23 (10)	1.37 ± 0.10 (10)	1.58 ± 0.13 (10)
<i>Stenelmis nipponica</i>	100.0 (17)	M	UMD	G	+++	2.82 ± 0.09 (20)	0.92 ± 0.04 (20)	1.17 ± 0.04 (20)
<i>Stenelmis vulgaris/S. miyamotoi</i>	38.0 (353)	D	MD	R (W)	+++			
Macropterous (<i>S. vulgaris</i>)						2.79 ± 0.13 (21)	0.70 ± 0.05 (22)	0.97 ± 0.06 (22)
Apterous (<i>S. miyamotoi</i>)						2.80 ± 0.15 (24)	0.73 ± 0.04 (24)	0.98 ± 0.05 (24)
<i>Ordobrevia gotoi</i>	100.0 (27)	M	HUM	G	–	1.73 ± 0.14 (17)	0.54 ± 0.04 (17)	0.62 ± 0.04 (17)
<i>Ordobrevia maculata</i>	100.0 (20)	M	HU	G	–	1.94 ± 0.12 (20)	0.65 ± 0.05 (18)	0.82 ± 0.04 (18)
<i>Ordobrevia foveicollis</i>	100.0 (15)	M	MD	S (W)	+++	3.19 ± 0.15 (20)	0.87 ± 0.05 (20)	1.16 ± 0.06 (20)
<i>Leptelmis gracilis/L. parallela</i>	24.2 (62)	D	MD/L	R (W)	+			
Macropterous (<i>L. parallela</i>)						2.68 ± 0.08 (10)	0.66 ± 0.02 (10)	1.00 ± 0.04 (10)
Apterous (<i>L. gracilis</i>)						2.64 ± 0.11 (16)	0.70 ± 0.03 (16)	1.03 ± 0.04 (16)
<i>Graphelmis shirahatai</i>	100.0 (15)	M	MD	W	+	3.21 ± 0.23 (20)	0.87 ± 0.07 (20)	1.20 ± 0.09 (20)
<i>Neorihelmis kurosawai</i>	100.0 (10)	M	U	G	–	3.60 ± 0.37 (8)	1.41 ± 0.15 (8)	1.69 ± 0.21 (8)
<i>Optioservus hagai</i>	2.4 (42)	B	HUM	S	–	2.19 ± 0.07 (20)	1.11 ± 0.04 (20)	1.27 ± 0.05 (20)
<i>Optioservus sp.</i>	100.0 (29)	M	HU	G	–	2.56 ± 0.10 (20)	1.05 ± 0.04 (20)	1.28 ± 0.04 (20)
<i>Optioservus maculatus</i>	100.0 (101)	M	HU	S	–	2.19 ± 0.09 (20)	0.94 ± 0.03 (20)	1.13 ± 0.04 (20)
<i>Optioservus nitidus</i>	100.0 (72)	M	UMD	S	–	1.58 ± 0.06 (20)	0.60 ± 0.02 (19)	0.79 ± 0.03 (19)
<i>Pseudamophilus japonicus</i>	100.0 (15)	M	UMD	W (R)	+	4.97 ± 0.16 (20)	1.63 ± 0.08 (20)	2.06 ± 0.07 (20)
<i>Grouvellinus marginatus</i>	100.0 (50)	M	MD	S (W)	+++	2.08 ± 0.11 (21)	0.71 ± 0.03 (21)	0.94 ± 0.04 (20)
<i>Grouvellinus nitidus</i>	100.0 (16)	M	UM	G (M)	–	2.16 ± 0.09 (21)	0.76 ± 0.03 (21)	1.00 ± 0.04 (21)
<i>Paramacronychus granulatus</i>	5.0 (20)	B	HU	G	–	2.32 ± 0.06 (19)	0.90 ± 0.03 (20)	1.12 ± 0.04 (20)
<i>Zaitzeviaria gotoi</i>	2.4 (41)	A	HUMD	G	–	1.22 ± 0.04 (20)	0.43 ± 0.02 (20)	0.52 ± 0.03 (20)
<i>Zaitzeviaria ovata</i>	0.4 (249)	A	HU	S	–	1.25 ± 0.06 (20)	0.47 ± 0.02 (20)	0.60 ± 0.03 (20)
<i>Zaitzeviaria brevis</i>	3.4 (29)	A	MD	S	–	1.26 ± 0.07 (20)	0.45 ± 0.03 (20)	0.56 ± 0.04 (20)
<i>Zaitzevia awana</i>	100.0 (85)	M	MD	G	+++	1.82 ± 0.08 (20)	0.60 ± 0.03 (20)	0.78 ± 0.05 (20)
<i>Zaitzevia nitida</i>	100.0 (14)	M	UM	G	–	1.96 ± 0.09 (10)	0.63 ± 0.03 (10)	0.79 ± 0.04 (10)
<i>Zaitzevia rivalis</i>	100.0 (38)	M	HU	G	–	1.99 ± 0.06 (15)	0.67 ± 0.020 (20)	0.88 ± 0.04 (20)

Wing type: M – macropterous; B – brachypterous; A – apterous; D – dimorphic. Habitat: H – head-stream; U – upper-stream; M – mid-stream; D – down-stream; L – lentic. Substrate: W – wood; G – gravel; S – sand; R – reed; M – moss. Parenthesized substrates are occasionally used. Tendency to fly to light: +++, many records; +, rare; –, not known.

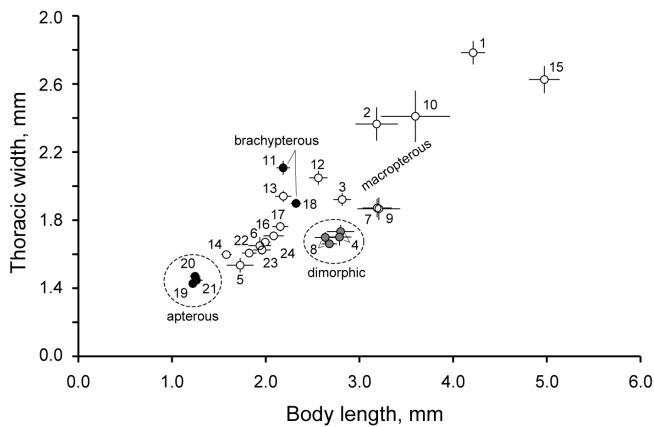


Fig. 3. Relationship between mean body length and mean thoracic width. Bars represent the standard deviation (SD). Numerals indicate species number as follows: 1 – *Dryopomorphus extraneus*; 2 – *Dryopomorphus nakanei*; 3 – *Stenelmis nipponica*; 4 – *Stenelmis vulgaris*/ *S. miyamotoi*; 5 – *Ordobrevia gotoi*; 6 – *Ordobrevia maculata*; 7 – *Ordobrevia foveicollis*; 8 – *Leptelmis gracilis*/ *L. parallela*; 9 – *Graphelmis shirahatai*; 10 – *Neorihelmis kurosawai*; 11 – *Optioservus hagai*; 12 – *Optioservus* sp.; 13 – *Optioservus maculatus*; 14 – *Optioservus nitidus*; 15 – *Pseudamophilus japonicus*; 16 – *Grouvellinus marginatus*; 17 – *Grouvellinus nitidus*; 18 – *Paramacronychus granulatus*; 19 – *Zaitzeviaria gotoi*; 20 – *Zaitzeviaria ovata*; 21 – *Zaitzeviaria brevis*; 22 – *Zaitzevia awana*; 23 – *Zaitzevia nitida*; 24 – *Zaitzevia rivalis*. Black circles indicate apterous or brachypterous species; grey circles, dimorphic species; open circles, macropterous species.

The other species pairs, *Optioservus maculatus* and *Optioservus* sp., and *Z. awana* and *Z. nitida* can be distinguished based on their external and genital morphology (SOI Figs S3–4; see also Hayashi, 2011; Kamite, 2011).

Hind wings

Among the 24 species studied, macroptery was common, occurring in 17 species of 9 genera (71% of 24 species; Table 2). Of the remaining 7 species, 2 belonging to 2 genera were brachypterous, 3 species from a single genus were apterous, and 2 species, *S. vulgaris* and *L. gracilis*, as mentioned above, had dimorphic hind wings (Table 2). Note that all of the brachypterous/apterous species sampled included at least one macropterous individual. Both macropterous and brachypterous/apterous species occurred in all the river habitats, while the two dimorphic species occurred in lower stream or still water habitats (Table 2). However, the occurrence of macropterous, dimorphic and brachypterous/apterous species did not differ statistically among the five habitats (Fisher's exact test, $P = 0.3209$). The five substrates used by larvae and adults were used differently by macropterous, dimorphic and brachypterous/apterous species (Table 2; Fisher's exact test, $P = 0.0472$; $P = 0.0109$ when occasionally used substrates were not considered). Macropterous species used all substrate types, whereas dimorphic species mostly used reeds and brachypterous/apterous species gravel and sand. Flying to light was observed in 35% of the macropterous species (Table 2).

Among the species studied, macropterous species showed a wide range of body sizes, whereas the three apterous species, all species of *Zaitzeviaria*, were the smallest (Fig. 3). Two brachypterous species and two dimorphic species were medium-sized. Among the dimorphic species, there were no significant differences in body lengths (\log_{10} -transformed) of macropterous and apterous individuals (t -test: *L. gracilis*, $df = 1, 24$, $t = 1.1$, $P = 0.2824$; *S. vulgaris*, $df = 1, 43$, $t = -0.272$, $P = 0.2824$). An analysis of covariance (ANCOVA) showed that in *L. gracilis*, macropterous individuals had wider thoraces and elytron widths than apterous individuals (\log_{10} -thoracic width: wing form effect, $F_{1,23} = 26.8$, $P < 0.0001$; \log_{10} -body length effect: $F_{1,23} = 51.4$, $P < 0.0001$; \log_{10} -elytron width: wing form effect, $F_{1,23} = 12.7$, $P = 0.0017$; \log_{10} -body length effect: $F_{1,23} = 26.8$, $P < 0.0001$). However, in *S. vulgaris*, the interaction effect between body length and wing form was significant, although macropterous individuals had wider thoraces on average (ANCOVA, \log_{10} -thoracic width: wing form effect, $F_{1,41} = 23.1$, $P < 0.0001$; \log_{10} -body length effect: $F_{1,41} = 179.9$, $P < 0.0001$; interaction effect, $F_{1,41} = 11.2$, $P = 0.0018$; \log_{10} -elytron width: wing form effect, $F_{1,41} = 0.345$, $P = 0.5602$; \log_{10} -body length effect: $F_{1,41} = 99.3$, $P < 0.0001$; interaction effect, $F_{1,41} = 7.2$, $P = 0.0107$).

DISCUSSION

The simultaneous analysis of mitochondrial and nuclear gene sequences revealed the monophyly of 9 of the 12 genera studied (Fig. 1). The remaining three genera, *Stenelmis*, *Leptelmis* and *Ordobrevia* were mixed in one clade in the molecular phylogeny, indicating that the classification of these genera should be reassessed. At the species level, our results suggest that *Stenelmis miyamotoi* Nomura & Nakane (1958) is a synonym of *S. vulgaris* Nomura (1958) and *Leptelmis parallela* Nomura (1962) a synonym of *L. gracilis* Sharp (1888). The formal synonymization of these species will be made elsewhere since this study is not intended to be a taxonomic revision. Here, we discuss the pattern of hind-wing degeneration based on our recognition of these two dimorphic species.

For the 24 species in this study, apterous species were found only in *Zaitzeviaria*, but brachypterous and dimorphic species occurred in four different genera in different clades of the molecular phylogeny (Fig. 1). Thus, hind wing degeneration has occurred repeatedly in these Japanese elmids (Fig. 1). Note that the apterous and brachypterous species reported here contained very few (< 5%) winged individuals, suggesting that alleles for macroptery are maintained in the populations. In holometabolous insects with one-locus two-allele system for wing dimorphism, macroptery is recessive to aptery/brachyptery (Roff & Fairbairn, 2007). Of the 25 species of Japanese elmids that were not included in this study, five, *Orientelmis parvula*, *Heterlimnius ater*, *Sinonychus satoi*, *S. tsujunensis* and *Zaitzeviaria kuriharai*, are apterous (Shepard, 1998; H. Yoshitomi & Y. Kamite, pers. com.), and one, *Heterlimnius hasegawai*, is dimorphic (Kamite,

2009, 2012). Thus, among all the species of Japanese elmids, the percentage of wingless species (apterous and brachypterous) is 20%, and it becomes 27% when dimorphic species are included. These percentages are relatively high among coleopteran families (Roff, 1990; <10% in temperate beetles). The loss of the ability to fly appears paradoxical for this water beetle family because they live in habitats with running water, where they are at risk of being washed away. Elmid beetles frequently drift downstream (Elliott, 2008), and therefore, the ability to fly is important for recolonizing upper stream habitats. Only *L. gracilis*, with hind-wing dimorphism, lives in still water in addition to running water, and only the apterous form is found in still water habitats (Satô, 1985; Yoshitomi & Satô, 2005). Thus, flightlessness can be favoured in stable habitats, but this cannot explain the occurrence of flightless forms in running water habitats.

Flightlessness may evolve in favor of a larger reproductive capacity, which is often associated with larger body sizes. However, among the species studied, apterous species were the smallest species, and brachypterous and dimorphic species were medium-sized. Among the dimorphic species, no difference in body length was observed between macropterous and apterous forms. Thus, a clear trade-off may not exist between flight capability and reproductive capacity (i.e., body size) in elmid beetles, although a more precise measurement of reproductive capacity (e.g., number of eggs) is needed. Our results suggest that wing dimorphism is associated with the use of reeds as substrates. The use of habitats with reeds may involve factors that promote the evolution of wingless forms. However, we need to study more species in order to critically assess this possibility.

In dimorphic species of elmids, wingless species have sloping shoulders (front part of the elytra) due to degeneration of the hind wings, whereas winged species have square shoulders (Fig. 2). Wing dimorphism accompanied by dimorphism in body shape may be rare, although such an association between elytral shape and hind-wing state is recorded as an inter-specific difference. Dimorphism in body shape, as in *S. vulgaris* and *L. gracilis*, is documented for two *Zeitzviaria* species (Ogata & Nakajima, 2006) and *H. hasegawai* (Kamite, 2009) in Japan, neither of which were included in the present study. Genetic and developmental factors that affect body shape and hind-wing length will be important subjects of future studies.

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APPENDIX ONLINE:

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