Phylogeographic analyses of the *Stenopsyche* caddisflies (Trichoptera: Stenopsychidae) of the Asian Region

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Abstract: Phylogeographic studies based on molecular information have been attracting attention because they have come to play a significant role in the elucidation of the population structure and phylogenetic relationships of species. Furthermore, molecular tools have helped to reveal evidence for cryptic biodiversity, including in many cases the discovery of new species. We focused on the phylogeography of stenopsychid caddisflies. The family Stenopsychidae has a distribution area mainly within the Southern Hemisphere, and only the *Stenopsyche* caddisflies have spread into parts of the Asian region of the Northern Hemisphere. They inhabit most of the major rivers/streams in Japan at relatively high density and are large and voluminous-bodied species among the benthic animals of Japanese rivers. Therefore, they are considered to be the most important species in the river ecosystem. We conducted phylogenetic analyses for 21 species of the genus *Stenopsyche*. The results of our genetic analyses inferred from the mitochondrial cytochrome c oxidase subunit I (COI) and nuclear elongation factor (EF)-1α strongly supported the previous morphologically based classification. *Stenopsyche* species inhabiting southern areas of the Asian region also have been evaluated as being lineages that diverged at a relatively earlier period than the species inhabiting northern areas of Asia. Therefore, a gradual trend has suggested an evolutionary history in which distributional expansion occurred from southern Asia toward northeastern Asia and was accompanied by corresponding species differentiation. Furthermore, newly observed genetically cryptic lineages not yet known to exist were found among the previously described species.

Key words: aquatic insect, benthos, cryptic species, geological history, hidden diversity, mitochondria DNA, nuclear DNA, phylogeny

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Stenopsychids have some of the largest body sizes (length: 30–52 mm) among net-spinning caddisflies, and they constitute a major component of the benthic macroinvertebrate fauna in tropical Asian streams (Holzenthal et al. 2007). The genus *Stenopsyche* is the most diverse group of Stenopsychidae and comprises >90 species distributed in the East Palearctic, Oriental, and Afrotropical regions (Xu et al. 2014, Morse 2016, Nozaki et al. 2016). Stenopsychid caddisflies have a distribution area mainly within the Southern Hemisphere, and only *Stenopsyche* has spread into parts of the Asian region of the Northern Hemisphere. Northeastern China, Mongolia, the Japanese islands including Hokkaido, Sakhalin, Russian Primorsky Territory, Khabarovsky Region, Siberia, and Kuril Islands correspond to the northern limit of the distribution for this caddisfly group (Ivanov 2011).

In Japan, 5 *Stenopsyche* caddisfly species have been recorded (Tanida 2005, Nozaki 2016). *Stenopsyche marmorata* Navás, 1920, is distributed widely throughout the Japanese Archipelago (Hokkaido, Honshu, Shikoku, and Kyushu in Japan), Sakhalin (Russia), and the Eurasian Continent (the Korean Peninsula, northeastern China, and Russian Primorsky Territory). *Stenopsyche sauteri* Ulmer, 1907, which is an endemic species to the Archipelago has been recorded in Honshu, Shikoku, and Kyushu. *Stenopsyche pallens* Nozaki, Arefina and Hayashi, 2008, inhabits only Hokkaido and Sakhalin. *Stenopsyche schmidi* Weaver, 1987, is an endemic species to the Ryukyu Islands (from Amami-Oshima to Yaeyama Islands). In addition, *Stenopsyche shinanoensis* Kobayashi, 1954, is described based on specimens from the Ina region of Nagano Prefecture (Kobayashi 1954). However, no reliable observation has been recorded since its initial description.

*Stenopsyche* caddisflies inhabit most of the major rivers/streets within Japan at a relatively high density. In the national project, the National Census on River Environments, conducted by the Japanese Ministry of Land Infrastructure and Transport, and intended to cover all of the Japanese Archipelago, *Stenopsyche* caddisflies were recorded in all 109 surveyed river systems. They were distributed across a wide range of environments, including both up- and downstream areas within each river basin. *Stenopsyche* caddisflies are a large and voluminous-bodied species among the benthic animals of Japanese rivers, and they are often dominant in number and total biomass. Therefore, *Stenopsyche* is considered to be the most important species in river ecosystems, i.e., the keystone species. They have been used as a human food called ‘Zaza-mushi’ in the Tenryu-gawa River Community of the Ina area in Nagano Prefecture, where licenses are sold to harvest them (Césard et al. 2015a, b), the only case of such special treatment among Japanese insects. *Stenopsyche* is a very important group especially in river ecosystems, but no significant phylogenetic analyses have yet been performed on them.

We conducted phylogenetic analyses for 21 species of the genus *Stenopsyche*. The species examined consisted of specimens we collected, and the genetic information on the remainder was acquired from GenBank. The results of our genetic analyses of *Stenopsyche* caddisflies strongly supported the previous morphologically based classification. Furthermore, we found newly observed genetically cryptic lineages not yet identified among the previously described species.

**METHODS**

**DNA extraction, amplification, sequencing and alignment**

We collected 159 specimens of 21 *Stenopsyche* caddisfly species from a wide area of the Asian, East Palearctic, and Oriental regions (Table S1, Fig. 1A–D). We identified each species by its key morphological characteristics. We were unable to identify 1 species from Myanmar. We extracted total genomic DNA from ethanol-preserved tissues of specimens and purified it with the aid of the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. We used total DNA to amplify fragments of mitochondrial DNA (mtDNA) cytochrome *c* oxidase subunit I (COI; 659 base pairs [bp]) and nuclear DNA (nDNA) elongation factor (EF)-1α (904 bp) by polymerase chain reaction (PCR). For COI, we used primers LCO1490: 5′-GG TCAAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′-TTAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al. 1994). The PCR protocol was: 94°C for 1 min; 35× (94°C for 1 min, 45°C for 2 min, 72°C for 1 min); 72°C for 7 min. For EF-1α, we used primers rcM4: 5′-ACACGC (CGA) AC (GT) GT (TC) TG (CT) CTCAT (AG) TC-3′ and M3: 5′-C-ACAGC (CGA) AC (GT) GC (AT) CT-3′ (Cho et al. 1995). The PCR protocol was: 95°C for 1 min; 35× (95°C for 1 min, 58°C for 1 min 10 s, 72°C for 1 min 30 s). We purified PCR products with the ExoSAP-IT or illustra™ ExoProStar Kits (GE Healthcare, Buckinghamshire, UK). We sequenced purified DNA fragments directly with a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) on an automated DNA Sequencer (ABI 3130xl DNA Analyzer; Applied Biosystems).

We submitted all DNA sequences to GenBank (see Table S1 for accession numbers and units). We performed sequence alignment and editing for each gene separately with MEGA (version 5.02; Tamura et al. 2011) and CLC Workbench software (CLC bio, Aarhus, Denmark). We aligned all sequence data using Clustal W (Thompson et al. 1994) instrumented with Mega (version 4.0; Rozas et al. 2003) prior to the phylogenetic analysis. At extremely few nucleotide sites in the DNA, heterozygous nucleotides (i.e., double peaks) were found within the sequence. We treated these sites as being undetermined bases and omitted them from our phylogenetic analysis.
Phylogenetic analyses

We used the maximum likelihood method (ML; Felsenstein 1981) to conduct phylogenetic analyses in MEGA with 1000 bootstrap replications. Prior to the ML phylogenetic estimations, we used MEGA to choose best-fit ML models based on Schwarz’s Bayesian Information (BIC) as follows: TN93 + G + I for mtDNA COI; T92 + G for nDNA EF-1α; GTR + G + I, for the combined mtDNA COI and nDNA EF-1α. In addition, we calculated genetic distances (p-distance) for pairwise combinations of haplotypes in MEGA.

Estimating divergence times

To estimate nodal divergence times, we used a relaxed Bayesian molecular clock analysis performed with BEAST (version 1.7.4; Drummond et al. 2012) on the mtDNA COI data set. The proposed insect molecular clock was adopted (COI substitution rate = 3.54%/1 × 10⁶ y; Papadopoulou et al. 2010). This substitution rate has been widely used in recent studies targeting insects (e.g., Sekiné et al. 2013, Macher et al. 2015, Tsuji et al. 2016). We used BEAUti (in BEAST) applying a relaxed uncorrelated log-normal molecular clock to create the BEAST input (.xml) file. We used the program Kakusan4 (Tanabe 2007) to select appropriate models based on Schwarz’s Bayesian Information (BIC) and the selected HKY + G + I sites model. We ran 2 independent Bayesian Markov Chain Monte Carlo (MCMC) simulations for 75 × 10⁶ generations, sampling every 2500 generations. We combined the results from the 2 runs with
LogCombiner (in BEAST). We checked the output files for convergence diagnostics after removing a 10% burn-in by examining effective sampling size (ESS) in Tracer (version 1.6; http://tree.bio.ed.ac.uk/software/tracer/). The results were summarized in Tree Annotator (in BEAST) before visualizing the resulting tree in FigTree (version 1.3.1; http://tree.bio.ed.ac.uk/software/figtree/). The times of the most recent common ancestor (tMRCA) for important nodes and main mitochondrial clades were reported as the mean value of node height with 95% highest posterior density (HPD).

RESULTS

Genetic analysis based on COI

Each of the 21 species, including an unidentified species from Myanmar (i.e., Stenopsyche sp. Myanmar), constituted a distinct monophyletic clade supported at a high level of reliability (Figs 2, 3). However, specimens identified as S. marmorata based on their morphological characteristics were split between 2 independent clades. We treated the clade containing the specimen collected at the type locality (Hokkaido, Japan) as S. marmorata. The 2nd clade consisted of 11 haplotypes observed from 43 S. marmorata specimens in collections from 16 localities and was clearly differentiated from the clade of S. marmorata. We treated the 2nd clade as Stenopsyche sp._alpine lineage. The localities at which the specimens consisting of the Stenopsyche sp._alpine lineage were collected were distributed mainly in the Central Mountainous Region (Nagano, Yamanashi, Gifu, and Toyama Prefectures, Honshu, Japan). Some of the specimens were collected from the high-altitude area around Ozegahara (or Oze Alpine Marshland; Fukushima, Honshu, Japan) (Table S1, Figs 2, 3).

Stenopsyche schmidtii had a larger degree of within-species genetic differentiation than did other Stenopsyche species. Three genetically differentiated subclades were observed within S. schmidtii: 1) a subclade consisting of the Amami-Oshima and Tokunoshima Island populations, 2) a subclade consisting of the Okinawa-jima Island populations, and 3) a subclade consisting of the Iriomote-jima Island populations (i.e., Yaeyama populations). The monophyly of each subclade was strongly supported. The genetic distance between subclade 1 and 2 was 0.018. The genetic distance among subclades 1, 2, and 3 was 0.044.

The monophyly of S. marmorata and S. pallens was strongly supported (ML bootstrap support and Bayesian posterior probability: 99/1.00; Figs 2, 3). The monophyly of those 2 species and a Stenopsyche sp._alpine lineage also was strongly supported (ML bootstrap support and Bayesian posterior probability: 85/1.00, Figs 2, 3). A 4th species, S. schmidtii, was relatively highly supported (ML bootstrap support and Bayesian posterior probability: 99/1.00; Figs 2, 3).

Genetic analysis based on EF-1α

The nDNA EF-1α region is highly conserved compared to the mtDNA COI region, and the observed level of genetic polymorphism was relatively small. However, the results obtained were consistent and in support of the analysis based on the sequence data for COI. The Stenopsyche sp._alpine lineage was genetically differentiated from S. marmorata. Furthermore, S. marmorata and Stenopsyche sp._alpine lineage specimens each constituted a monophyletic clade, and each was supported by high bootstrap values (Fig. 4). The interspecies relationships among S. schmidtii based on sequence data for EF-1α indicated 2 subclades: 1) a subclade consisting of the populations on the Amami-Oshima, Tokunoshima, and Okinawa-jima Islands, and 2) a subclade consisting of the Iriomote-jima Island (i.e., Yaeyama populations). The monophyly of each subclade was supported by high bootstrap values (Fig. 4).

Genetic analysis based on the concatenated COI and EF-1α data

The ML phylogram strongly supported the monophyly of each species, and the Stenopsyche sp._alpine lineage also was supported by a high bootstrap value (Fig. 5). The position of each species in the phylogenetic tree was consistent with the separate analyses of the COI and EF-1α regions.

Estimation of divergence time

The estimated divergence times revealed that the differentiation between S. marmorata and S. pallens may have occurred 2.12 Ma (95% HPD: 3.21–1.18 Ma) and between Stenopsyche sp._alpine lineage and S. marmorata and S. pallens 4.37 Ma (95% HPD: 6.41–2.62 Ma) (Fig. 3). The estimated divergence times within S. schmidtii revealed that the differentiation between the Amami-Tokunoshima populations and the Okinawa-jima populations may have occurred 1.20 Ma (95% HPD: 1.97–0.55 Ma) and between the Yaeyama populations and the Central Ryukyu populations (i.e. Amami-Tokunoshima + Okinawa-jima populations) 2.51 Ma (95% HPD: 3.92–1.22 Ma) (Fig. 3).

DISCUSSION

Our study has greatly increased genetic information accumulated for Stenopsyche caddisflies in the Asian region. This caddisfly group has a large total biomass in the Asian region and is an important group as an ecological keystone species. Thus, our findings can be considered to be an achievement of significant value. We are unable to undertake a robust discussion with respect to relationships at the interspecies or at higher taxonomic levels, but Stenopsyche species inhabiting southern areas of the Asian region have been evaluated as species that diverged progressively earlier than species found further south. On the other hand, S. pallens and S. marmorata inhabiting the northernmost distribution limits of Stenopsyche caddisflies (i.e., Hokkaido, Sakhalin, Russian Primorsky Territory, Khabarovsk Region, Siberia and Kuril Islands) were evaluated as species that diverged
Figure 2. Maximum likelihood (ML) phylogram of *Stenopsyche* caddisfly species based on the sequence data of the mitochondrial DNA cytochrome c oxidase subunit I (COI) region (569 base pairs). The numbers around major nodes indicate ML bootstrap support >80%. The operational taxonomic units (OTUs) indicate haplotype names with the number of specimens (in parentheses) and the collection sites (Table S1, Fig. 1A–D).
comparatively recently from related species. We hypothesize that the gradual trend observed suggests an evolutionary history in which distributional expansion occurred from southern Asia toward northeastern Asia and was accompanied by corresponding species differentiation. However, we were able to analyze only ~23% of the *Stenopsyche* species group. In particular, we have not analyzed specimens of the *Stenopsyche* species group from the South Asia region. In the future, we would like to undertake a more robust study, including a number of analyzed specimens from this area.

Our study revealed a cryptic species within *S. marmorata*. This cryptic clade, *Stenopsyche* sp._alpine lineage, was the only one that consisted of specimens collected from a high-altitude mountainous area (Table S1), and we assume that this undescribed species is extremely well adapted to cold-water environments. We are undertaking taxonomic examinations with respect to their morphological characteristics. In the headwater areas of the Chikuma-gawa River basin (Kawakami-mura, Nagano Prefecture, Japan), *S. marmorata* and the *Stenopsyche* sp._alpine lineage are clearly...
segregated by altitude, with ~1450 m as a boundary. Therefore, they exhibit niche differentiation on an extremely fine geographical scale. In the future, it will be desirable to conduct a comparison and evaluation of their physiological and ecological characteristics as associated with their unique adaptation to cold-water environments.

Yaegashi et al. (2014) suggested the existence of a genetically differentiated lineage within *S. marmorata*, based on their microsatellite analysis. We analyzed the mtDNA COI region to identify any specimens of the cryptic lineage (inhabiting the upstream region of northeastern Honshu), which was revealed by Yaegashi’s research. The COI analysis shows that this lineage is positioned within the *S. marmorata* species despite the confirmation in the microsatellite analysis that the specimens of the lineage were greatly genetically differentiated. In contrast, the *Stenopsyche* sp._alpine lineage, treated as a cryptic species in our study, is a more widely differentiated lineage. Furthermore, the high-altitude zone inhabited by the *Stenopsyche* sp._alpine lineage is at a much higher elevation than the watershed inhabited by the lineage examined by Yaegashi et al. (2014). Yaegashi et al. (2014) detected a genetically differentiated

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**Figure 4.** Maximum likelihood (ML) phylogram of *Stenopsyche* caddisfly species based on the sequence data of the nuclear DNA elongation factor (EF)-α region (904 base pairs). The numbers around major nodes indicate ML bootstrap support. Only values >80% are shown. The operational taxonomic units (OTUs) indicate haplotype names with the number of specimens (in parentheses) and the collection sites (Table S1, Fig. 1A–D). Polymerase chain reaction (PCR) products could not be amplified with our primers for *S. siamensis*, *S. similis*, *S. laminate*, and *S. himalayana*. 

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lineage within *S. marmorata*, but *Stenopsyche* sp._alpine lineage appears to be genetically differentiated beyond the species level. Thus, we have detected a cryptic lineage, largely genetically differentiated from the *S. pallens* + *S. marmorata* clade.

Discovery of cryptic species has become common during phylogenetic/phylogeographic research in many animal groups (Karanovic et al. 2016). Most often, cryptic species complexes are ascertained on the basis of molecular data and are defined by population exclusivity (e.g., Stockman and Bond 2007). For this reason, phylogeographic information is an effective tool to help define cryptic species (Rissler and Apodaca 2007). Phylogeographic researchers targeting various animals have clearly demonstrated the presence of cryptic species; e.g., the monogonont rotifer, *Synchaeta pectinate* Ehrenberg 1832 (Kimpel et al. 2015); the snail, *Semisulcospira libertina* Gould, 1859 (Hsu et al. 2014); the sow bug, *Asellus aquaticus* Linnaeus 1758 (Sworobowicz et al. 2015); and the frog, *Rana schmackeri* Boettger 1892 (Li et al. 2015). The phylogeographic study of *Isonychia japonica* Ulmer, 1919 by Saito and Tojo (2016a) and by Saito et al. (2016), revealed that *I. japonica* collected from the Japanese Archipelago is segregated environmentally, with specimens constituting 1 lineage occurring in small-scale rivers of upstream regions and specimens constituting another lineage occurring in large-scale rivers of downstream regions. Genetic differentiation or speciation between upstream and downstream along river courses are reported relatively frequently in aquatic insects (e.g., Baggiano et al. 2011, Ogittani et al. 2011, Leys et al. 2016). Given the distributional pattern observed between *S. marmorata* and the *Stenopsyche* sp. alpine lineage in our study, we can speculate that
ecological or physiological traits may differ between these 2 lineages. The estimated differentiation time of the Stenopsyche sp._alpine lineage falls just within the initial period of orogenic activity in the Central Mountainous Areas (northern Japan Alps). This mountain-forming activity probably was associated with their genetic differentiation.

Three subclades of S. schmidi were observed based on COI, and 2 subclades were observed based on EF-1α. We found genetic differentiation across the Kerama Strait (i.e., the Hachisuka Line; Fig. 1C) that corresponds with the analysis of both genetic regions (i.e., the Central Ryukyu Islands vs the South Ryukyu Islands). Furthermore, within the Central Ryukyu Islands, S. schmidi was further subdivided based on the analysis results of the COI region. These results are reasonable and consistent with the geological history of the Ryukyu Islands. We are conducting a further evaluation of their morphological characteristics with respect to the taxonomic handling of these subclades to assess whether they should be treated as a subspecies of S. schmidi, or whether the Yaeyama (i.e., Iriomote-jima) populations should be treated as an independent species (R. B. Kuranishi, Prefectural Natural History Museum and Institute Chiba, personal communication).

After the Miocene, the Ryukyu Islands were repeatedly connected and separated as a result of fluctuations in the sea level caused by glacial–interglacial cycles (Kimura 2002, Tojo et al. 2017). We could not ascertain the relevant details of the geohistory that contributed to the genetic differentiation within S. schmidt, but the location is clearly within the region of the present Kerama Strait, which formed a deep strait at a time similar to the estimated age of their genetic differentiation. The Ryukyu Islands contain the boundary between the Holarctic and the Oriental regions, so biogeographic studies targeting this archipelago are interesting. In particular, studies on organisms living in freshwater habitats are important, so we are conducting various related research projects in parallel.

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**LITERATURE CITED**

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Avise, J. C. 2004. Molecular markers, natural history and evolution. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Baggiano, O., D. J. Schmidt, F. Sheldon, and J. M. Hughes. 2011. The role of altitude and associated habitat stability in determining patterns of population genetic structure in two species of Atalophlebia (Ephemeroptera: Leptophlebiidae). Freshwater Biology 56:230–249.

Céard, N., S. Komatsu, and A. Iwata. 2015a. Les zazamushi: pêche et consommation des larves de Trichoptères au Japon. Insectes 176:9–12.

Céard, N., S. Komatsu, and A. Iwata. 2015b. Processing insect abundance: trading and fishing of zazamushi in Central Japan (Nagano Prefecture, Honshu Island). Journal of Ethnobiology and Ethnomedicine 11:78.

Cho, S., A. Mitchell, J. C. Regier, C. Mitter, R. W. Poole, T. P. Friedlander, and S. Zhao. 1995. A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1α recovers morphology-based tree for heliothine moths. Molecular Biology and Evolution 12:650–656.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29:1969–1973.

Ellis, J. S., M. E. Knight, B. Darvill, and D. Goulson. 2006. Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, Bombus sylvarum (Hymenoptera: Apidae). Molecular Ecology 15:4375–4386.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17:368–376.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–299.

Holzenthal, R. W., R. J. Blahnik, A. L. Prather, and K. M. Kjer. 2007. Order Trichoptera Kirby, 1813 (Insecta), Caddisflies. Zoo-taxa 1668:639–698.

Hsu, K. C., H. Bor, H. D. Lin, P. H. Kuo, M. S. Tan, and Y. W. Chiu. 2014. Mitochondrial DNA phylogeography of Semisulcospira libertina (Gastropoda: Cerithioidea: Pleuroceridae): implications of the history of landform changes in Taiwan. Molecular Biology Reports 41:3733–3743.

Hwang, J. M., T. J. Yoon, K. I. Suh, and Y. J. Bae. 2013. Molecular phylogeny evidence of altitudinal distribution and habitat adaptation in Korean Ephemerida species (Ephemeroptera: Ephemeridae). Entomological Research 43:40–46.
and genetic relationship of two dipteromimid mayflies inferred from mitochondrial 16S rRNA gene sequences. Zoological Science 20:1249–1259.

Tojo, K., K. Sekiné, T. Suzuki, R. Saito, and M. Takenaka. 2016. The species and genetic diversities of insects in Japan, with special reference to the aquatic insects. Pages 229–247 in M. Motokawa and H. Kajihara (editors). Species diversity of animals in Japan. Springer, Tokyo, Japan.

Tojo, K., K. Sekiné, M. Takenaka, Y. Isaka, S. Komaki, T. Suzuki, and S. D. Schoville. 2017. Species diversity of insects in Japan: their origins and diversification processes. Entomological Science 20:357–381.

Tsuji, K., M. Hori, M. H. Phyu, H. Liang, and T. Sota. 2016. Colorful patterns indicate common ancestry in diverged tiger beetle taxa: molecular phylogeny, biogeography, and evolution of elytral coloration of the genus Cicindela subgenus Sophio-
dela and its allies. Molecular Phylogenetics and Evolution 95:1–10.

Unmack, P. J., and T. E. Dowling. 2010. Biogeography of the genus Craterocephalus (Teleostei: Atherinidae) in Australia. Molecular Phylogenetics and Evolution 55:968–984.

Williams, H. C., S. J. Ormerod, and M. W. Bruford. 2006. Molecular systematics and phylogeography of the cryptic species complex Baetis rhodani (Ephemeroptera, Baetidae). Molecular Phylogenetics and Evolution 37:625–643.

Xu, J. H., B. X. Wang, and C. H. Sun. 2014. The Stenopsyche simplex species group from China with descriptions of three new species (Trichoptera: Stenopsychidae). Zootaxa 3785:217–230.

Yaegashi, S., K. Watanabe, M. T. Monaghan, and T. Omura. 2014. Fine-scale dispersal in a stream caddisfly inferred from spatial autocorrelation of microsatellite markers. Freshwater Science 33:172–180.