

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

Rie Saito<sup>1,7</sup>, Shinya Kato<sup>2</sup>, Ryoichi B. Kuranishi<sup>3,8</sup>, Takao Nozaki<sup>4,9</sup>,  
Takeshi Fujino<sup>5,10</sup>, and Koji Tojo<sup>1,2,6,11</sup>

<sup>1</sup>Division of Mountain and Environmental Science, Interdisciplinary Graduate School of Science and Technology,  
Shinshu University, Matsumoto, Japan

<sup>2</sup>Department of Biology, Faculty of Science, Shinshu University, Matsumoto, Japan

<sup>3</sup>Natural History Museum and Institute Chiba, Chiba, Japan

<sup>4</sup>Ninomiya-machi, Naka-gun, Kanagawa, Japan

<sup>5</sup>Graduate School of Science and Engineering, Saitama University, Saitama, Japan

<sup>6</sup>Institute of Mountain Science, Shinshu University, Matsumoto, Japan

**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)-1 $\alpha$  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

Phylogeographic studies based on molecular information have come to play a significant role in the elucidation of population structure and phylogenetic relationships among species (Avisé 1994, 2004, Ellis et al. 2006, Williams et al. 2006, Múrria and Hughes 2008, Kellermann et al. 2009, Hwang et al. 2013, Suzuki et al. 2014, Saito and Tojo 2016a, b, Tojo et al. 2016, 2017). Furthermore, molecular tools have revealed evidence for cryptic biodiversity, including the dis-

covery of new species (Tojo and Matsukawa 2003, Avisé 2004, Unmack and Dowling 2010, Saito and Tojo 2016a, b). We focused on the phylogeography of stenopsychid caddisflies because they have interesting distributional patterns when viewed from a global perspective.

Stenopsychidae comprises 3 genera: *Stenopsyche* McLachlan, 1866, *Pseudostenopsyche* Döhler, 1915, and *Stenopsychodes* Ulmer, 1916 (Morse 1997, 2016, Holzenthal et al.

E-mail addresses: <sup>7</sup>manmaru.cocoro@gmail.com; <sup>8</sup>kuranishi@chiba-muse.or.jp; <sup>9</sup>takao.nozaki@nifty.com; <sup>10</sup>fujino@mail.saitama-u.ac.jp; <sup>11</sup>To whom correspondence should be addressed, ktojo@shinshu-u.ac.jp

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2007, Xu et al. 2014). 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 Palaearctic, 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, Khabarovsk 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 Japanese 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/streams 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; 569 base pairs [bp]) and nuclear DNA (nDNA) elongation factor (EF)-1 $\alpha$  (904 bp) by polymerase chain reaction (PCR). For COI, we used primers LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The PCR protocol was: 94°C for 1 min; 35 $\times$  (94°C for 1 min, 45°C for 2 min, 72°C for 1 min); 72°C for 7 min. For EF-1 $\alpha$ , we used primers rcM4: 5'-ACAGC (CGA) AC (GT) GT (TC) TG (CT) CTCAT (AG) TC-3', and M3: 5'-CACAT (CT) AACATTGTCGT (CG) AT (CT) GC-3' (Cho et al. 1995). The PCR protocol was: 95°C for 1 min; 35 $\times$  (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™ Exo-ProStar 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. We aligned unique haplotypes and genotypes with the aid of DnaSP (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.

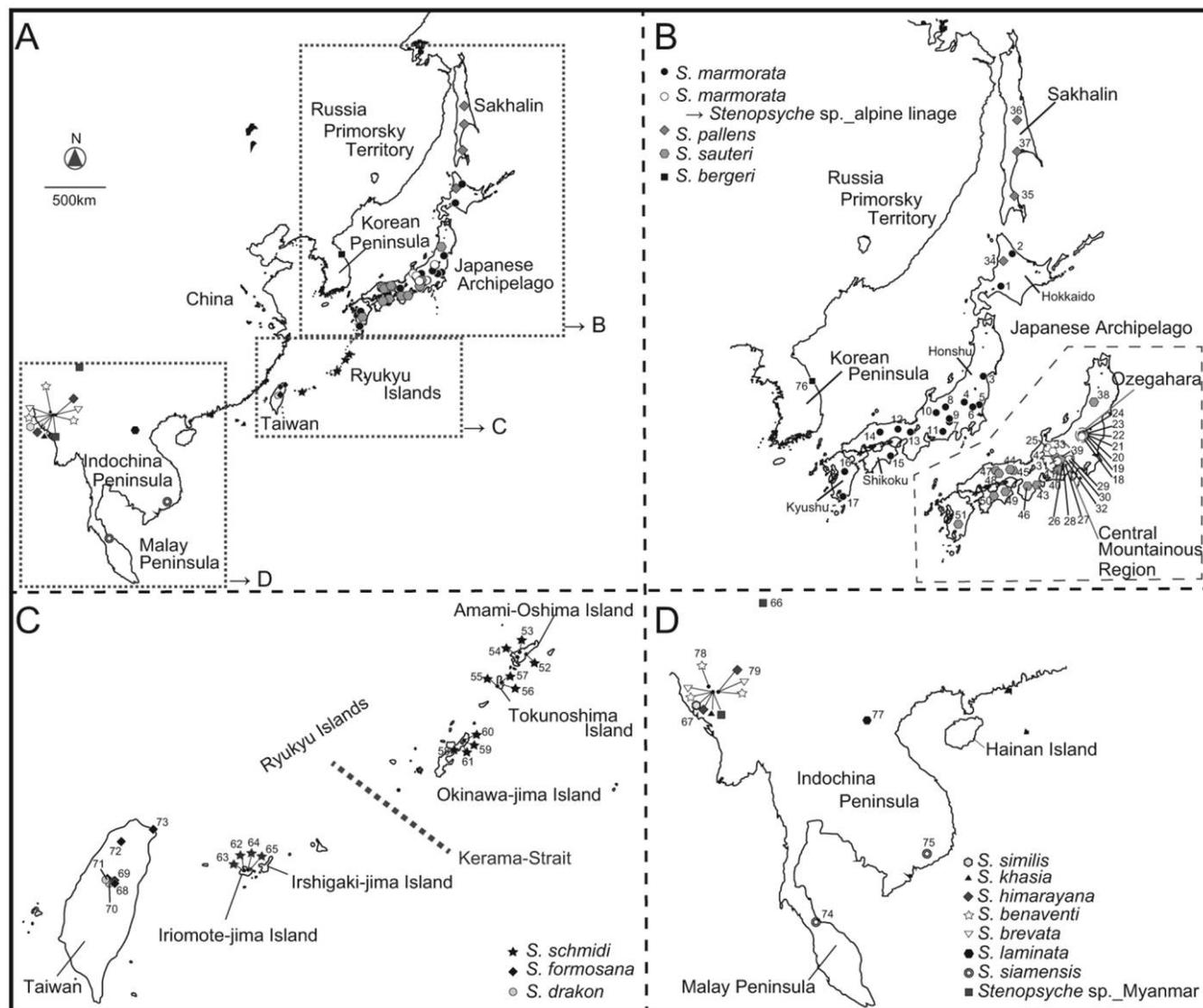


Figure 1. Sampling localities of *Stenopsyche* caddisfly species. A.—Map of the entire area from which all specimens examined in our study were collected. B–D.—Enlarged map of each local area shown in panel A.

### 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-1 $\alpha$ ; GTR + G + I, for the combined mtDNA COI and nDNA EF-1 $\alpha$ . 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  $\times$  10<sup>6</sup> y; Papadopoulos 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  $\times$  10<sup>6</sup> 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 2<sup>nd</sup> 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 2<sup>nd</sup> 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 schmidi* had a larger degree of within-species genetic differentiation than did other *Stenopsyche* species. Three genetically differentiated subclades were observed within *S. schmidi*: 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 relatively 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 4<sup>th</sup> species, *S. schmidi*, was relatively highly supported (ML bootstrap support and Bayesian posterior probability: 99/1.00; Figs 2, 3).

### Genetic analysis based on EF-1 $\alpha$

The nDNA EF-1 $\alpha$  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 intraspecific relationships among *S. schmidi* based on sequence data for EF-1 $\alpha$  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-1 $\alpha$ 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-1 $\alpha$  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. schmidi* 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).

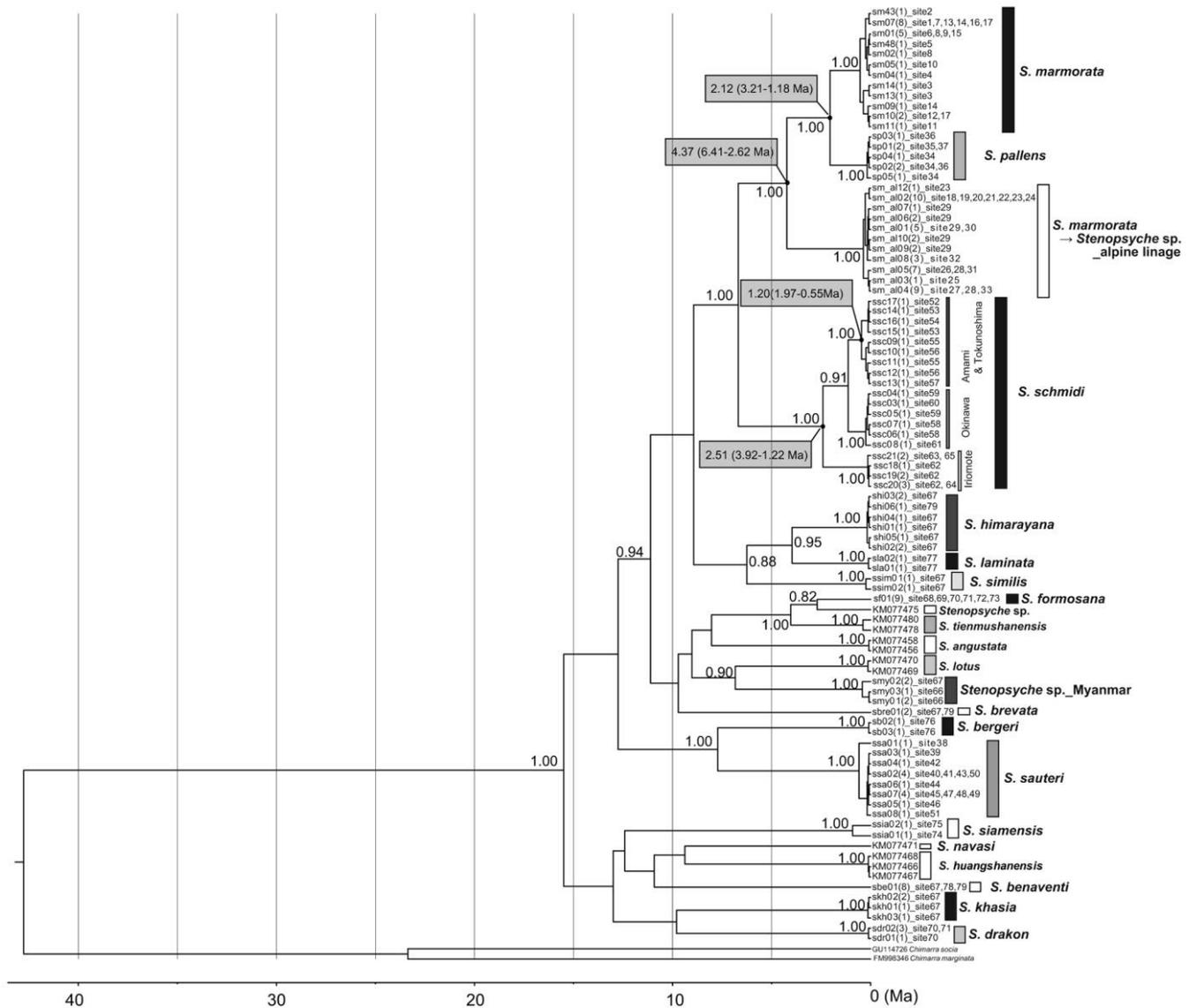


Figure 3. Estimated Bayesian phylogenetic relationships of *Stenopsyche* caddisflies based on the mitochondrial DNA cytochrome *c* oxidase subunit I (COI) region (569 base pairs). The numbers around major nodes indicate the Bayesian posterior probabilities >0.80. The numbers in gray boxes indicate the estimated divergence time and 95% credibility estimates for node ages. The operational taxonomic units (OTUs) indicate numbers of haplotype names with the number of specimens (in parentheses) and the collection sites (Table S1, Figs 1A–D, 2).

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



Figure 4. Maximum likelihood (ML) phylogram of *Stenopsyche* caddisfly species based on the sequence data of the nuclear DNA elongation factor (EF)-1 $\alpha$  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*.

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

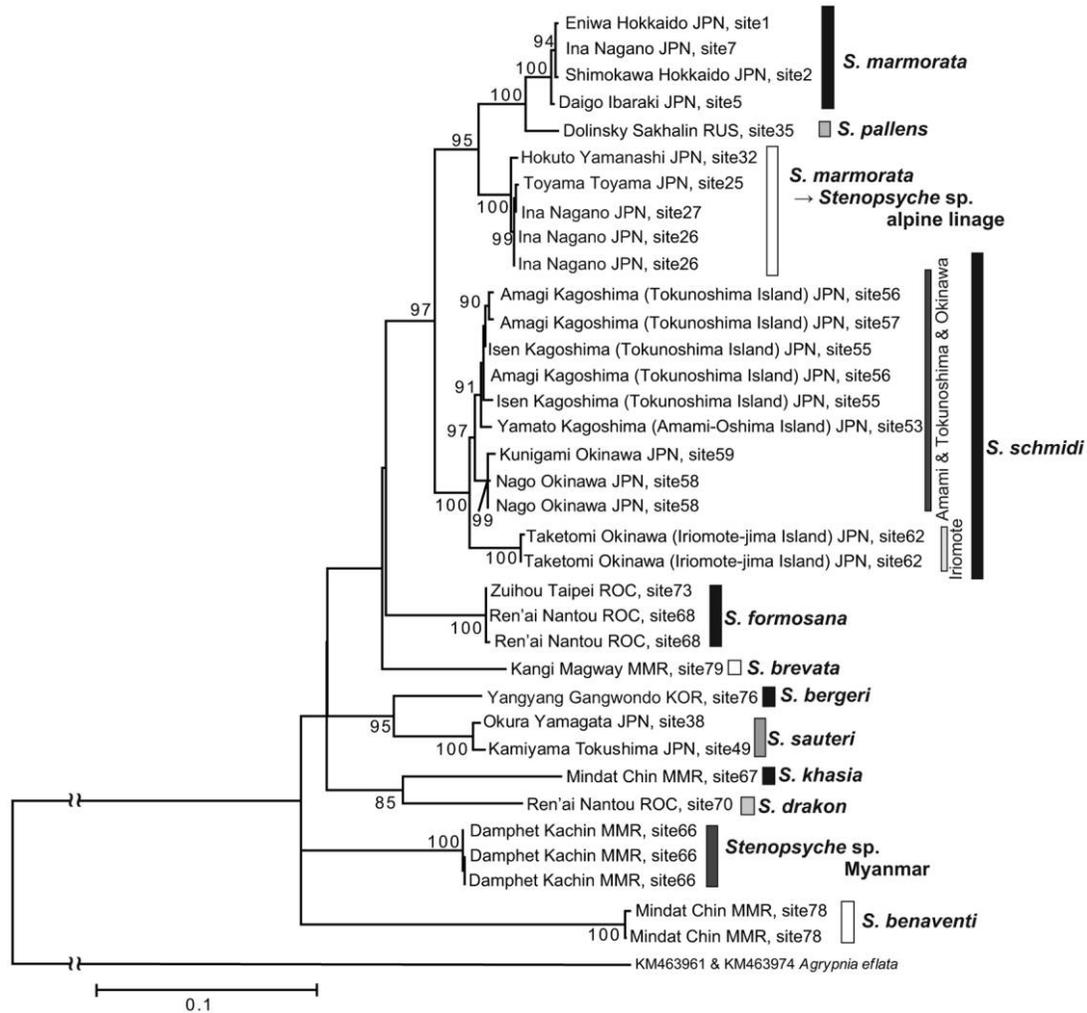


Figure 5. Maximum likelihood (ML) phylogram of *Stenopsyche* caddisfly species based on the concatenated sequence data of the mitochondrial DNA cytochrome *c* oxidase subunit I (COI) region (569 base pairs) and the nuclear DNA elongation factor (EF)-1 $\alpha$  region (904 base pairs). The numbers around 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).

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 libertine* 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 up- and downstream along river courses are reported relatively frequently in aquatic insects (e.g., Baggiano et al. 2011, Ogitan 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-1 $\alpha$ . 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. schmidi*, 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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Author contributions: KT and RS conceived and designed the research. RS, SK, RBK, TN, TF, and KT assisted with sampling. TN and RBK carried out morphological classification. RS and SK conducted genetic analyses, and the subsequent construction of their phylogenetic relationships. All the authors discussed the composition of the paper, which was mainly written by RS and KT.

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Table S1. Specimen information on *Stenopsyche* caddisfly species used for molecular phylogenetic analyses. COI = cytochrome *c* oxidase subunit I, EF-1 $\alpha$  = elongation factor-1 $\alpha$

Locality		Larva/Pupa/Adult		Haplotype		GenBank accession number	
Site no.	Prefecture/Province	City/town/village	Latitude (N)	Longitude (E)	Altitude (m)	COI	EF-1 $\alpha$
<b><i>Stenopsyche marmorata</i></b>							
<b>Japanese Archipelago</b>							
1	Hokkaido	Eniwa	42.90	141.59	21	Larva sm07	LC385327 -
2	Hokkaido	Shimokawa	44.45	142.69	283	Larva Adult sm43	LC385328 LC385486 LC385329
3	Miyagi	Kawasaki	38.20	140.59	263	Larva sm13	LC385330 -
4	Fukushima	Hinoemata	36.97	139.41	1229	Larva Adult sm14	LC385331 -
5	Ibaraki	Daigo	36.77	140.36	101	Larva Adult sm04	LC385332 -
6	Tochigi	Nakagawa	36.78	140.12	119	Larva sm01	LC385333 LC385488
7	Nagano	Ina	35.81	138.15	1258	Larva sm07	LC385334 LC385335
8	Nagano	Chikuma	36.50	138.14	370	Larva sm02	LC385336 -
9	Nagano	Fujimi	35.93	138.21	906	Larva Adult sm01	LC385337 -
10	Gifu	Takayama	36.28	137.38	521	Larva sm05	LC385338 LC385339
11	Shizuoka	Hamamatsu	35.09	137.80	123	Larva sm11	LC385340 LC385341
12	Hyogo	Asago	35.34	134.86	73	Adult sm10	LC385342 -
13	Kyoto	Kyoto	35.01	135.68	34	Larva sm07	LC385343 -
14	Okayama	Shinjyo	35.23	133.58	634	Adult sm07	LC385344 -
						sm09	LC385345 -

15	Tokushima	Yoshinogawa	34.03	134.25	115	Adult	sm01	LC385346	—
16	Kumamoto	Yamato	32.67	131.01	368	Larva	sm07	LC385347	—
17	Kagoshima	Kinko	31.16	130.86	197	Adult	sm07	LC385348	—
						Adult	sm10	LC385349	—
						Adult	sm07	LC385350	—

*Stenopsyche marmorata* → *Stenopsyche* sp.\_alpine lineage

**Japanese Archipelago**

18	Fukushima	Minamiaizu	36.98	139.50	1398	Larva	sm_al02	LC385351	—
						Larva	sm_al02	LC385352	—
19	Fukushima	Hinoemata	36.97	139.41	1230	Larva	sm_al02	LC385353	—
						Larva	sm_al02	LC385354	—
20	Fukushima	Hinoemata	36.97	139.41	1220	Larva	sm_al02	LC385355	—
21	Fukushima	Hinoemata	36.99	139.39	1090	Larva	sm_al02	LC385356	—
22	Fukushima	Hinoemata	37.00	139.39	1050	Larva	sm_al02	LC385357	—
23	Fukushima	Hinoemata	37.03	139.38	1100	Larva	sm_al02	LC385358	—
						Larva	sm_al12	LC385359	—
24	Fukushima	Hinoemata	37.03	139.39	980	Larva	sm_al02	LC385360	—
						Larva	sm_al02	LC385361	—
25	Toyama	Toyama	36.48	137.23	260	Larva	sm_al03	LC385362	LC385490
26	Nagano	Ina	35.86	138.15	1588	Larva	sm_al05	LC385363	LC385491
						Larva	sm_al05	LC385364	LC385492
27	Nagano	Ina	35.81	138.15	1259	Larva	sm_al04	LC385365	LC385493
28	Nagano	Ina	35.83	137.86	1261	Adult	sm_al04	LC385366	—
						Adult	sm_al05	LC385367	—

29	Nagano	Kawakami	35.95	138.71	1455	Larva	sm_al04	LC385368	-
						Larva	sm_al04	LC385369	-
						Larva	sm_al05	LC385370	-
						Larva	sm_al05	LC385371	-
						Larva	sm_al05	LC385372	-
						Pupa	sm_al04	LC385373	-
						Adult	sm_al04	LC385374	-
						Adult	sm_al04	LC385375	-
						Larva	sm_al06	LC385376	-
						Larva	sm_al10	LC385377	-
30	Nagano	Kawakami	35.94	138.68	1491	Adult	sm_al10	LC385378	-
						Larva	sm_al06	LC385379	-
						Adult	sm_al01	LC385380	-
						Adult	sm_al01	LC385381	-
						Adult	sm_al07	LC385382	-
						Adult	sm_al09	LC385383	-
						Larva	sm_al09	LC385384	-
						Larva	sm_al01	LC385385	-
						Larva	sm_al01	LC385386	-
						Larva	sm_al01	LC385387	-
31	Nagano	Kiso	35.88	137.78	1095	Larva	sm_al05	LC385388	-
						Larva	sm_al08	LC385389	-
32	Yamanashi	Hokuto	35.93	138.41	1348	Larva	sm_al08	LC385390	LC385494
						Larva	sm_al08	LC385391	-
33	Gifu	Takayama	36.22	137.55	1158	Larva	sm_al04	LC385392	-
						Larva	sm_al04	LC385392	-



49	Tokushima	Kamiyama	34.03	134.40	45	Adult	ssa07	LC385412	LC385497
50	Kochi	Motokawa	33.75	133.21	718	Adult	ssa02	LC385413	–
51	Kumamoto	Asagiri	32.13	130.92	521	Adult	ssa08	LC385414	–

*Stenopsyche schmidti*

**Japanese Archipelago**

52	Kagoshima (Amami-Oshima Island)	Amami	28.30	129.48	213	Adult	ssc17	LC385415	–
53	Kagoshima (Amami-Oshima Island)	Yamato	28.32	129.35	216	Adult	ssc15	LC385416	LC385498
						Adult	ssc14	LC385417	–
54	Kagoshima (Amami-Oshima Island)	Uken	28.30	129.30	117	Adult	ssc16	LC385418	–
55	Kagoshima (Tokusnoshima Island)	Isen	27.72	128.93	99	Adult	ssc09	LC385419	LC385499
						Adult	ssc11	LC385420	LC385500
56	Kagoshima (Tokusnoshima Island)	Amagi	27.76	128.97	325	Adult	ssc12	LC385421	LC385501
						Adult	ssc10	LC385422	LC385502
57	Kagoshima (Tokusnoshima Island)	Amagi	27.78	128.97	315	Adult	ssc13	LC385423	LC385503
58	Okinawa	Nago	26.58	128.03	83	Larva	ssc06	LC385424	LC385504
						Larva	ssc07	LC385425	LC385505
59	Okinawa	Kunigami	26.72	128.21	282	Pupa	ssc04	LC385426	–
						Larva	ssc05	LC385427	LC385506
60	Okinawa	Kunigami	26.77	128.27	285	Adult	ssc03	LC385428	–
61	Okinawa	Kunigami	26.71	128.19	115	Adult	ssc08	LC385429	–
62	Okinawa (Iriomote-jima)	Taketomi	24.36	123.80	60	Adult	ssc18	LC385430	LC385507
						Adult	ssc19	LC385431	LC385508
						Adult	ssc19	LC385432	–
						Adult	ssc20	LC385433	–

63	Okinawa (Iriomote-jima)	Taketomi	24.36	123.79	25	Larva	ssc21	LC385434	–
64	Okinawa (Iriomote-jima)	Taketomi	24.38	123.88	60	Larva	ssc20	LC385435	–
65	Okinawa (Iriomote-jima)	Taketomi	24.39	123.87	35	Larva	ssc21	LC385437	–

***Stenopsyche* sp.\_Myanmar**

**Myanmar**

66	Kachin	Damphet	25.71	97.49	144	Larva	smy01	LC385438	LC385509
						Larva	smy01	LC385439	LC385510
67	Chin	Mindat	21.36	93.94	764	Larva	smy03	LC385440	LC385511
						Larva	smy02	LC385441	–
						Adult	smy02	LC385442	–

***Stenopsyche formosana***

**Taiwan**

68	Nantou	Ren'ai	24.08	121.01	697	Larva	sf01	LC385443	LC385512
						Larva	sf01	LC385444	LC385513
69	Nantou	Ren'ai	24.09	121.03	706	Adult	sf01	LC385445	–
70	Nantou	Ren'ai	24.08	121.01	497	Adult	sf01	LC385446	–
71	Nantou	Ren'ai	24.08	120.95	495	Adult	sf01	LC385447	–
						Larva	sf01	LC385448	–
						Larva	sf01	LC385449	–
72	Taoyuan	Longtan	24.84	121.26	124	Larva	sf01	LC385450	–
73	Taipei	Zuihou	25.12	121.89	25	Larva	sf01	LC385451	LC385514

*Stenopsyche drakon*

**Taiwan**

70	Nantou	Ren'ai	24.08	121.01	497	Adult	sdr01	LC385452	LC385515
						Larva	sdr02	LC385453	-
						Adult	sdr02	LC385454	-
71	Nantou	Ren'ai	24.08	120.95	495	Adult	sdr02	LC385455	-

*Stenopsyche siamensis*

**Thailand**

74	Hatyai	Ton Nga Chang	6.95	100.24	98	Larva	ssia01	LC385456	-
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**Vietnam**

75	Dak Nong	Nam Nung	12.38	107.93	423	Adult	ssia02	LC385457	-
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*Stenopsyche bergeri*

**Korean Peninsula**

76	Gangwondo	Yangyang	38.08	128.63	9	Adult	sb02	LC385458	LC385516
						Larva	sb03	LC385459	-

*Stenopsyche similis*

**Myanmar**

67	Chin	Mindat	21.36	93.94	764	Adult	ssim02	LC385460	-
						Adult	ssim01	LC385461	-

*Stenopsyche laminata*

**Laos**

77	Houaphanh	Nam-et	20.11	103.33	812	Adult	sla01	LC385462	-
						Adult	sla02	LC385463	-
<i>Stenopsyche benaventi</i>									
<b>Myanmar</b>									
78	Chin	Mindat	21.17	94.15	382	Larva	sbe01	LC385464	LC385517
						Larva	sbe01	LC385465	-
						Pupa	sbe01	LC385466	-
						Larva	sbe01	LC385467	LC385518
67	Chin	Mindat	21.36	93.94	764	Adult	sbe01	LC385468	-
						Adult	sbe01	LC385469	-
79	Magway	Kangi	21.36	94.09	430	Larva	sbe01	LC385470	-
						Pupa	sbe01	LC385471	-
<i>Stenopsyche himalayana</i>									
<b>Myanmar</b>									
67	Chin	Mindat	21.36	93.94	764	Larva	shi02	LC385472	-
						Larva	shi03	LC385473	-
						Larva	shi03	LC385474	-
						Larva	shi01	LC385475	-
						Pupa	shi04	LC385476	-
						Pupa	shi05	LC385477	-
						Adult	shi02	LC385478	-
79	Magway	Kangi	21.36	94.09	430	Pupa	shi06	LC385479	-

*Stenopsyche brevata*

**Myanmar**

67	Chin,	Mindat	21.36	93.94	764	Pupa	sbre01	LC385480	–
79	Magway	Kangi	21.36	94.09	430	Pupa	sbre01	LC385481	LC385519

*Stenopsyche khasia*

**Myanmar**

67	Chin	Mindat	21.36	93.94	764	Larva	skh03	LC385482	–
						Adult	skh01	LC385483	LC385520
						Adult	skh02	LC385484	–
						Larva	skh02	LC385485	–

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