# DNA-based identification of South Korean Megaloptera larvae with taxonomic notes

Sang Woo Jung, Tatyana S. Vshivkova, Yeon Jae Bae[1]

**Abstract**—Mitochondrial DNA (mtDNA) sequences, which serve as DNA barcodes, have been used to associate immature and adult stages of insects and to delineate species. The partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences for South Korean Megaloptera (all known in the adult stage) were tested to identify undetermined larvae as a rapid and effective method from 31 specimens (16 adults and 15 larvae). The COI gene sequences distinguished all six known Megaloptera species, with a low genetic distance between larvae and adults ($0.50 \pm 0.21\%$). Based on the COI gene sequences, we associated five types of larvae with known adults including the following four species of newly described larval stages: *Sialis annae* Vshivkova, 1979 (Megaloptera: Sialidae); *Neochauliodes formosanus* (Okamoto, 1910) (Megaloptera: Corydalidae); *Parachauliodes asahinai* Liu *et al*., 2008 (Megaloptera: Corydalidae); and *Protohermes xanthodes* Navás, 1913 (Megaloptera: Corydalidae). The known larval stage of *Sialis koreana* Jung and Bae, 2012 (Megaloptera: Sialidae) was confirmed, and the morphological variation in the male genitalia of *Sialis longidens* Klingstedt, 1932 (Megaloptera: Sialidae) is discussed. A larval key to the six South Korean species of Megaloptera is provided.

## Introduction

The Megaloptera, which was most diverse in the late Permian period, contains ~350 extant species in the world (Wang *et al.* 2012; Yang *et al.* 2012). Currently, there are 35 species belonging to eight genera (Corydalidae: *Acanthacorydalis* Weele, *Eochauliodes* Liu, Wang, Shih, Ren, and Yang, *Jurochauliodes* Wang and Zhang, *Neochauliodes* Weele, *Parachauliodes* Weele, *Protohermes* Weele; Sialidae: *Nipponosialis* Kuwayama, *Sialis* Latreille) from the eastern Palaearctic region, with seven fossil species (Oswald 2013). The larval stage is exclusively aquatic inhabiting diverse lotic and lentic freshwater habitats, whereas the pupal and adult stages are terrestrial. The larvae prey on small aquatic invertebrates but the adults normally do not feed while they live for a week or two in their one-year to three-year life span (Takeuchi and Hoshiba 2012, 2013). The larvae are frequently used in freshwater biomonitoring programmes (Yang and Yang 1995; Liu and Yang 2004; Flint *et al.* 2008).

The order contains two families, Corydalidae and Sialidae, each of which includes two subfamilies, Corydalinae and Chauliodinae in Corydalidae and Sialidinae and Sharasialinae in Sialidae (Liu *et al.* 2014). The order has been classified as either monophyletic or paraphyletic based on adult morphology or DNA sequences (Cameron *et al.* 2009; Beutel *et al.* 2010; Aspöck *et al.* 2012; Liu *et al.* 2012). Megaloptera larvae can be easily distinguished by morphological characters. Species of Corydalidae can be characterised by the colour pattern of the head and pronotum, respiratory tubes on the abdominal segment VIII, spiracle size and arrangement, macrosetae on the abdomen, and shape of the submentum of the labium (Neunzig and Baker 1991; Contreras-Ramos and Harris 1998). Species of Sialidae can be characterised by the colour

Received 30 September 2014. Accepted 11 February 2015. First published online 26 June 2015.

**S.W. Jung,** Department of Life Science, Graduate School, Korea University, Seoul 136-713, South Korea
**T.S. Vshivkova,** Institute of Biology and Soil Science, Russian Academy of Sciences, Far East Branch, Vladivostok 690022, Russia
**Y.J. Bae,**[1] Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, South Korea

[1]Corresponding author (e-mail: yjbae@korea.ac.kr).
Subject editor: Christopher Buddle
doi:10.4039/tce.2015.44

pattern on the head and pronotum, postoccipital sutures, and colour pattern on the abdominal tergum (Elliott 1996; Meinander 1996).

Despite their large body size and importance in aquatic ecosystems, larval stages are poorly known since Megaloptera taxonomy is largely based on adult morphology. Six species of Korean Megaloptera belonging to four genera in two families were recorded based on male adults (Jung and Bae 2012). The larvae, however, have not been formally recorded in South Korea, except for one species, *Sialis koreana* Jung and Bae, which was described recently in both the adult and larval stages.

Mitochondrial DNA (mtDNA) sequences such as partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences have been widely used for species level identification in different life stages with cryptic species. Previous studies showed that the COI gene sequences can be effectively used as a DNA barcode to identify or associate immature stages with identified adult stages (Miller *et al.* 2007; Ruiter *et al.* 2013) and for resolving evolutionary relationships among closely related species of insects (Lunt *et al.* 1996; Hwang *et al.* 2013).

The purposes of this study were to apply DNA barcoding for Megaloptera and associate unknown larvae of South Korean Megaloptera with taxonomically identified adults using COI gene sequences; and to provide keys to the known larvae of South Korean Megaloptera.

## Materials and methods

**Taxonomy.** All larval materials were collected using a hand net (mesh size 1.0 mm) or a Surber net (30 × 30 cm or 50 × 50 cm, mesh size 0.2 mm) in streams and wetlands, whereas adults were collected with a sweeping net or a light trap (particularly for Corydalidae) near streams, wetlands, and mountains. Dorsal larvae whole bodies were photographed with a digital camera (Nikon D90, Tokyo, Japan). Male genitalia and larvae were illustrated using a dissecting microscope with an image analyser (Carl Zeiss Discovery V12 with AxioCam I Cc 1, Oberkochen, Germany). The tip of the abdomen and larvae was dissected and cleared with 10% KOH for two days for examination, and then preserved in microtube with glycerin. The morphological terminology for larvae generally follows Neunzig and Baker (1991). Province abbreviations used in this study are as follows: CB, Chungcheongbuk-do; CN, Chungcheongnam-do; GB, Gyeongsangbuk-do; GG, Gyeonggi-do; GN, Gyeongsangnam-do; GW, Gangwon-do; JB, Jeollabuk-do; JN, Jeollanam-do. All materials are preserved in 80–90% ethanol and deposited in the Entomological Museum of Korea University, Seoul, South Korea (KU). One adult male of *Parachauliodes asahinai* Liu, Hayashi, and Yang is deposited in the Entomological Museum of China Agricultural University, Beijing, China (CAU).

**DNA sequencing.** A piece of tissue from the thorax (for larvae) or femur (for adults) was taken from 31 specimens (16 adults and 15 larvae) and preserved in 80% ethanol (Table 1). Total DNA extraction was performed using the DNeasy Tissue Kit (Qiagen, Valencia, California, United States of America) extraction protocol and the DNA was stored at −20 °C. The DNA barcoding region of ~660 base pairs (bp) of the COI gene was amplified using the primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCA AAAAATCA-3′) described by Folmer *et al.* (1994). Each 50 µL polymerase chain reaction (PCR) contained ~40–100 ng of template DNA, 0.5 U of Taq DNA polymerase with 1X Taq buffer containing 20 mM Tris-HCL (pH 8.2) and 50 mM KCl, 4 mM $MgCl_2$, 200 µM total dNTP, and each primer at 1 µM. Thermal cycling started with incubation at 94 °C for one minute, followed by 35 cycles at 94 °C for one minute, annealing at 47 °C for 30 seconds, and extension at 72 °C for one minute, with a final extension step at 72 °C for five minutes. All PCR products were visualised on 1.5% agarose gels using GelRed (Biotium Inc, Hayward, California, United States of America). Gel purified PCR products were acquired using a Gel Extraction Kit (Koma Biotech Inc, Seoul, South Korea) and sequenced on an ABI 3730 Automated Sequencer (Macrogen Inc., Seoul, South Korea). All sequences were submitted to GenBank (accession numbers: KJ127477-KJ127500, KJ155793-KJ155799) (Table 1) and BOLD systems (http://www.boldsystems.org) wherein voucher data can be accessed in the public dataset "Megaloptera in Korea" (KMDS001-14–KMDS031-14).

**Table 1.** Specimen number, species used in analysis, collecting site and date, and GenBank accession numbers for COI sequences.

| Specimen number | Species | Collecting site | Collecting date (date-month-year) | COI (GenBank accession number) |
|---|---|---|---|---|
| 003 (M) | *Sialis koreana* Jung and Bae (paratype) | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 10 June 2012 | KJ127478 |
| 012 (L) | *Sialis koreana* Jung and Bae | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 16 August 2011 | KJ127480 |
| 002 (M) | *Sialis koreana* Jung and Bae | South Korea, GW, Inje-gun, Seohwa-myeon, Simjeok-ri, Suribong | 3 June 2009 | KJ127477 |
| 004 (F) | *Sialis koreana* Jung and Bae (paratype) | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 10 June 2012 | KJ127479 |
| 013 (L) | *Sialis koreana* Jung and Bae | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 16 August 2011 | KJ127481 |
| 014 (L) | *Sialis koreana* Jung and Bae | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 16 August 2011 | KJ127482 |
| 015 (L) | *Sialis koreana* Jung and Bae | South Korea, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup | 16 August 2011 | KJ127483 |
| 030 (L) | *Sialis* species (Type A) | South Korea, GB, Gimcheon-si, Buchang-myeon, Picheon-ri | 22 September 2011 | KJ127486 |
| 028 (L) | *Sialis* species (Type A) | South Korea, GB, Gimcheon-si, Buchang-myeon, Picheon-ri | 22 September 2011 | KJ127484 |
| 029 (L) | *Sialis* species (Type A) | South Korea, GB, Gimcheon-si, Buchang-myeon, Picheon-ri | 22 September 2011 | KJ127485 |
| 008 (M) | *Sialis annae* Vshivkova | South Korea, GG, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Seungcheonsa | 20-May-2010 | KJ127487 |
| 009 (L) | *Sialis* species (Type B) | South Korea, GG, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Garymgyo | 26 February 2012 | KJ127488 |
| 010 (L) | *Sialis* species (Type B) | South Korea, GG, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Garymgyo | 26 February 2012 | KJ127489 |
| 011 (L) | *Sialis* species (Type B) | South Korea, GG, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Garymgyo | 26 February 2012 | KJ127490 |
| 005 (M) | *Sialis longidens* Klingstedt | South Korea, JN, Naju-si, Noan-myeon, Yugok-ri | 16 April 2009 | KJ127491 |
| 033 (M) | *Sialis longidens* Klingstedt | South Korea, JN, Naju-si, Noan-myeon, Yugok-ri | 16 April 2009 | KJ127492 |
| 031 (M) | *Sialis longidens* Klingstedt | South Korea, JN, Naju-si, Noan-myeon, Yugok-ri | 16 April 2009 | KJ127496 |
| 034 (F) | *Sialis longidens* Klingstedt | South Korea, CB, Yeongdong-gun, Hwanggan-myeon, Nangok-ri | 13 April 2011 | KJ127497 |
| 025 (M) | *Sialis longidens* Klingstedt | South Korea, CB, Yeongdong-gun, Hwanggan-myeon, Nangok-ri | 13 April 2011 | KJ127493 |
| 026 (M) | *Sialis longidens* Klingstedt | South Korea, CB, Yeongdong-gun, Hwanggan-myeon, Nangok-ri | 13 April 2011 | KJ127494 |
| 027 (M) | *Sialis longidens* Klingstedt | South Korea, CB, Yeongdong-gun, Hwanggan-myeon, Nangok-ri | 13 April 2011 | KJ127495 |
| 016 (F) | *Neochauliodes formosanus* (Okamoto) | South Korea, GW, Samcheok-si, Miro-myeon, Hwalgi-ri | 30 August 2011 | KJ127499 |
| 001 (M) | *Parachauliodes asahinai* Liu, Hayashi, and Yang | South Korea, GG, Gapyeong-gun, Buk-myeon, Jeongmok-ri, Garimgyo | 19 May 2012 | KJ127498 |
| 023 (F) | *Protohermes xanthodes* Navás | South Korea, GW, Hongcheon-gun, Seo-myeon, Dumi-ri, Dumigyo | 20 June 2011 | KJ127500 |
| 006 (L) | Corydalidae species (Type E) | South Korea, GB, Gimcheon-si, Jirye-myeon, Dogok-ri | 18 April 2012 | KJ155793 |
| 007 (L) | Corydalidae species (Type E) | South Korea, GB, Gimcheon-si, Jirye-myeon, Dogok-ri | 18 April 2012 | KJ155794 |
| 017 (L) | Corydalidae species (Type C) | South Korea, GG, Yeoju-gun, Geumsa-myeon, Geumsa-ri, Geumsa2gyo | 13 May 2010 | KJ155795 |
| 018 (L) | Corydalidae species (Type D) | South Korea, JN, Yeongam-gun, Yeongam-eup, Gaesin-ri | 6 March 2010 | KJ155796 |
| 020 (F) | *Protohermes xanthodes* Navás | South Korea, GW, Hongcheon-gun, Seo-myeon, Dumi-ri, Dumigyo | 20 June 2011 | KJ155797 |
| 021 (F) | *Protohermes xanthodes* Navás | South Korea, GW, Hongcheon-gun, Seo-myeon, Dumi-ri, Dumigyo | 20 June 2011 | KJ155798 |
| 024 (L) | Corydalidae species (Type E) | South Korea, GG, Yeoju-gun, Geumsa-myeon, Geumsa-ri, Geumsa2gyo | 13 May 2010 | KJ155799 |

**Note:** L, larva; M, male adult; F, female adult; CB, Chungcheongbuk-do; GB, Gyeongsangbuk-do; GG, Gyeonggi-do; GW, Gangwon-do; JN, Jeollanam-do.

Sequences (660 bp) were aligned using CLUSTAL X version 1.81 (Thompson *et al.* 1997). Maximum parsimony analyses of 31 nucleotide sequences were conducted in MEGA 6.0 (Tamura *et al.* 2013) and sequence divergence was calculated using the Kimura 2-parameter (K2P) model (Kimura 1980). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The maximum parsimony (MP) tree was obtained using the tree-bisection-regrafting (Nei and Kumar 2000) algorithm with random addition of sequences (10 replicates). The species of *Parainocellia bicolor* (Costa) (Raphidioptera: Inocelliidae) was used to root the trees as an outgroup species (sequence available in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under accession number EU839733).

## Results and discussion

### Morphological types of larvae and their DNA-based identification

In total, 15 larval specimens for DNA extraction were examined based on diagnostic external morphology in this study. Megalopteran larvae were separated by family levels: Sialidae (Figs. 1A–1D) has small body size (less 20 mm), sickle-shaped mandibles, and abdominal segment X with caudal filament; whereas Corydalidae (Figs. 1E–1G) has large body size (above 40 mm), robust mandibles, and abdominal segment X with a pair of anal prolegs. Within two families, all materials were classified into the following five types (Fig. 1) morphologically.

**Type A** (Fig. 1A). Family Sialidae. Body elongate; labrum subtriangular; prothorax wider than mesothorax and metathorax. Abdomen dark brown to black, with white longitudinal stripe dorsally and transverse dark stripes ventrally (Fig. 1B). The specimens belonging to this type were the larvae from the type locality of *S. koreana* Jung and Bae (2012) and additional larvae from other localities that were morphologically identical to the larvae of *S. koreana* (Table 1).
**Type B** (Fig. 1C). Family Sialidae. Body elongate; labrum distinctly protruding; prothorax as wide as mesothorax and metathorax. Abdomen dorsally pink, without white longitudinal stripe.
**Type C** (Fig. 1E). Family Corydalidae. Body grey, flattened, with long respiratory tubes on abdominal segment VIII.

**Fig. 1.** (A–G) Larval habitus: (A) *Sialis koreana* Jung and Bae, (B) abdomen of *S. koreana*, ventral view, (C) *Sialis annae* Vshivkova, (D) abdomen of *S. annae*, ventral view, (E) *Neochauliodes formosanus* (Okamoto), (F) *Parachauliodes asahinai* Liu, Hayashi, and Yang, (G) *Protohermes xanthodes* Navás.

**Type D** (Fig. 1F). Family Corydalidae. Body green, flattened, with short and stout respiratory tubes on abdominal segment VIII.
**Type E** (Fig. 1G). Family Corydalidae. Body brown, flattened, without respiratory tubes on abdominal segment VIII (Fig. 2).

Thirty-one specimens (16 adults and 15 larvae) of South Korean Megaloptera were sequenced successfully, resulting in identification of larvae with adults through the high bootstrap support ($\geqslant 90\%$) by MP tree (Fig. 3), and there was a low genetic distance (K2P model) between larvae and adults ($0.50 \pm 0.21\%$). A MP tree using the COI sequence also supported the monophyly of Megaloptera (family Sialidae + family Corydalidae (Chauliodinae + Corydalinae)) (Wang *et al*. 2012). The average intraspecific K2P distances of the 31 specimens belonging to the families Sialidae and Corydalidae were 0.6% (range, 0.1–1.0%) and 0.3% (range, 0–0.5%), respectively, whereas the average interspecific K2P distances were 10.1% (7.3–11.6%) and 16.6% (15.0–18.5%), respectively. Furthermore, the average maximum intraspecific K2P distance of the compared species was 1.5% within the species of *Sialis longidens* Klingstedt, whereas the minimum interspecies distance was 7.3% between *S. koreana* and *Sialis annae* Vshivkova. High intraspecific divergence was revealed for *S. longidens* and *S. koreana*, which had the COI gene sequence variations ranging from 4–11 and 2–7 bp, respectively.

As a result, the larval types were associated with the known species as follows: Type A (*S. koreana* Jung and Bae), Type B (*S. annae* Vshivkova), Type C (*Neochauliodes formosanus* (Okamoto), Type D (*P. asahinai* Liu, Hayashi, and Yang), and Type E (*Protohermes xanthodes* Navás). The larva of *S. longidens* was not sequenced in this study (see the species account below).

## Taxonomic accounts: Corydalidae

### *Neochauliodes formosanus* (Okamoto, 1910)

(Figs. 1E, 4A–4G)

*Chauliodes formosanus* Okamoto, 1910: 263.
*Chauliodes kawarayamanus* Okamoto, 1910: 262.
*Neochauliodes formosanus* (Okamoto): Liu *et al.* 2007: 35; Jung and Bae 2012: 4.
*Parachauliodes continentalis* Weele: Yoon 1988: 429 (Fig. 4) (misidentification).

**Material examined. Korea:** 1 larva, GG, Pocheon-si, Ildong-myeon, Suip-ri, Sampalgyo, 14.

**Fig. 2.** (A–E) Larval habitats of Megaloptera in South Korea: (A) Seomjin river (*Protohermes xanthodes*), (B) Ganmun stream (*Neochauliodes formosanus*), (C) Geumsa stream (*Parachauliodes asahinai*), (D) Yongnup swamp (*Sialis koreana*), (E) Garim stream (*Sialis annae*).

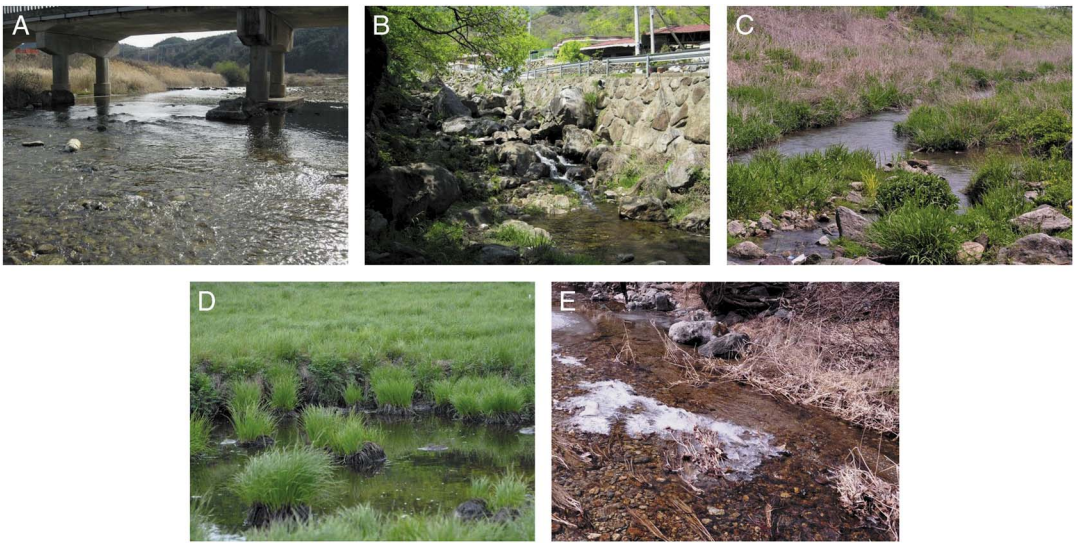
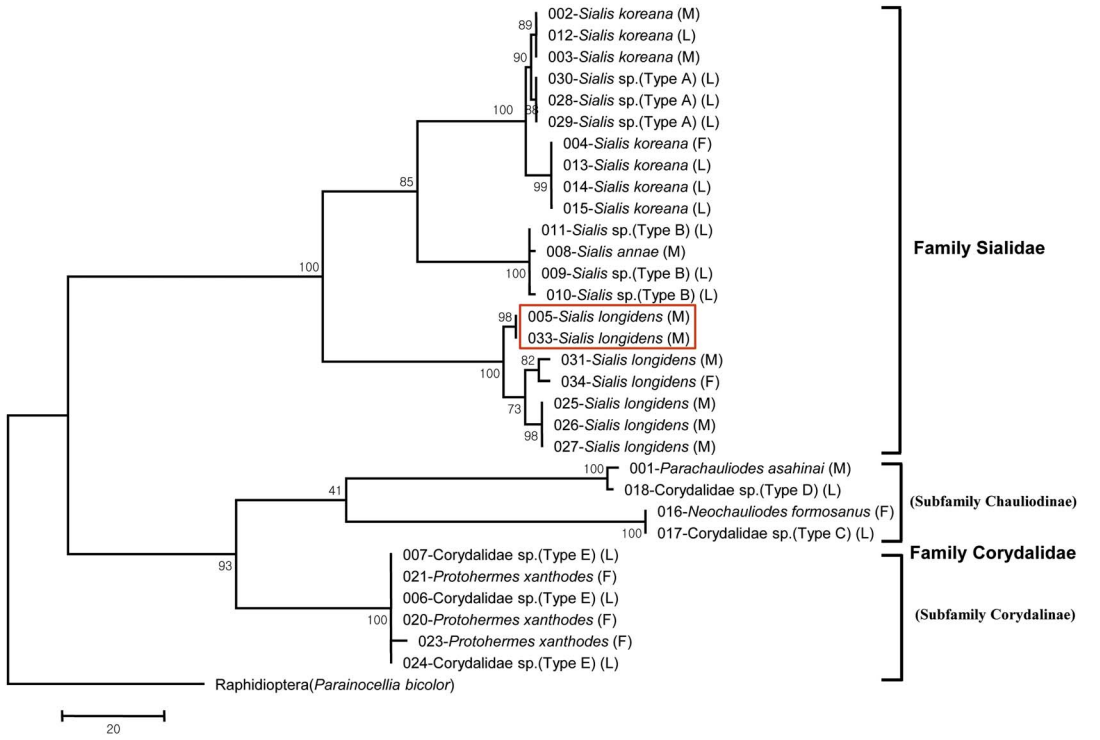


**Fig. 3.** Maximum-parsimony tree of 31 cytochrome *c* oxidase I sequences from six species (adults and larvae) of Corydalidae and Sialidae (length = 425, consistency index = 0.715, retention index = 0.926). The red-boxed specimens represent a genital variation of *Sialis longidens* Klingstedt. Nodal support values are bootstrap values (percentage of 1000 replicate). The tree is drawn to scale with branch lengths calculated using the average pathway method and represents the number of changes over the whole sequence. Scale bar indicates 20 nucleotide substitutions. L, larva; M, male adult; F, female adult.



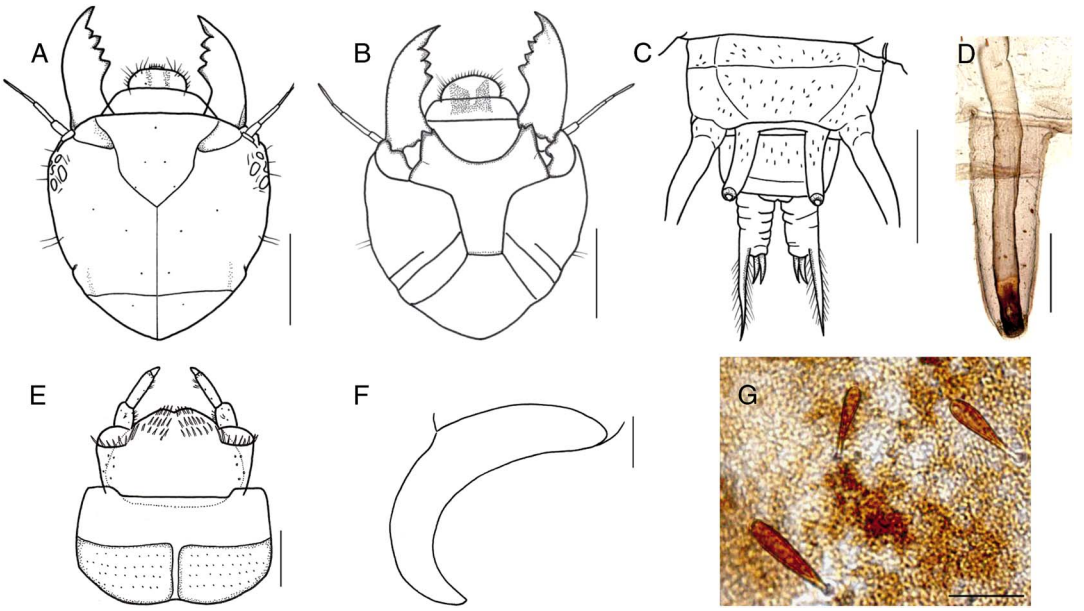

x.1997, Y.J. Bae (KU); 1 larva, GG, Yeoju-gun, Geumsa-myeon, Dogok-ri, 13.v.2010, S.W. Jung, DNA voucher: 017-PCL01 (# KJ155795) (KU); 2 larvae, GG, Suwon-si, Changan-gu, 5.vi.2012, S.W. Jung, (KU); 1 larva, JB, Jinan-gun, Bugwi-myeon, Suhang-ri, 1.x.1984, Y.J. Bae (KU); 1 larva, JN, Yeongam-gun, Yeongam-eup, Gaesin-ri, 6.iii.2010, S.W. Jung (KU); 1 female, GW, Samcheok-si, Miro-myeon, Hwalgi-ri, 30. viii.2011, S.W. Jung, DNA voucher: 016-PCa01 (# KJ127499) (KU); 1 larva, CB, Chungju-si, Eomjeong-myeon, Geodong-ri, 2.vi.2012, S.W. Jung (KU); 1 larva, CB, Jecheon-si, Geumseong-myeon, Seongnae-ri, 13.vi.2012, S.W. Jung (KU).

**Description. Adult (male and female).** See Liu *et al.* (2007) for full description.

**Larva.** Body length 42.4–47.6 mm ($n$ = 3), elongate and distinctly flattened. Head, prothorax reddish brown without pale markings. Mandibles, labium dark brown. Mesothorax and metathorax, legs brown. Abdomen grey with small yellow markings and lateral filaments.

Head (Fig. 4A) 5.0–5.7 mm in length, 5.0–5.5 mm in width ($n$ = 3), subquadrate but more or less round, narrowing posteriorly without markings; frontal suture lyriform, epicranial suture distinct, 3/5 × length of head; genal carinae distinct and short. Antennae long and slender, about 2.3 mm in length, with five antennomeres; gradually slender apically; approximate ratio of antennomeres ($n$ = 1) as 1.0:3.2:7.5:2.2:5.0. Labrum round, slightly prominent medially with two thick darkish brown markings and several setae in anterior margin. Clypeus three times wider than long, brown without pale markings. Mandibles asymmetrical, dark or reddish brown,

**Fig. 4.** Larva of *Neochauliodes formosanus*. (A) Head, dorsal view; (B) head, ventral view; (C) abdominal segment VIII, dorsal view; (D) respiratory tube; (E) labium, ventral view; (F) anal claw; (G) macrosetae on abdominal segment VIII. Scale bars = 2 mm for A–C; 0.5 mm for D, E; 0.2 mm for F; 0.05 mm for G.




well developed, left mandible with four teeth; third tooth more or less long but blunt, right mandible with three teeth; second tooth wide and round. Maxillae reddish brown, cardo small; stipes elongate with several strong setae on inner margin; galea with three galeomeres; galeomere 1 wide; galeomere 2 stout, smaller (about 3/5) than galeomere 3; galeomere 3 long and slender; maxillary with five palpomeres; palpomere 3 shortest; palpomere 4 over 3 × length of palpomere 3; approximate ratio of palpomeres ($n$ = 1) as 5.0:1.5:1.0:3.2:1.2. Labium (Fig. 4B) pale brown submentum brown, but anterolateral margin black, more or less narrow in posterior part; mentum entirely brown, but laterally blackish brown, and medially yellow; prementum blackish brown with several setae anteriorly; ligula concave at apex; labial palpomere (Fig. 4E), blackish brown; approximate ratio of palpomere ($n$ = 1) as 2.5:4.5:1.0.

Thorax. Prothorax slightly wider than long, 5.0–5.5 mm in length, 5.0–5.8 mm in width ($n$ = 3). Mesothorax and metathorax nearly equal to length and width, 2.2–2.5 mm in length, 5.0–6.0 mm in width ($n$ = 3), brown with several dark brown markings in dorsum. Legs brown, with six tarsomeres, with two claws and arolium; tarsi nearly equal to length of tibiae; prothoracic and mesothoracic claws with different in length, short outer and long inner side; metathoracic claws nearly equal to length.

Abdomen elongate and flattened, 10-segmented, with thin sparse microsetae (Fig. 4G) on dorsal part. Lateral filaments on abdominal segments I–VIII without setae; abdominal segments I–VII with round spiracles on lateral portion; abdominal segment VIII (Fig. 4C) with respiratory tubes on posterior margin; respiratory tubes (Fig. 4D) about 1.5 mm in length, slender and long with spiracle at apex; abdominal segment X with a pair of anal prolegs, each bearing two elongate claws (Fig. 4F) and anal proleg filaments with sparse setae.

**Distribution.** South Korea, China, Japan

**Remarks.** The larvae of *N. formosanus* were collected from the riffle areas from the midstream to downstream reaches (Fig. 2B) and they often occurred with *P. asahinai.* The larvae can be distinguished from *P. asahinai* by the combination of the following characters: generally grey body

with reddish brown head and pronotum, narrow clypeus without pale marking, and presence of long respiratory tubes on the abdomen apex.

Based on the examination of preserved specimens at KU, the previous recording of larva of *Parachauliodes continentalis* Weele by Yoon (1988) was a misidentification, in reality these larvae belong to *N. formosanus*. The long and slender respiratory tubes on the abdominal segment VII (Yoon 1988: 429, Fig. 4) are characteristic of the genus *Neochauliodes*.

### *Parachauliodes asahinai* Liu *et al.*, 2008
(Figs. 1F, 5A–5H)

*Parachauliodes asahinai* Liu *et al.,* 2008: 563; Jung and Bae 2012: 5.

**Material examined. Korea:** 3 larvae, JB, Jinan-gun, Bugwi-myeon, Suhang-ri, 1.x.1984, Y.J. Bae (KU); 1 larva, JN, Yeongam-gun, Yeongam-eup, Gaesin-ri, 6.iii.2010, S.W. Jung, DNA voucher: 018-PCL02 (# KJ155796) (KU); 1 larva, GN, Changwon-si, Dong-eup, Museong-ri, 22.vi.2011, S.W. Jung (KU); 1 male, GG, Gapyeong-gun, Buk-myeon, Jeokmok-ri, 19.v.2012, S.W. Jung and Y.J. Bae, DNA voucher: 001-MPA01 (# KJ127498) (CAU); 1 larva, JN, Gangjin-gun, Seongjeon-myeon, Wolpyeong-ri (34°42'16.0"N, 126°40'41.4"E), 18.vii.2013, M.C. Kim (KU).

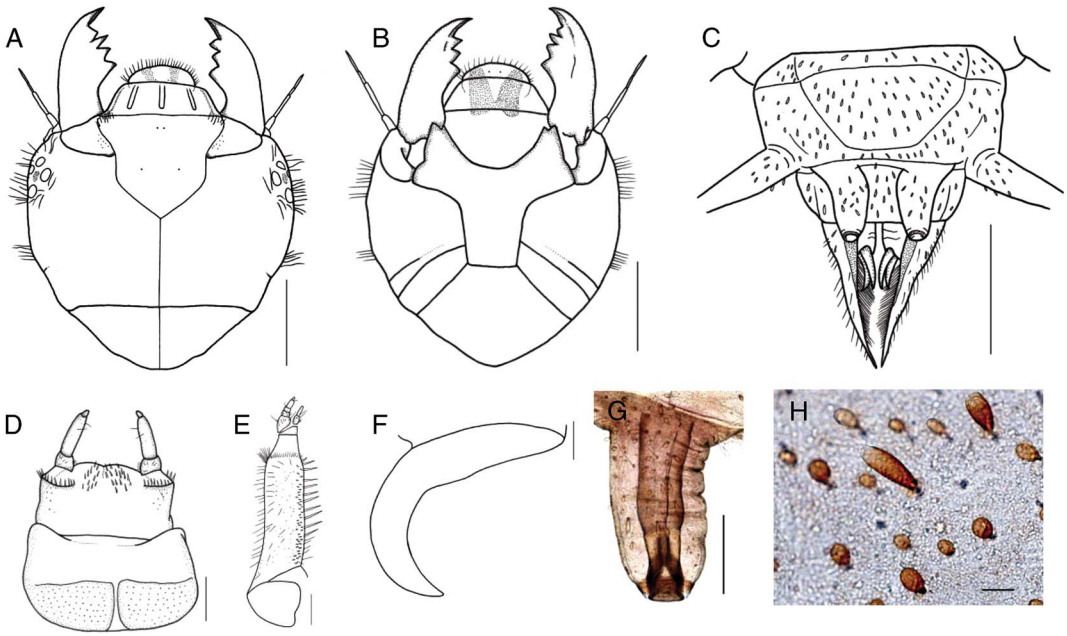
**Description. Adult (male and female).** See Liu *et al.* (2008) for full description.

**Larva.** Body length 45.3–49.2 mm ($n = 3$), elongate and distinctly flattened.

Head, mandibles, and prothorax dark brown without pale markings. Antennae, mouth parts and legs brown. Abdomen grey with green brindle markings and lateral filaments.

Head (Fig. 5A) 5.5–6.0 mm in length, 5.2–6.0 mm in width ($n = 3$), subquadrate but more or less round and wide posteriorly without markings; frontal suture lyriform, epicranial suture distinct, 3/5 × length of head; genal carinae distinct and short. Antennae long and slender, about 2.3 mm in length, with five antennomeres; antennomere 1 short and wide; antennomere 2 thicker than antennomere 3; antennomere 3 much thinner and longest, 2 × length of antennomere 2; approximate ratio of antennomeres ($n = 1$) as

**Fig. 5.** Larva of *Parachauliodes asahinai*. (A) Head, dorsal view; (B) head, ventral view; (C) abdominal segment VIII, dorsal view; (D) labium, ventral view; (E) left maxilla; (F) anal claw; (G) respiratory tube; (H) macrosetae on abdominal segment VIII. Scale bars = 2 mm for A–C; 0.5 mm for D, E, G; 0.2 mm for F; 0.05 mm for H.



1.0:4.0:8.0:2.5:5.0. Labrum round, reddish brown with several setae in anterior margin. Clypeus three times wider than long, brown with three longitudinal yellow markings, 2 × length of labrum. Mandibles asymmetrical, dark or reddish brown, well developed, left mandible with four teeth; teeth 1, 2, and 4 wide; third tooth long and sharp, right mandible with three teeth; third tooth widest; second tooth smallest. Maxillae (Fig. 5E) blackish brown, cardo small; stipes elongate with several strong setae on inner margin; galea with three galeomeres; galeomere 1 wide and short; galeomere 2 stout, smaller than galeomere 3; galeomere 3 long and slender; maxillary with five palpomere; palpomere 4 much longer than palpomere 3; approximate ratio of palpomeres ($n = 1$) as 5.0:1.0:1.0:2.5:1.0. Labium (Fig. 5B) pale brown submentum brown, but anterolateral margin black, more or less wide in posterior part; mentum entirely yellow, laterally blackish brown, but medially yellow; prementum blackish brown with several setae anteriorly; ligula broad margin, slightly concave at apex; labium with three palpomeres (Fig. 5D), blackish brown; approximate ratio of palpomeres ($n = 1$) as 2.5:6.0:1.0.

Thorax. Prothorax slightly wider than long, 5.5 mm in length, 6.0–6.8 mm in width ($n = 3$), blackish brown with brindle markings in posterior margin. Mesothorax and metathorax nearly equal to length and width, 2.8–3.0 mm in length, 6.0–6.5 mm in width ($n = 3$), brown with several dark brown markings in dorsal part and brindle markings in anterior and posterior margin. Legs brown, with six tarsomeres, with two claws and arolium; tarsi nearly equal to length of tibiae; prothoracic and mesothoracic claws with different in length, short outer and long inner side; metathoracic claws nearly equal to length.

Abdomen elongate and flattened, 10-segmented, with short and long microsetae (Fig. 5H) densely scattered on dorsal part. Lateral filaments on abdominal segments I–VIII without setae; abdominal segments I–VII with round spiracles on lateral portion, abdominal segment VIII (Fig. 5C) with respiratory tubes on posterior margin; respiratory tubes (Fig. 5G) about 1.2 mm in length, more or less stout with spiracle at apex; abdominal segment X with a pair of anal prolegs, each bearing two elongate claws (Fig. 5F) and anal proleg filaments covered with dense setae.

**Distribution.** South Korea, Japan.

**Remarks.** The larval stage is described for the first time here. This species inhabits streams in rhithral zones preferring habitats with aquatic vegetation (Fig. 2C). The larvae of *P. asahinai* are morphologically similar to those of *P. continentalis* (Hayashi, 2005: 381, figs. 2–4) in having clypeus with three longitudinal yellow markings, and short and stout respiratory tubes on abdominal segment VIII. However, *P. asahinai* differs from this species and all other described Korean Corydalidae by the following characters: wider head and pronotum, broad and round labrum, maxillary palpomere 4 much longer than palpomere 3, labial palpomere 2 about three times longer than first palpomere, abdomen with microsetae (elongate shape with smooth surface), stout and closely located respiratory tubes on the abdominal segment VIII, and proleg filaments covered with dense setae on the inner side.

### *Protohermes xanthodes* Navás, 1913
(Figs. 1G, 6A–6F)

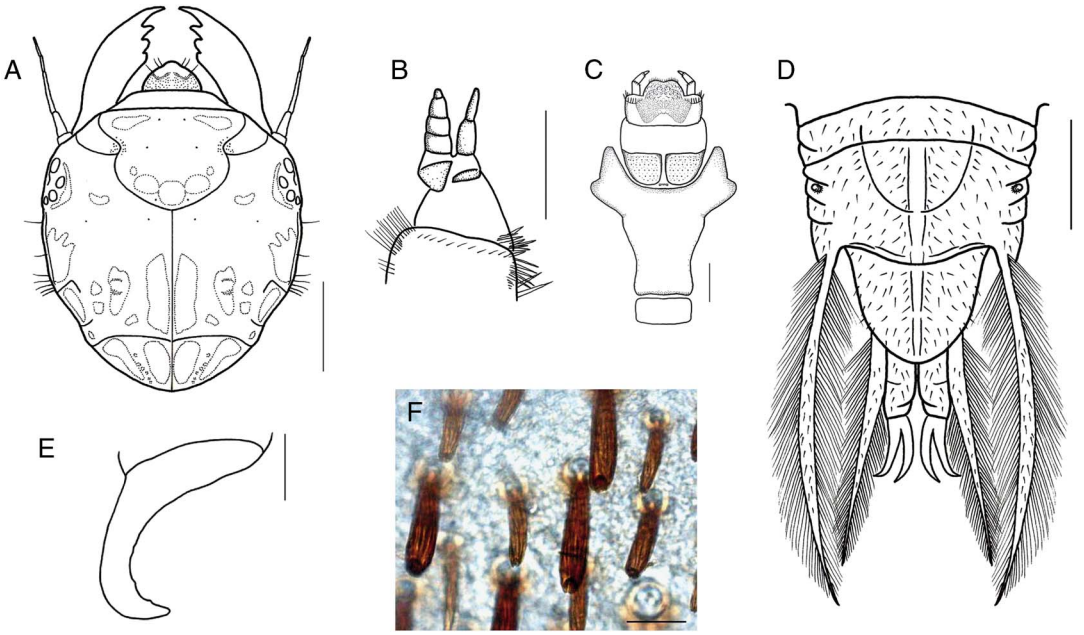
*Protohermes xanthodes* Navás, 1913: 427; Liu *et al.* 2006: 405; Jung and Bae 2012: 7.
*Parachauliodes grandis* (Thunberg): Yoon 1988: 428 (Fig. 3) (misidentification).

**Material examined. Korea:** 3 larvae, GG, Gapyeong-gun, Ha-myeon, Sinsang-ri, 11.vii.1993, Y.J. Bae (KU); 2 larvae, GG Gapyeong-gun, Buk-myeon, Dodae-ri, 10.vii.1995, Y.J. Bae (KU); 1 larva, GG, Yeoju-gun, Geumsa-myeon, Geumsa-ri, Geumsa 2-gyo (Br.), 13.v.2010, DNA voucher: 024-PGL01 (# KJ155799) (KU); 2 larvae, CB, Jechoen-si, Bongyang-eup, Jupo-ri, 6.x.2010, S.W. Jung and J.M. Hwang (KU); 3 females, 1 larva, GW, Hongcheon-gun, Seo-myeon, Dumi-ri, 20.vi.2011, S.W. Jung, DNA voucher: 020-PGa02 (# KJ155797), 021-PGa03 (# KJ155798), 023-PGa05 (# KJ127500) (KU); 4 larvae, GB, Gimcheon-si, Jirye-myeon, Dogok-ri, 18.iv.2012, S.W. Jung, DNA voucher: 006-MPL01 (# KJ155793), 007-MPL03 (# KJ155794) (KU); 2 larvae, GB, Uljin-gun, Seo-myeon, Wangpi-ri, 26.iv.2012, S.W. Jung (KU).

**Description. Adult (male and female).** See Liu *et al.* (2006) for full description.

**Larva.** Body length 52.0–55.8 mm ($n = 3$), elongate and distinctly flattened. Head and thorax sclerotised, dark or reddish brown with several

**Fig. 6.** Larva of *Protohermes xanthodes*. (A) Head, dorsal view; (B) left maxilla; (C) labium, ventral view; (D) abdominal segment VIII, dorsal view; (E) anal claw; (F) macrosetae on abdominal segment VIII. Scale bars = 2 mm for A, D; 1 mm for C; 0.5 mm for B, E; 0.05 mm for F.




yellow markings, legs and abdomen brown, with lateral filaments.

Head (Fig. 6A) 6.2–7.0 mm in length, 5.5–6.5 mm in width ($n = 3$), subquadrate, with several yellow markings and spots in dorsal and lateral view; frontal suture lyriform, more or less round laterally, epicranial suture distinct, 2/3 times as long as head; genal carinae distinct and long; frons with three large round yellow markings posteriorly, two oblong markings anteriorly; postoccipital suture slightly curved laterally. Antennae long and slender, 2.8 mm in length, with five antennomeres; antennomere 1 short and wide; antennomere 2 thicker than antennomere 3; antennomeres 4 and 5 much thinner and nearly equal in length; approximate ratio of antennomeres ($n = 1$) as 1.0:3.5:5.5:2.0:2.0. Labrum protruding anteriorly, reddish brown, with paler anterior margin bearing two long setae laterally. Clypeus pale, 1/2 × length of labrum. Mandibles dark or reddish brown, well developed, with three teeth; apical tooth blunt, twice length of third tooth; first and second teeth wide and round. Maxillae (Fig. 6B) blackish brown, cardo small; stipes elongate with several strong setae on inner margin; galea with three galeomeres; galeomere 1 wide and short; galeomere 2 stout; galeomere 3 slender and equal length of galeomere 2; maxillary with five palpomeres; palpomere 4 more or less longer than palpomere 3; approximate ratio of palpomeres ($n = 1$) as 2.6:1.0:1.0:1.3:1.0. Labium (Fig. 6C) from pale brown to yellow, submentum brown, but with black margin, mentum entirely yellow, laterally blackish brown, prementum blackish brown; labium with three palpomeres, blackish brown; approximate ratio of palpomeres ($n = 1$) as 2.0:1.6:1.0.

Thorax. Prothorax nearly as long as wide, 6.0–6.5 mm in length, 5.8–6.5 mm in width ($n = 3$), blackish brown with yellow marking, laterally with dense short setae and long strong setae. Mesothorax and metathorax nearly equal to length and width, 2.8 mm in length, 5.5 mm in width ($n = 3$), blackish brown with a few yellow markings. Legs yellow, with six tarsomeres, with two claws and triangular arolium; protarsi 1/2 × length of tibiae with a row of protuberances on outer side, two curved claws with different length; mesotarsi and metatarsi as long as tibiae, two claws equal in length and strongly curved with one tooth located basally.

Abdomen elongate and flattened, 10-segmented, with dense microsetae (Fig. 6F) on dorsal part. Lateral filaments on abdominal segments I–VIII covered with setae; lateral filaments 7 and 8 longer than other and denser setae (Fig. 6D); abdominal segments I–VII ventrally bearing tufts of tracheal gills; abdominal segments I–VIII with round spiracle; abdominal segment X with a pair of anal prolegs, each bearing two elongate claws (Fig. 6E) and anal proleg filaments covered with long and dense setae.

**Distribution.** South Korea, China

**Remarks.** The larvae of *P. xanthodes* are common and widespread in the riffles of downstream reaches (Fig. 2A). The larvae resemble *P. grandis* Thunberg (Hayashi 2005: 381, figs. 2–3) in having ventral gill tufts on abdominal segments I–VII and absence of respiratory tubes on abdominal segments VIII, but can be distinguished from the latter by their generally reddish brown head and pronotum with light markings, wider pronotum, short labial palpomere 2, thick and distinct gular suture, lower number of setae on the lateral filaments, and denser setation of the proleg filaments. Based on the examination of preserved specimens at KU, the previous recording of larva of *P. grandis* by Yoon (1988) was a misidentification of *P. xanthodes*.

The close species *Parachauliodes martynovae* Vshivkova is recorded from the south of the Russian Far East: Primorye Territory (the last record is dated 1960) and recently from Khabarovsky District, Bolshekhekhtsyrskii Federal Nature Reserve (1 male and 1 female). Liu *et al.* (2006) considered this species as a junior synonym of *P. xantodes*. In order to solve the problem of *P. martynovae* taxonomic status it is necessary to carry out careful comparative study of species morphology (wing venation, non-genital, and genital structure) as well as the DNA data. (Vshivkova and Dubatolov 2010). Also the geographical data should be revised along with reidentification of *P. xantodes* material from different areas.

## Taxonomic accounts: Sialidae

### *Sialis annae* Vshivkova, 1979
(Figs. 1C, 1D, 7A–7E)

*Sialis annae* Vshivkova, 1979: 79; Vshivkova 1995: 33; Vshivkova 2001: 379, 779 (Fig. 8); Jung and Bae 2012: 11.

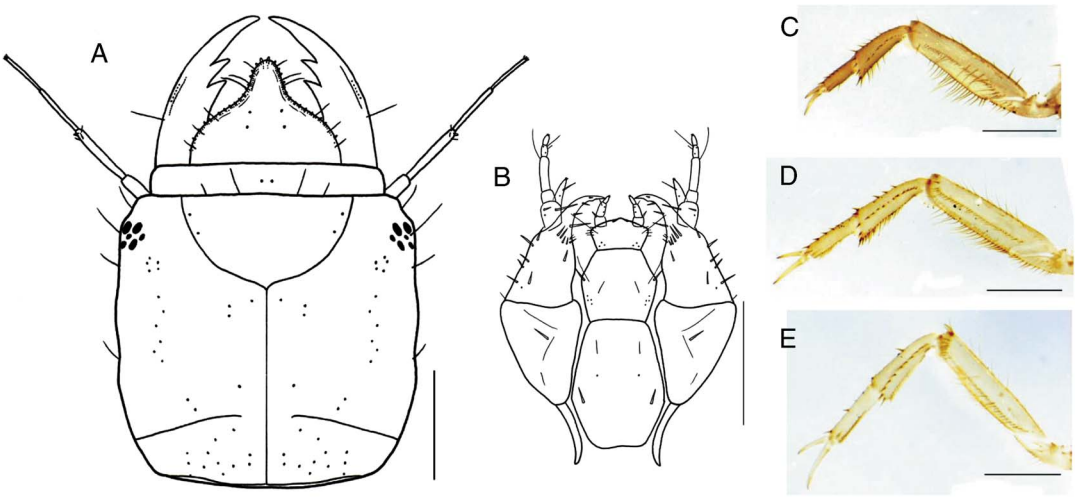
**Material examined. Korea:** 1 male, GG, Gapyeong-gun, Buk-myeon, Jeokmok-ri, Seungcheonsa (Temple), 20.v.2010, S.W. Jung, DNA voucher: 008-SSa04 (# KJ127487) (KU); 3 larvae, GG, Gapyeong-gun, Buk-myeon, Jeokmok-ri, 26.ii.2012, S.W. Jung, DNA voucher: 009-SSL04 (# KJ127488), 010-SSL05 (# KJ127489), 011-SSL06 (# KJ127490) (KU).

**Description. Adult (male and female).** See Vshivkova (1979) for full description.

**Larva.** Body length 16.1–19.1 mm ($n = 3$); caudal filaments length 5.1–5.2 mm ($n = 3$). Body elongate, slightly flattened. Head and thorax sclerotised, brown with yellowish several markings, legs yellowish brown (without medial longitudinal light stripe), abdomen soft and pink brown, with pale lateral filaments.

Head (Fig. 7A) 2.1–2.8 mm in length, 2.5–3.1 mm in width ($n = 3$), subquadrate, with several yellow markings and spots in dorsal and lateral view; Y-formed epicranial suture distinct; frons with four setae laterally; postoccipital suture slightly arched, interrupted at coronal suture. Antennae long and slender, 1.8 mm in length, with four antennomeres; antennomere 1 more or less stout; antennomere 2 thicker than antennomeres 3 and 4, with four short setae posteriorly, terminal antennomere with four sensorial appendages; approximate ratio of antennomeres ($n = 1$) as 1.5:4.3:3.5:3.0. Labrum subtriangular and protruding anteriorly with anterior margin crenulate, abruptly narrow at 1/3 apical part. Mandibles well developed, with two teeth, long setae on outer margin. Maxillae (Fig. 7B) with large cardo; stipes slightly smaller than cardo; galea conical; lacinia falciform, with two strong long setae posteriorly and two strong small setae anteriorly; maxillary with five palpomeres; palpomere 1 stout, with two setae; palpomere 3 slender and longest, with long seta anteriorly; palpomere 4 short, with long seta; terminal palpomere with sensorial appendages; approximate ratio of palpomeres ($n = 1$) as 1.6:1.1:1.9:0.7:0.6. Labium elongate; submentum large, with two strong setae posteriorly and two fine setae anteriorly, widest at posterior 2/5 part; mentum widest at middle part, as long as half submentum, with strong setae laterally; prementum short, subtriangular between palpi; labial palp short, with three palpomeres; terminal palpomere with sensorial appendages.

**Fig. 7.** Larva of *Sialis annae*. (A) Head, dorsal view; (B) mouth parts, ventral view; (C) left proleg; (D) left mesoleg; (E) left metaleg. Scale bars = 1 mm.




Thorax yellowish brown; prothorax large, 2.1–2.2 mm in length, about twice as long as mesothorax; mesothorax 1.2 mm in length, 2.5–3.1 mm in width; metathorax partially sclerotised, 1.1–1.3 mm in length, 2.4–2.8 mm in width ($n = 3$). Legs (Figs. 7C–7E) long and slender, with six tarsomeres, with two claws in different length.

Abdomen elongate, 10-segemented, with 7 lateral filaments (from I to VII segment). Abdominal segment X small and emarginate at apex, with long caudal filament. Abdominal terga dorsally uniformly pink each with two distinct pale submedial bracket-like figures, which apically became smaller and stick-like; sublaterally at the base of lateral filament in each tergite there are slightly curved pale stripes. Sternites uniformly pink-brown, without any black pattern (Fig. 1D).

**Distribution.** South Korea, northeast China, Far East Russia (Primorye Territory)

**Remarks.** Adults of this species were recently recorded by Jung and Bae (2012) from South Korea. We collected immature larvae in a water-filled pool area with fallen leaves near a mountain stream (Fig. 2E). The substrate was composed mostly of fine sand or silt (< 0.5 mm). Aquatic insects such as Chironomidae (Diptera) and the mayfly *Siphlonurus chankae* Tshernova (Ephemeroptera: Siphlonuridae) were dominant there; the caddisfly *Semblis phalaenoides* Linnaeus (Trichoptera: Phryganeidae) was also common. The larvae of *S. annae* are similar to the larvae of *S. longidens* and *S. koreana* in general, but can easily be distinguished from these species by slightly arched postoccipital suture, antennomere 3 as long as antennomere 2, protruding labrum, smaller sclerotisation of metathorax with yellow markings, more remarkably pink abdominal tergites and sternites without any obvious black longitudinal or transverse stripes or pattern.

### *Sialis koreana* Jung and Bae, 2012
(Figs. 1A, 1B)

*Sialis koreana* Jung and Bae, 2012: 8

**Material examined. Korea:** 3 larvae, GB, Gimcheon-si, Buchang-myeon, Picheon-ri, 22.ix.2011, S.W. Jung, DNA voucher: 028-SSL01 (# KJ127484), 029-SSL02 (# KJ127485), 030-SSL03 (# KJ127486) (KU); 1 male, GW, Inje-gun, Seohwa-myeon, Simjeok-ri, Suribong, 3.vi.2009, J.K. Choi and H.M. Lim, DNA voucher: 002-MSK01 (# KJ127477) (KU); 4 larvae, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup (swamp), 16.viii.2011, M.C. Kim and D.G. Kim, DNA voucher: 012-SSL07 (# KJ127480), 013-SSL08 (# KJ127481), 014-SSL09 (# KJ127482), 015-SSL10 (# KJ127483) (KU); 4 larvae, GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup (swamp), 1.vi.2012, Y.J. Bae and S.W. Jung (KU); 1 male (paratype),

1 female (paratype), GW, Inje-gun, Seohwa-myeon, Seoheung-ri, Yongneup (swamp), 10. vi.2012, Y.J. Bae and S.W. Jung, DNA voucher: 003-MSK02 (# KJ127478), 004-MSK03 (# KJ127479) (KU).

**Description. Adult (male and female).** See Jung and Bae (2012) for full description.

**Larva.** See Jung and Bae (2012) for full description.

**Diagnosis.** The larva (Fig. 1A) of this species can be distinguished from congeners by the following characters: elongate body, almost square head with several yellow markings, distinct Y-shaped epicranial suture, slightly round postoccipital suture disconnected to the coronal suture, apically more or less emarginate abdominal segment X, and abdominal terga with light median longitudinal stripe and light paired small lateral spots; The important distinguish characters are also: the characteristic pattern of sternites, which on the periphery bordered with intense black stripe so that the centre of the sternum is presenting an oblong light field. This pattern would indicate that it is in the *Sialis sibirica* species group, however, *S. koreana* does not have two sublateral black round spots on sternum (Fig. 1B), which is different from the *S. sibirica* species group (Vshivkova 2001: 379, 779, plate 196, fig. 6).

**Distribution.** South Korea.

**Remarks.** This species is endemic to South Korea (Gangwon Province) and found only in high mountain swamps (above 1200 m altitude) (Fig. 2D) near the demilitarised zone (DMZ). The larval specimens from the southeastern area (Gyeongsangbuk Province) of the Korean Peninsula and the specimens collected together with the holotype adult of *S. koreana* (Jung and Bae 2012) were confirmed to be conspecific based on the COI gene sequences (Table 1, Fig. 3). It is interesting to note that the separation the speciation process in the genus *Sialis* in some cases was caused by altitude factor: for example, in Caucasus two sister species *S. abchasica* Vshivkova and *S. zhiltzovae* Vshivkova in their distribution divided by altitude – one species is found in the highlands, the second inhabits foothills waters.

### *Sialis longidens* Klingstedt, 1932
(Figs. 8A–8J)

*Sialis longidens* Klingstedt, 1932: 1; Hayashi and Suda 1995: 6; Vshivkova and Ito 1993: 108; Vshivkova 1995: 33; Vshivkova 2001: 380, 769 (Figs. 1, 4, larva final stage), 773 (Figs. 1–4, larva first stage), 775 (Fig. 1, pupa); Liu and Yang 2006: 396; Jung and Bae 2012: 11.

*Sialis* KUa: Yoon 1988: 427 (Fig. 2).

**Material examined. Korea:** 3 males, JN, Naju-si, Noan-myeon, Gyelim-ri (35°03'52.77"N, 126°42'50.98"E), 16.iv.2009, J.K. Choi and D.H. Lee, DNA voucher: 005-MSA01 (# KJ127491), 031-MSA02 (# KJ127496), 033-MSA04 (# KJ127492) (KU); 3 males, 1 female, CB, Yeongdong-gun, Hwanggan-myeon, Nangok-ri (36°14'50.96"N, 127°57'19.92"E), 13.iv.2011, S.W. Jung, DNA voucher: 025-SSa01 (# KJ127493), 026-SSa02 (# KJ127494), 027-SSa03 (# KJ127495), 034-MSA05 (# KJ127497) (KU). **Russia:** 3 males, Primorye Territory, Lazovsky Nature Reserve, Kordon Sokolchi, 33. vi.1978, G. Zapolina and T.S.Vshivkova (KU).

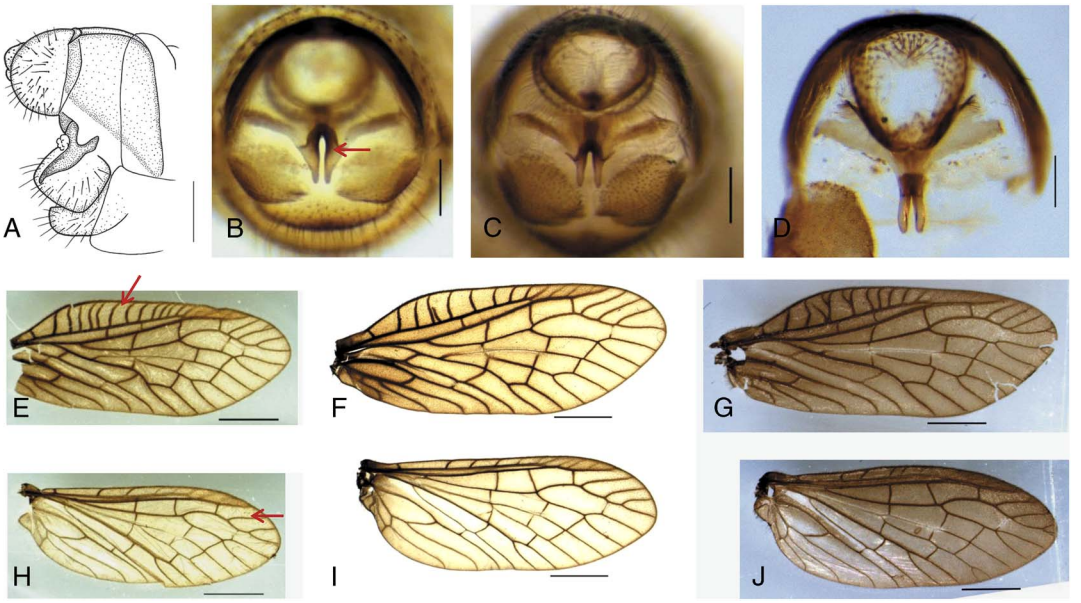
**Description. Adult (male and female).** See Klingstedt (1932) for full description.

**Larva.** See Vshivkova (1995, 2001), Yoon (1988), and Hayashi (2005) for diagnostic characters with habitus drawing and photograph.

**Diagnosis.** Adults of *S. longidens* can be distinguished from all the other *Sialis* species by the following combination of characters: black pronotum without markings, somewhat broad brown wings; round tergum X which is as long as 2/3 of the width of tergum IX and subquadrate from the lateral view, elongate sternum X, and subquadrate gonostylus IX. The larvae are similar to those of *S. koreana*, but can be distinguished from the latter by having abdominal terga with wider light median longitudinal stripe (Vshivkova 1995: 17, fig. 4–1; Vshivkova 2001: 769, figs. 1, 4) or without distinct median stripe; if medial stripe present, lateral margins of median stripe straight (Hayashi 2005: 383, fig. 5).

**Variations.** The populations of *S. longidens* distributed in the Korean Peninsula and the Russian Far East show variations in male genitalia. The specimens from the southwestern part of the Korean Peninsula (Naju-si) have a basally broader and apically gradually tapering sternum X (Figs. 8A, 8B) compared with the specimens from the central part of the Korean Peninsula (Yeongdong-gun) (Fig. 8C) or those from the Russian Far East (Fig. 8D). Some other variations are also found in the pattern of several yellow markings on the head, antennomeres (35–39), and

**Fig. 8.** Male of *Sialis longidens*. (A) Genitalia, lateral view (South Korea, JN); (B) genitalia, caudal view (South Korea, JN); (C) genitalia, caudal view (South Korea, CB); (D) genitalia caudal view (Russia); (E) fore wing, right (South Korea, JN); (F) fore wing, right (South Korea, CB); (G) fore wing, right (Russia); (H) hind wing, right (South Korea, JN); (I) hind wing, right (South Korea, CB); (J) Hind wing, right (Russia). Scale bars = 0.2 mm for A–D; 2 mm for E–J.




wing venation (*e.g.*, number of costal crossveins in the forewings and R2 and R3 branches in the hindwings) (Figs. 8E–8J). The larvae also show geographical variations in abdominal terga with a wide light median longitudinal stripe (Russia) or without a median longitudinal stripe (Japan). The status of Japanese species "*S. longidens* having larvae without longitudinal black stripes" should be confirmed on DNA level, probably it may be another sister species of *S. longidens* species group.

**Distribution.** South Korea, northern China, Japan (Hokkaido), Mongolia, eastern Russia (southern Siberia and southern Russian Far East).

**Remarks.** This species is widely distributed in Japan (Hayashi and Suda 1995), China (Liu and Yang 2006), Russia, and Mongolia (Vshivkova 1980, 2001; Vshivkova and Ito 1993), and was first recorded in the middle and northern parts of South Korea by Jung and Bae (2012). Although the male adults collected from the southwestern part of the Korean Peninsula (Naju-si) showed variations in the male genitalia, their genetic distance based on COI gene sequences was relatively low (< 2.0%).

Hayashi and Suda (1995) showed that two *Sialis* species (*S. melania melania* Nakahara and *S. sinensis* Banks) from Japan, China, and Taiwan showed geographic variations in male genitalia; the sternum X is pointed to a greater degree at the apex or slightly narrower among conspecific individuals. According to Bowles and Mathis (1992), the genital plate of the male of *Neohermes concolor* (Davis) differs in size and shape (rounded to pointed) among conspecific individuals. From this evidence, we concluded that the genital plate of the male is probably flexible and weakly sclerotised with a broad range of phenetic variation in this species.

Although we were unable to examine fresh larval specimens of this species in South Korea, we identified the undetermined larva of *Sialis* KUa in Yoon (1988: 427, fig. 2) as *S. longidens*, as it has distinct smooth dark paired longitudinal stripes on the abdomen. This larva will be sequenced and compared to adult COI barcode references available in GenBank (Table 1) in order to confirm the conspecificity of the larva and adult. Future study should be focussed on the geographical variation in this species using extensive adult and larval materials and, if required, additional gene markers. Probably, some so-called "*S. longidens*" in reality may represent a complex of closely related sister species.

**Key to the larvae of Megaloptera (Corydalidae and Sialidae) in South Korea**

1. Body large (42–55 mm), 8 lateral filaments on abdomen with anal prolegs (Fig. 1E) (Corydalidae) . . . . . 2
– Body small (12–19 mm), 7 lateral filaments on abdomen without anal prolegs (Fig. 1A) (Sialidae). . . . . 4

2. Abdominal segments VIII without respiratory tubes (Fig. 6D), ventral gill tufts present. . . . . *Protohermes xanthodes* Navás
– Abdominal segments VIII with respiratory tubes, ventral gill tufts absent . . . . . 3

3. Respiratory tubes stout and short (Fig. 5G), clypeus with three longitudinal yellow markings . . . . . *Parachauliodes asahinai* Liu, Hayashi, and Yang
– Respiratory tubes slender and long (Fig. 4D), clypeus without markings. . . . . *Neochauliodes formosanus* (Okamoto)

4. Abdomen uniformly pink dorsally and ventrally (Fig. 1C) without dark stripes in ventral part (Fig. 1D) . . . . . *Sialis annae* Vshivkova
– Abdomen black dorsally with medial longitudinal light stripe. . . . . 5

5. Abdominal terga with narrow light median longitudinal stripe (Fig. 1A); light stripe typically rhomboid-shaped on each tergum (Jung and Bae 2012: 9, fig. 7D); with sternites bordered on the periphery with intense black stripe and light oblong field in the centre of sternum (Fig. 1B) . . . . . *Sialis koreana* Jung and Bae
– Abdominal terga with wide light median longitudinal stripe (Vshivkova 1995: 17, fig. 4–1); variation (or sibling species) without distinct median stripe (Hayashi 2005: 383, fig. 5) . . . . . *Sialis longidens* Klingstedt

## Acknowledgements

The authors thank Dr. Xingyue Liu (China Agricultural University) for valuable comments on the variation in Megaloptera; Dr. Fumio Hayashi (Tokyo Metropolitan University) for the information on the larvae of *Sialis longidens*; Dr. Ahn Soo-Jeong (Erang Bio-Environment Research System) for providing an excellent photograph of *Parachauliodes asahinai*. This research was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR 201401203).

## References

Aspöck, U., Haring, E., and Aspöck, H. 2012. The phylogeny of the Neuropterida: long lasting and current controversies and challenges (Insecta: Endopterygota). Arthropod Systematics & Phylogeny, **70**: 119–129.

Beutel, R.G., Friedrich, F., Hörnschemeyer, T., Pohl, H., Hünefeld, F., Beckmann, F., *et al.* 2010. Morphological and molecular evidence converge upon a robust phylogeny of the megadiverse Holometabola. Cladistics, **26**: 1–15.

Bowles, D.E. and Mathis, M.L. 1992. Variation in the terminalia of *Neohermes concolor* with a key to males of *Neohermes* in eastern North America (Megaloptera: Corydalidae: Chauliodinae). Insecta Mundi, **6**: 145–149.

Cameron, S.L., Sullivan, J., Song, H., Miller, K.B., and Whiting, M.F. 2009. A mitochondrial genome phylogeny of the Neuropterida (lace-wings, alderflies and snakeflies) and their relationship to the other holometabolous insect orders. Zoologica Scripta, **38**: 575–590.

Contreras-Ramos, A. and Harris, S.C. 1998. The immature stages of *Platyneuromus* (Corydalidae), with a key to the genera of larval Megaloptera of Mexico. Journal of the North American Benthological Society, **17**: 489–517.

Elliott, J.M. 1996. British freshwater Megaloptera and Neuroptera: a key with ecological notes. Freshwater Biological Association Scientific Publication, **54**: 1–69.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.

Flint, O.S., Evans, E.D., and Neunzig, H.H. 2008. Megaloptera and aquatic Neuroptera. *In* An introduction to the aquatic insects of North America. 4th edition. *Edited by* R.W. Merritt, K.W. Cummins, and M.B. Berg. Kendall/Hunt Publishing Company, Dubuque, Iowa, United States of America. Pp. 425–438.

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

Hayashi, F. 2005. Megaloptera. *In* Aquatic insects of Japan: manual with keys and illustrations. *Edited by* R. Kawai and K. Tanida. Tokai University Press, Kanagawa, Japan. Pp. 379–386.

Hayashi, F. and Suda, S. 1995. Sialidae (Megaloptera) of Japan. Aquatic Insects, **17**: 1–15.

Hwang, J.M., Yoon, T.J., Suh, K.I., and Bae, Y.J. 2013. Molecular phylogeny evidence of altitudinal distribution and habitat adaptation in Korean *Ephemera* species (Ephemeroptera: Ephemeridae). Entomological Research, **43**: 40–46.

Jung, S.W. and Bae, Y.J. 2012. Taxonomic review of the Korean Megaloptera with description of *Sialis koreana*, new species. Entomological Research Bulletin, **28**: 3–13.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, **16**: 111–120.

Klingstedt, H. 1932. *Sialis longidens* n. sp. Aus dem sudlichen Zentral-Sibirien. Memoranda Societatis pro Fauna et Flora Fennica, **8**: 1–3.

Liu, X., Hayashi, F., and Yang, D. 2006. Systematics of the *Protohermes xanthodes* species-group in eastern Asia (Megaloptera: Corydalidae). Entomological Science, **9**: 399–409.

Liu, X., Hayashi, F., and Yang, D. 2007. Revision of the *Neochauliodes sinensis* species-group (Megaloptera: Corydalidae: Chauliodinae). Zootaxa, **1511**: 29–54.

Liu, X., Hayashi, F., and Yang, D. 2008. Systematics and biogeography of the fishfly genus *Parachauliodes* (Megaloptera: Corydalidae) endemic to the east Asian island. Systematic Entomology, **33**: 560–578.

Liu, X., Hayashi, F., and Yang, D. 2014. Phylogeny of the family Sialidae (Insecta: Megaloptera) inferred from morphological data, with implications for generic classification and historical biogeography. Cladistics, **2014**: 1–32.

Liu, X., Wang, Y., Shih, C., Ren, D., and Yang, D. 2012. Early evolution and historical biogeography of fishflies (Megaloptera: Chauliodinae): implications from a phylogeny combining fossil and extant taxa. Public Library of Science One, **7**: 1–12.

Liu, X. and Yang, D. 2004. A revision of the genus *Neoneuromus* in China (Megaloptera: Corydalidae). Hydrobiologia, **517**: 147–159.

Liu, X. and Yang, D. 2006. The genus *Sialis* Latreille, 1802 (Megaloptera: Sialidae) in Palaearctic China, with description of a new species. Entomologica Fennica, **17**: 394–399.

Lunt, D.H., Zhang, D.X., Szymura, J.M., and Hewitt, G.M. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Molecular Biology, **5**: 153–165.

Meinander, M. 1996. Megaloptera Sialidae, alder flies. *In* Aquatic insects of North Europe: a taxonomic handbook. *Edited by* A.N. Nilsson. Apollo Books, Stenstrup, Denmark. Pp. 105–110.

Miller, K.B., Alarie, Y., and Whiting, M.F. 2007. Description of the larva of *Notaticus fasciatus* (Coleoptera: Dytiscidae) associated with adults using DNA sequence data. Annals of the Entomological Society of America, **100**: 787–797.

Navás, L. 1913. Neuroptera Asiatica. II series. Revue Russe d'Entomologie, **13**: 424–430.

Nei, M. and Kumar, S. 2000. Molecular evolution and phylogenetics. Oxford University Press, New York, New York, United States of America.

Neunzig, H.H. and Baker, J.R. 1991. Order Megaloptera. *In* Immature insects. Volume 2. *Edited by* F.W. Stehr. Kendall-Hunt, Dubuque, Iowa, United States of America. Pp. 112–122.

Okamoto, H. 1910. Die Sialiden Japan. Wiener Entomologische Zeitung, **29**: 255–263.

Oswald, J.D. 2013. A catalogue of the species-group names of the extant and fossil Neuroptera, Megaloptera, Raphidioptera and Glosselytrodea (Insecta: Neuropterida) of the world [online]. Available from http://lacewing.tamu.edu/Species-Catalogue/index.html [accessed 22 December 2014].

Ruiter, D.E., Boyle, E.E., and Zhou, X. 2013. DNA barcoding facilitates associations and diagnoses for Trichoptera larvae of the Churchill (Manitoba, Canada) area. BMC Ecology, **13**: 5.

Takeuchi, Y. and Hoshiba, H. 2012. The life histories of three species of Corydalidae (Megaloptera) from Japan. Aquatic Insects, **34**: 55–63.

Takeuchi, Y. and Hoshiba, H. 2013. The life histories of two species of Sialidae (Megaloptera) from Japan. Aquatic Insects, **34**: 205–216.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, **30**: 2725–2729.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, **25**: 4876–4882.

Vshivkova, T.S. 1979. New data on the systematics and distribution of Megaloptera (Insecta) of the Far East. *In* Systematics and ecology of continental reservoirs of the Far East. *Edited by* V.Y. Levanidov, I.M. Levanidova, I.A. Chereshnev, and E.A. Makarchenko. Akademiya nauk SSSR, Vladivostok, Russia. Pp. 78–85.

Vshivkova, T.S. 1980. A new species of *Sialis* (Megaloptera: Sialidae) and preliminary data on the distribution of Far Eastern species. *In* Freshwater fauna of the Far East. *Edited by* V.Y. Levanidov. Akademiya nauk SSSR, Vladivostok, Russia. Pp. 75–79.

Vshivkova, T.S. 1995. Order. Megaloptera. *In* Key to the insects of Russian Far East. Volume IV. *Edited by* M.A. Kozlov and E.A. Makarchenko. Nauka, St. Petersburg, Russia. Pp. 9–34.

Vshivkova, T.S. 2001. Megaloptera. Dobsonflies and Alderflies. *In* Key to freshwater invertebrates of Russia and adjacent lands. *Edited by* S.J. Tsalolikhin. Nauka, St. Petersburg, Russia. Pp. 374–380. plates 191–196 (pp. 768–779).

Vshivkova, T.S. and Dubatolov, V.V. 2010. New records of *Protohermes* species (Megaloptera, Corydalidae, Corydalinae) from the south of the Russian Far East. Euroasian Entomological Journal, **9**: 345–351.

Vshivkova, T.S. and Ito, T. 1993. A new record of *Sialis longidens* Klingstedt (Megaloptera, Sialidae) from Hokkaido, Northern Japan. Japanese Journal of Entomology, **61**: 108.

Wang, Y., Liu, X., Winterton, L., and Yang, D. 2012. The first mitochondrial genome for the fishfly subfamily Chauliodinae and implications for the higher phylogeny of Megaloptera. Public Library of Science One, **7**: 1–13.

Yang, D. and Yang, C.K. 1995. Megaloptera and aquatic Neuroptera. *In* Aquatic insects of China useful for monitoring water quality. *Edited by* L.F. Yang, L.X. Tian, and J.C. Morse. Hohai University Press, Nanjing, China. Pp. 250–259.

Yang, Q., Makarkin, V.N., Winterton, S.L., Khramov, A.V., and Ren, D. 2012. A remarkable new family of Jurassic insects (Neuroptera) with primitive wing venation and its phylogenetic position in Neuropterida. Public Library of Science One, **7**: 1–38.

Yoon, I.B. 1988. Illustrated encyclopedia of fauna and flora of Korea. Aquatic Insects. Ministry of Education of Korea, Seoul, South Korea. Pp. 423–429.