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ORIGINAL ARTICLE

DNA barcoding of the genus *Dichopygina*, with a new species from China (Diptera: Sciaridae)

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Abstract Species of the genus *Dichopygina* Vilkamaa, Hippa & Komarova, 2004 has been morphologically cryptic. In this paper, eight species of the genus were clearly defined by DNA barcodes. Based on both molecular and morphological evidences, *D. bernhardi* Vilkamaa, Hippa & Komarova, 2004, **stat. rev.** is separated from *D. perfecta* (Pettey, 1918); a closely allied new species, *D. tibetana* Leng, Heller & Huang, **sp. nov.**, is described from Tibet, China. Detailed descriptions and figures of the two species are presented.

Key words Cryptic species, COI, new records.

1 Introduction

The monophyletic genus *Dichopygina* Vilkamaa, Hippa & Komarova, 2004, a small to medium-sized Sciaridae, was established by Vilkamaa *et al*. (2004) for the former *Corynoptera nigrohalteralis* group in a new, redefined concept, including seven species. The genus distinguishes from other similar groups by the presence of a basal sclerotized medial stripe (septum) between the gonocoxites, the unusually long basal part and the lack of a whiplash-like seta on the gonostylus. It has a wide Holarctic distribution in Finland, Sweden, Russia, Canada, USA, Japan, Czech Republic (Vilkamaa *et al*., 2004). The distribution was extended to the Oriental region, as *D. nigrohalteralis* Rudzinski, 2008 was recorded from Taiwan, China (Rudzinski, 2008). However, there are many morphologically cryptic species within *Dichopygina*, which have similar genital structures (Vilkamaa *et al*., 2004; Rudzinski, 2008; Mohrig *et al*., 2013), as in *D. duplicis*, *D. bernhardi*, *D. ramosa* and *D. stricta*, the four species described by Vilkamaa *et al.* (2004). Mohrig *et al.* (2013) transferred *Neosciara perfecta* Pettey, 1918 to *Dichopygina*, and treated *D. bernhardi* Vilkamaa, Hippa & Komarova, 2004 as the junjor synonym of *D. perfecta* (Pettey, 1918), only conditionally based on the comparison of the poorly preserved holotype. Anyway, it is a challenge to separate *Dichopygina* species only by morphology. The treatment is doubtful.

DNA barcoding provides significant support for the rapid and accurate identification of morphologically cryptic species (Shin *et al.*, 2014). During the study of Chinese *Dichopygina*, we examined the DNA barcoded specimens from Canada and Northern Europe. Several individuals are better match with the description of *D. perfecta* and are different from the true *D. bernhardi*. In this study, morphological classification and COI barcodes are combined for identifying cryptic species.

2 Materials and methods

2.1 Taxon sampling

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Table 1. Materials morphologically examined and their COI sequences analyzed in this study, with BOLD process ID and BIN.

#	Species	Collecting sites	Process ID	BIN BOLD
1	*Dichopygina aculeata*	Canada	DARC596-11	AAH4002
2	*Dichopygina aculeata*	Norway	SCINO1252-16	AAH4002
3	*Dichopygina aculeata*	Norway	SCINO1253-16	AAH4002
4	*Dichopygina bernhardi*	China, Liaoning	SCILA007-16	AAP9901
5	*Dichopygina bernhardi*	China, Shaanxi	SCILA008-16	AAP9901
6	*Dichopygina bernhardi*	Norway	SCINO736-15	AAP9901
7	*Dichopygina bernhardi*	Canada	GMORK2686-15	AAP9901
8	*Dichopygina duplicis*	Canada	CNPKF225-14	ACK7781
10	*Dichopygina duplicis*	Canada	CNPKF597-14	ACK7781
9	*Dichopygina duplicis*	Canada	CNJAB852-12	AAH4007
11	*Dichopygina nigrohalteralis*	Norway	SCINO031-14	ACM6664
12	*Dichopygina nigrohalteralis*	Norway	SCINO192-15	ACM6664
13	*Dichopygina nigrohalteralis*	Norway	GMNWF813-14	ACM6664
14	*Dichopygina perfecta*	Canada	CNFDK107-14	ACK5904
15	*Dichopygina perfecta*	Canada	CNKOS809-14	ACK5904
16	*Dichopygina perfecta*	Finland	SCILA009-16	ADC5642
17	*Dichopygina perfecta*	Finland	SCILA010-16	ADC5642
19	*Dichopygina ramosa*	Finland	SCFI572-16*	ACD9510
18	*Dichopygina ramosa*	Finland	SCFI733-16*	ACD9510
21	*Dichopygina ramosa*	Norway	SCINO235-15	ACD9510
20	*Dichopygina ramosa*	Norway	SCINO497-15	ACD9510
22	*Dichopygina stricta*	Canada	CNEIC1928-12	ACA9566
23	*Dichopygina stricta*	Canada	CNEIC2206-12	ACJ4304
24	*Dichopygina stricta*	Canada	SSPAB4831-13	ACJ4574
25	*Dichopygina tibetana*	China, Tibet	SCILA001-16	ADB9658
26	*Dichopygina tibetana*	China, Tibet	SCILA002-16	ADB9658
27	*Dichopygina tibetana*	China, Tibet	SCILA003-16	ADB9658
28	*Dichopygina tibetana*	China, Tibet	SCILA004-16	ADB9658
29	*Dichopygina tibetana*	China, Tibet	SCILA005-16	ADB9658
30	*Dichopygina tibetana*	China, Tibet	SCILA006-16	ADB9658
31	*Dichopygina* sp.1	Canada	CNPEO837-14	ACA4507
32	*Dichopygina* sp.1	Canada	CNPEE1632-14	ACA4507
33	*Dichopygina* sp.1	Canada	CNTID1923-15	ACU6173
34	*Dichopygina* sp.1	Canada	CNWAD2010-14	ACS1671
35	*Dichopygina* sp.2	Canada	CNFDS170-14	ABA1241
36	*Dichopygina* sp.2	Canada	CNSLB025-12	ACA4748
37	*Dichopygina* sp.3	Canada	SSKUA5462-15	ACD2567
38	*Dichopygina* sp.3	Canada	CNRME3622-12	ACC7171
39	*Dichopygina* sp.4	Canada	CNKLC1238-14	AAP8780
40	*Dichopygina* sp.5	Canada	BBDCP025-10	AAN6438
41	*Dichopygina* sp.5	Canada	BBDCM738-10	AAN6438
42	*Masakimyia pustulae*	South Korea	JQ613784	ACD7488

*Materials examined by photos of genitalia.

All the sequenced materials were morphologically examined in this study, which marked by BOLD process ID of COI (Table 1). Specimens were collected by sweeping net, light trap, Malaise trap and yellow-pan trap in the field, then preserved in 100% ethanol. Eight Chinese specimens were mounted on Euparal microscope slides after DNA extraction. The

preparations were made under a Nikon SMZ1500 stereo microscope (Nikon, Tokyo, Japan). Chinese specimens were observed, measured and photographed under a Leica DM2500 microscope (Leica Camera AG, Wetzlar, Germany) with NIS-Elements D4.00.00 and Helicon Focus software®. *Dichopygina perfecta* were observed and photographed under ISO0990 microscope and MCA-510 Camera with TSView 7.1.1.3, combining ZP and GIMP software. The morphological work was done based on males only, while females were not studied. Materials and voucher specimens in this study were deposited at the Institute of Forest Protection, Zhejiang A&F University, Hangzhou, Zhejiang Province, China (ZAFU), Biodiversity Institute of Ontario, Guelph, Canada (BIOG), collection of BioFokus, Oslo, Norway (CBFO), and Alexander Koenig Museum, Bonn, Germany (ZFMK), respectively.

2.2 DNA extraction, PCR amplification, and sequencing

For Chinese materials, a DNeasy® Blood and Tissue kit (Qiagen, Inc., Hilden, Germany) was used to extract genomic DNA from single individuals. A non-destructive DNA extraction method was used following Shin *et al.* (2014). Briefly, the head, wings, legs, and genitalia were dissected and mounted on microscope slides, while the thorax and abdomen were used for DNA extraction. The cleared cuticle was mounted together with the rest of the body on the same slide as the voucher specimen. All genomic DNA samples were stored at −20°C. The primer pair, LCO1490 (5'-GGTCAACAAATCATAAAGA TATTGG-3') / HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994) was used to amplify cytochrome oxidase I (COI). The TaKaRa Taq™ (with Mg^{2+}-free buffer) system (Takara Biomedical Technology Co., Ltd., Beijing, China) was used for PCR amplification. Each reaction contained 0.13 μL TaKaRa Taq, 1.5 μL 10x buffer, 1.5 μL Mg^{2+}, 1.5 μL dNTPs, 1 μL genomic DNA template, and 0.3 μL each primer in a final volume of 15 μL. The thermal cycle parameters were as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, annealing at 45°C for 40 s, and extension at 72°C for 50 s, with a final extension at 72°C for 10 min.

For the other materials, DNA extracts and partial COI gene sequences were generated using standard primers and bi-directional Sanger sequencing with BigDye 3.1 termination at the Canadian Centre for DNA Barcoding in Biodiversity Institute of Ontario, Guelph (Heller & Rulik, 2016). All the sequences in this study were uploaded to Barcode of Life Data Systems (BOLD; http://dx.doi.org/10.5883/ DS-SCILA1) (Table 1) and deposited in GenBank under accessions KY079364 to KY079386.

2.3 Sequence alignment and phylogenetic analysis

We analyzed 658 bp of the COI sequence. The alignment was performed using MEGA version 6.0 (Tamura *et al*., 2013), as well as pairwise distances, numbers of substitutions (transitions and transversions), and measured nucleotide compositions based on the Kimura two-parameter (K2P) model (Kimura, 1980). Neighbor-joining (NJ) (Saitou & Nei, 1987) bootstrap support analysis (1,000 replicates) was performed using MEGA 6.0.

3 Results and Discussion

3.1 Species delimitation with morphological characteristics

Including *D. bernhardi*, eight species are described in this genus. *Dichopygina intermedia* (Mohrig & Krivosheina, 1982) is morphologically unique by having the dorsal mesial margin of the gonostylus subapically curved laterad and reaching the lateral margin of the gonostylus well before the apex (Vilkamaa *et al*., 2004). All the other recorded species share a different gonostylus struncture, since this margin is only gently curved and reaches the apex of gonostylus. The type species *D. nigrohalteralis* (Frey, 1948) is easily recognized by its triangular tegmen and its gonostylar megasetae placed on the mesial side of the gonostylus (SCINO031-14, SCINO192-15, GMNWF813-14). Apparently, *D. aculeata* Vilkamaa, Hippa & Komarova, 2004 differs in its gonostylus basally much broader than apically and its very long megasetae (SCINO1252-16, SCINO1253-16, DARC596-11).

However, due to the similar genital structures, the remaining species are morphologically cryptic, including *D. bernhardi*, *D. duplicis*, *D. ramosa*, *D. stricta* and *D. perfecta*. These five species share similar form of gonostylus and tegmen, their differences mainly in the number, location and length of megasetae on gonostylus. These characters may change due to different aspects of specimens prepared in the slides. Meanwhile, considerable intraspecific variations are found in individuals from different localities. Anyway, the former four species resemble each other in having a rather straight gonostylus with all megasetae at the apical half of the gonostylus. *D. bernhardi* and *D. duplicis* are similar in having the

basalmost megasetae longer than the others, while the basalmost megasetae about as long as or shorter than the others in *D. ramosa* and *D. stricta*. *Dichopygina bernhardi* (SCILA007-16, SCILA008-16, SCINO736-15, GMORK2686-15) is larger than *D. duplicis* (CNPKF225-14, CNPKF597-14, CNJAB852-12) (wing length 1.4–1.7 mm versus 1.2–1.4 mm) and also differs from *D. duplicis* in having more numerous megasetae (10–13 versus 6–8). *Dichopygina ramosa* (SCFI572-16, SCFI733-16, SCINO235-15, SCINO497-15) is distinguished in having its gonostylus apically rather tumid and all gonostylar megasetae very short. Furthermore, *D. ramosa* has a basal group of three megasetae on the gonostylus, whereas in *D. stricta* (CNEIC1928-12, CNEIC2206-12, SSPAB4831-13) the most basal megaseta is separated from and shorter than the subbasal megasetae.

For *D. perfecta*, its original description did not provide clear diagnostic characteristics. Due to the poorly preserved holotype and lack of further materials around the type locality, Mohrig *et al.* (2013) redescribed the species, treating *D. bernhardi* as a junior synonym, since *D. perfecta* having numerous gonostylar megasetae just like *D. bernhardi* (10 versus 10–13). However, here we found additional materials from Canada and Finland (CNFDK107-14, CNKOS809-14, SCILA009-16, SCILA010-16) that perfectly match with figure 28 of *D. perfecta* (Mohrig *et al*., 2013), and shows clear characteristics different from *D. bernhardi*. The species has 7–9 subapical megasetae in one group that equally in length, while it having 10–12 megasetae at the apical half of the gonostylus and the basalmost megasetae longer than others in *D. bernhardi*. Therefore, we suppose *D. bernhardi* is another species as *D. perfecta*.

Furthermore, six individuals from Tibet, China (SCILA001-16, SCILA002-16, SCILA003-16, SCILA004-16, SCILA005-16, SCILA006-16) resemble *D. bernhardi* but have fewer gonostylar megasetae (6–8 versus 10–13), larger wings (wing length 1.6–1.8 mm versus 1.4–1.7 mm), which indicate that they might to be a separate new species. However, since most described species are all very similar and at the same time intraspecifically variable, the correctness of above hypothesis and other previous identifications need to be verified in the light of the modern technique of DNA barcoding.

3.2 Species delimitation with NJ tree of COI gene

Totally, sequences from 41 *Dichopygina* samples were obtained for molecular analysis (Table 1). *Masakimyia pustulae* Yukawa & Sunose, 1976 in Cecidomyiidae was included as outgroup, since the family is related to Sciaridae based on molecular result (Ševčík *et al.*, 2016). A NJ tree based on the K2P model of DNA substitution showed that most species of the genus form well-supported, cohesive groups, which indicating an agreement between barcode identifications and morphological identifications of voucher specimens (Fig. 1, Table 1).

COI barcodes of all the recorded species were provided except *D. intermedia*, due to the lack of fresh materials. For the recorded species, the K2P distance between congeneric species was 10.43%–19.48% (average 15.01%), while that within species was 0.13%–1.57% (average 0.93%) calculated by MEGA 6.0. There is a significant ‘barcode gap’ (Hebert *et al.*, 2004). The morphologically well recognized species, *D. nigrohalteralis* and *D. aculeata* show a distance of 15.06%–19.48% and 13.71%–19.48% to the other species, respectively. Among the morphologically cryptic species including *D. duplicis*, *D. ramosa* and *D. stricta*, the three species show close genetic distance from each other of 10.43%–14.36%, smaller than the average. However, most of the cryptic species complexes, which have been studied to date, morphological differences could only be clearly detected when genetic distances were larger than 4.0% (Heller *et al.*, 2016). Accordingly, there are at least five undescribed species in the additional materials from Canada, with a nearest neighbor distance larger than 6.90%. Among them, *Dichopygina* sp.1, *Dichopygina* sp.2, and *Dichopygina* sp.3 are closely allied to this cryptic species complex. Whereas, another morphologically close species, *D. bernhardi* shows a distance larger than 11.62% to the species complex.

Our presumptive *D. perfecta* individuals (BOLD: ACK5904 and ADC5642) revealed that they were assigned to a clearly different cluster from *D. bernhardi* (BOLD: AAP9901), as shown in the molecular tree (Fig. 1), with a distance of 12.39%. Considering the NJ tree and the morphological differences, we suggest treating specimens (BIN BOLD: AAP9901) as *D. bernhardi* and the other ones (BIN BOLD: ACK5904 and ADC5642) as *D. perfecta*. For those reasons, we are again treating *D. bernhardi* **stat. rev.** as a valid species. For *D. perfecta* materials from Canada and Finland, an intraspecific distance of 1.64% is evolved between the two populations, while no obvious morphological variation observed. It appears that most *Dichopygina* species show a genetic variation, which becomes manifest in different, closely related BINs.

The morphologically different Tibet specimens (BOLD: ADB9658), being separated from the Canadian-Norwegian-Chinese cluster of *D. bernhardi* (BOLD: AAP9901), have a nearest neighbor distance of 2.67%. The subtle morphological and genetic differences may reflect adaptations to special habitat in Tibetan Plateau, indicating an incipient speciation process. However, a genetic distance of 2.67% is usually an acceptable intraspecific distance range. Therefore, considering the morphological differences and genetic distance, we are treating these Tibet specimens as a separate new species *Dichopygina tibetana* Leng, Heller & Huang, **sp. nov.**

As shown by the BOLD data (Table 1), many species of the genus *Dichopygina*, particularly *D. bernhardi* seem to

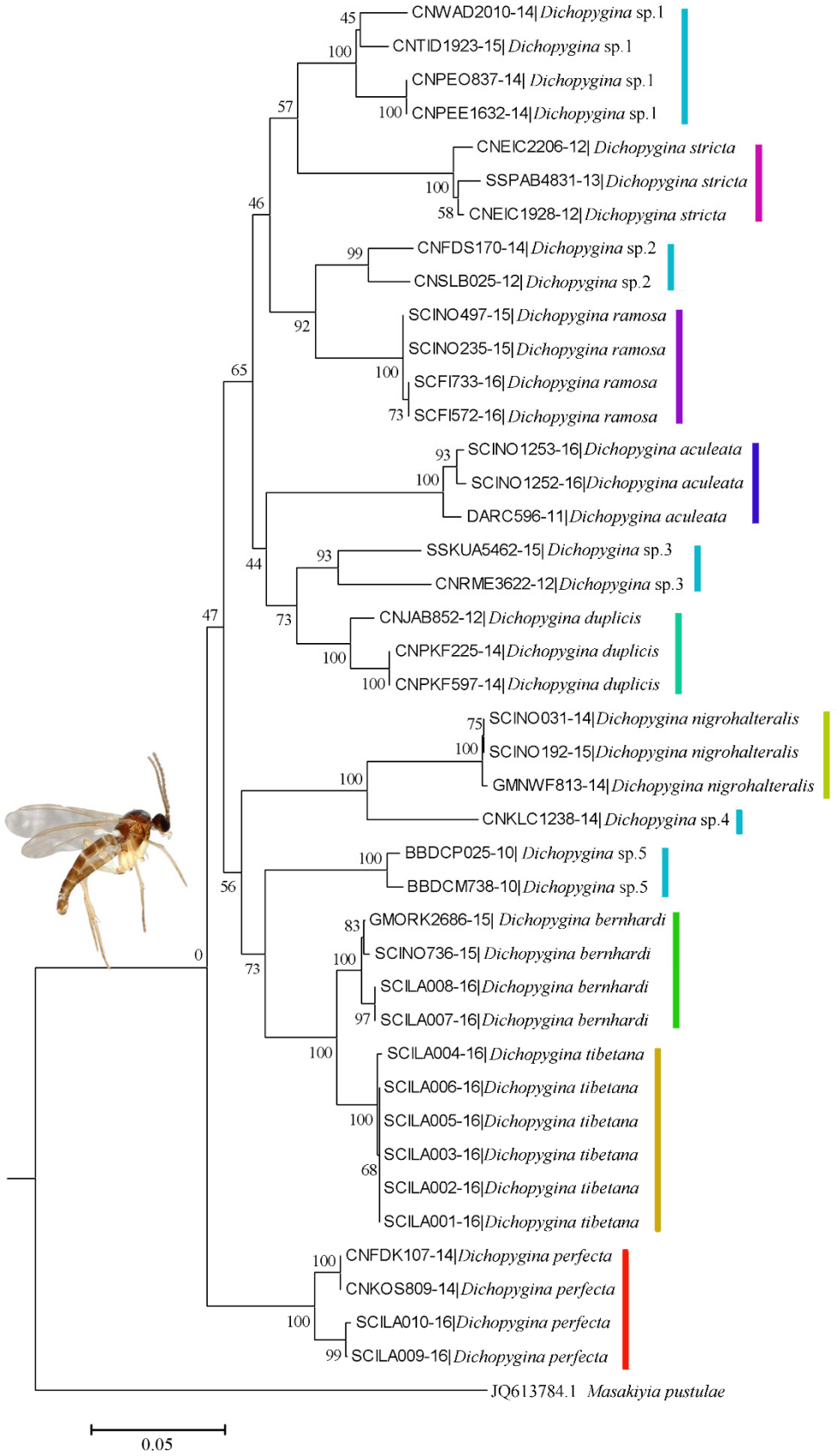


Figure 1. Neighbor Joining (NJ) tree of *Dichopygina* species COI gene. The alignment was performed based on the Kimura two-parameter (K2P) model, with substitutions of transitions and transversions. Each sequence is numbered by its BOLD process ID, with voucher materials in Table 1. The habitus photo is *Dichopygina tibetana* Leng, Heller & Huang, **sp. nov.**

have a wide range of distribution, but they are mainly restricted to the far northern latitudes of the Holarctic Region. Even our new Chinese records come from the high altitudes of the Palearctic Region. The only record from the Oriental Region remains *D. nigrohalteralis* from the high mountain in Taiwan, China (Rudzinski, 2008), from which we do not have a DNA barcode.

4 Conclusion

***Dichopygina bernhardi* Vilkamaa, Hippa & Komarova, 2004, stat. rev.**

Dichopygina bernhardi Vilkamaa, Hippa & Komarova, 2004: 110 (key), 113 (figs 5A–H), 115. Type locality: Japan, Hokkaido, Tomakomai.

Material examined. China. 1♂, Liaoning, Laotudingzi Nature Reserve, sweep net, 13 August 2015, leg. Feilong Chen (SCILA007-16) (ZAFU); 1♂, Shaanxi, Zhouzhi, Houzhenzi, Old Town of Protected Areas, Jingyang Guesthouse (33°48' 09″N, 107°44'49″E; elev. 1797 m), light trap, 19 August 2014, leg. Lan Ye (SCILA008-16) (ZAFU). Norway. 1♂, Hedmark, Elverum, S Starmoen, yellow-pan trap, 01–06 September 2014, leg. Kjell Magne Olsen (SCINO736-15) (CBFO). Canada. 1♂, Northwest Territories (65.2791°N, 126.83°W), 12 August 2014, leg. S. Behrens & R. Popko (GMORK2686-15) (BIOG).

Diagnosis. The species is distinguished by the longer basalmost megasetae with 10–13 megasetae and larger wing length of 1.4–1.7 mm (Vilkamaa *et al*., 2004).

Description. See Vilkamaa *et al.*, 2004.

Remarks. This species has a wide distribution, which was originally recorded in Japan and is new to China and Norway. The Chinese specimens examined, show some slight differences from the original description that length/width of 4th flagellomere 2.08–2.13, R1/R 0.61–0.72 in the Chinese specimen, while length/width of 4th flagellomere 1.65–1.90 and R1/R 0.75–0.95 in the type series.

Distribution. China (Shaanxi, Liaoning), Norway, Czech Republic, Sweden, Russia, Japan, Canada (Vilkamaa *et al*., 2004; Heller *et al*., 2009).

***Dichopygina perfecta* (Pettey, 1918)** (Figs 2–6)

Neosciara perfecta Pettey, 1918: 325, 341, figs 30, 61. Type locality: USA, Maryland, Montgomery Co., Plummers Island.
Bradysia perfecta (Pettey, 1918): Stone & Laffoon, 1965: 234.
Corynoptera perfecta (Pettey, 1918): Steffan, 1966: 49, 54.
Dichopygina perfecta (Pettey, 1918): Mohrig *et al.*, 2013: 200–201, fig. 28.

Material examined. Canada. 1♂, New Brunswick, Fundy National Park, Devil`s Halfacre Road (45°35'22″N, 64°57' 20″W; elev. 61 m), 21 May 2013, leg. Shirley Butland (CNFDK107-14) (BIOG); 1♂, New Brunswick, Kouchibouguac National Park, Near Park Compound, behind Research House (46°46'15″N, 65°00'23″W; elev. 61 m), malaise trap, 26 August 2013, leg. Bernard Martin (CNKOS809-14) (BIOG). Finland. 2♂, Lapland, Rovaniemi, Sorvanulkki, herb-rich, old-growth boreal forest, malaise trap, 28 July 2014, leg. Jukka Salmela (SCILA010-16, SCILA009-16) (BIOG).

Diagnosis. The morphological differences between *D. perfecta* and *D. bernhardi* are hereby confirmed. The gonostylus of *D. perfecta* has almost equal 7–9 thin and straight subapical megasetae in one group, whereas *D. bernhardi* has 10–13 megasetae at the apical half of the gonostylus and the basalmost megasetae longer than others. In addition, the tegmen of *D. perfecta* is equally rounded, while it is flatter in *D. bernhardi*.

Redescription. Colour. Thorax bright brown; abdomen, hypopygium brown; legs yellow; wing hyaline or slightly darkened. Antenna unicolour and yellowish brown.

Head. Eye bridge 2–3 facets wide. Antennal setae fine, dense, shorter than segment width. Length/width of flagellomere 4 of antenna 1.40–1.80; transition of basal part to neck pronounced (Fig. 3). Neck length/segment width 0.30–0.40. Maxillary palpus bright and 3-segmented; basal segment with 1 bristles; 2nd segment short, oval; 3rd segment as long as basal segment; sensillae present.

Thorax. Notum brown. Thoracic setae weak, white. Posterior pronotum bare. Mesothoracic sclerites bare. Wings (Fig. 4). Length 1.6–1.9 mm. bM, r-m bare; R1/R 0.60–0.80; c/w 0.63–0.72. Membrane without macrotrichia; venation weak, with faint stM; M-fork of normal shape; R1 ending clearly before base of M-fork. Halter bright. Legs. Foretibia with dense patch of setae and curved margin; claws untoothed. Hind coxa of same colour as femora. Hind tibia 0.80–0.90 mm; Tibial spurs of equal length. Abdomen. Abdominal setae weak, sparse. Tergal setae white; sternal setae white.

Hypopygium (Fig. 5). Hypopygium 0.5–0.7 times as long as wide. Base of gonocoxites with normal, weak hairs;

gonocoxites narrowly separated; inner margin of gonocoxites U-shaped; inner membrane of hypopygium bare; gonostylus elongate, narrowed and curved (Fig. 2); 1.1–1.5 times longer than wide; inner margin straight, or concave in ventral view; apical part of gonostylus tapered. Apical tooth without internal structure, shorter than subapical megasetae; 1.1–1.7 times longer than broad. Awl-like setae absent. Innerside of gonostylus with 7–9 thin and straight subapical megasetae in one group. Position of basalmost megaseta 55–67% from apex. Whiplash-hair absent. Tegmen 0.6–0.7 times as long as broad; equally rounded; without special structures; central process absent (Fig. 6). Aeadeagal apical structure and teeth absent.

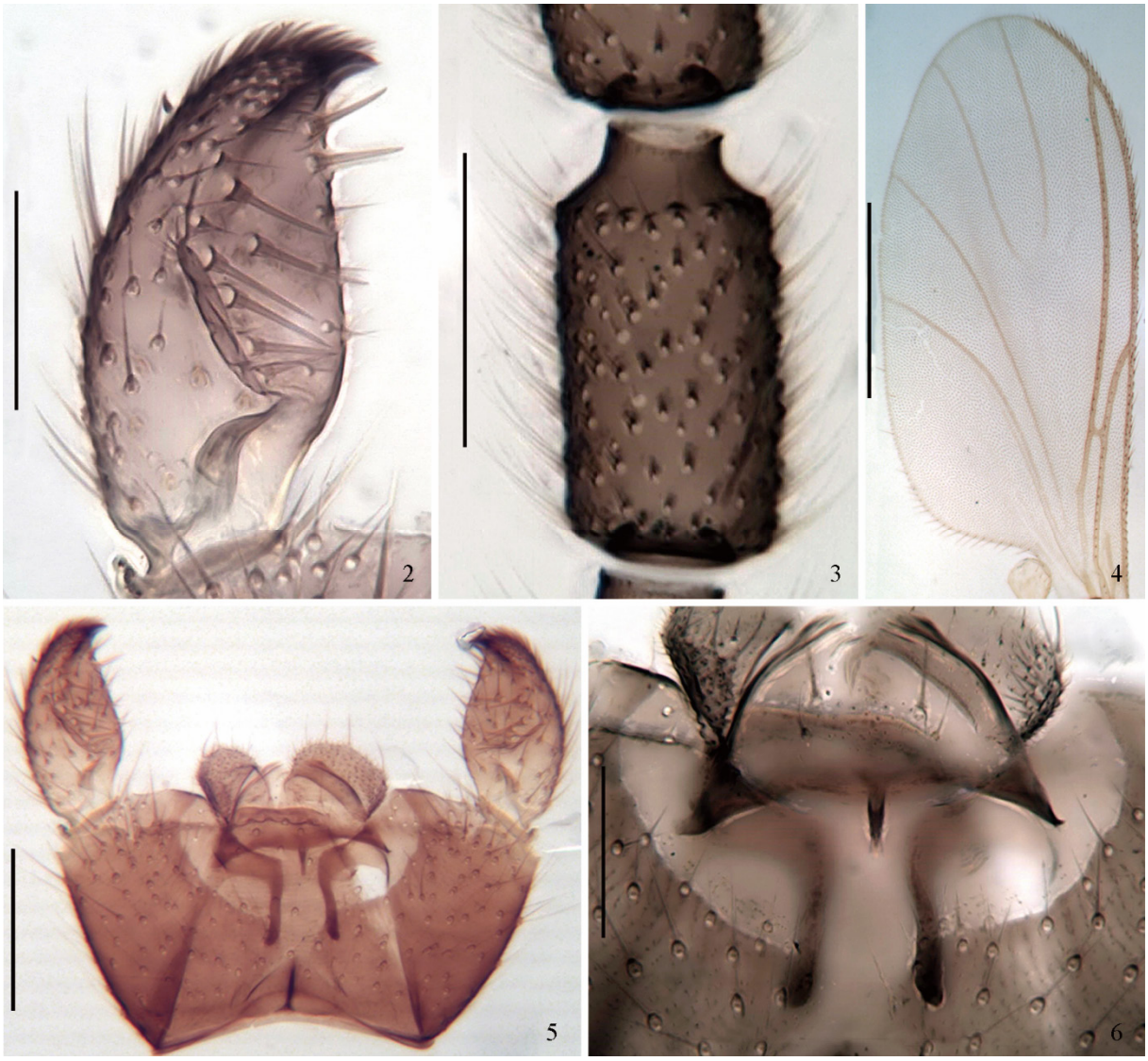
Remarks. The species is firstly recorded in Finland and Canada.

Distribution. Finland, Canada, USA (Mohrig *et al.*, 2013).

***Dichopygina tibetana* Leng, Heller & Huang, sp. nov.** (Figs. 7–12)

Material examined. Holotype, 1♂. China. Tibet, Bomi, Ganjing Guesthouse, light trap, 18 July 2014, leg. Jun Xu/Mei Qin (SM02765) (SCILA001-16) (ZAFU). Paratypes. 5♂, the same data as holotype (SM02766–67, SM02818–20) (SCILA002-16, SCILA003-16, SCILA004-16, SCILA005-16, SCILA006-16) (ZAFU].

Diagnosis. The new species and *D. bernhardi* are similar to each other in having a rather straight gonostylus with all megasetae at the apical half of the gonostylus and the flatter top of tegmen. However, *D. tibetana* differs in having larger wings (wing length 1.6–1.8 mm versus 1.4–1.7 mm) and fewer gonostylar megasetae (6–8 versus 10–13). By its number of



Figures 2–6. *Dichopygina perfecta* (Pettey, 1918), male, (BIN BOLD: ACK5904). 2. Left gonostylus, ventral view. 3. The 4th flagellomere, lateral view. 4. Wing, dorsal view. 5. Genitalia, ventral view. 6. Inner margin of gonocoxites and tegmen with aedeagus, ventral view. Scale bars: 2–3, 5–6 = 0.1 mm; 4 = 1.0 mm.

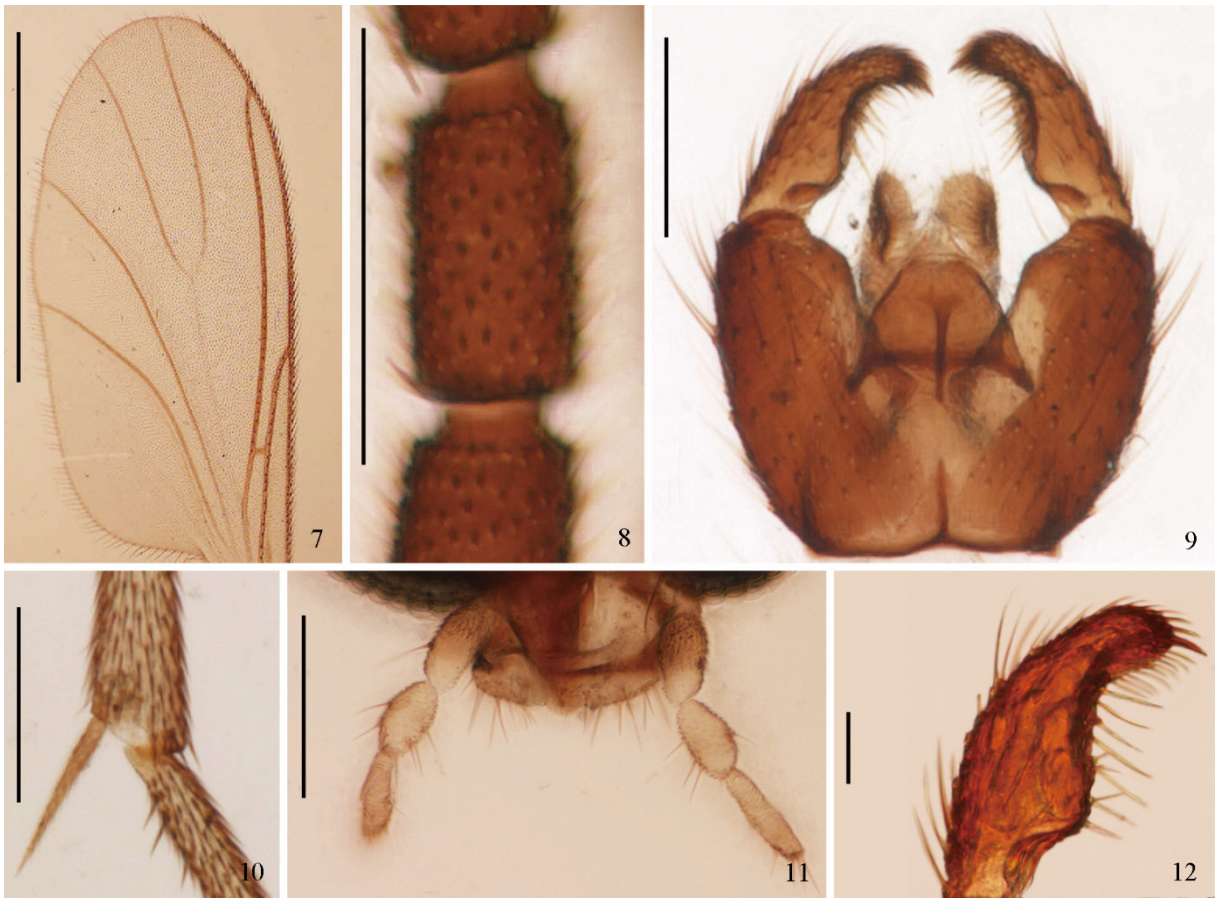
gonostylar megasetae and basalmost megasetae longer than the others, *D. tibetana* also resembles *D. duplics*. However, the new species can be distinguished by its larger wings (wing length 1.6–1.8 mm versus 1.2–1.4 mm) and broader eye bridge (facets wide 4 versus 3). Furthermore, *D. tibetana* resembles *D. perfecta* by sharing the very similar arrangement of the gonostylar megasetae, while the later species may easily be recognized by its equally rounded tegmen.

Description. Colour. Thorax, abdomen, antennae and hypopygium brown; legs, palpus and wing veins yellowish brown; wings fumose. Antenna unicolour and yellowish brown.

Head. Eye bridge 4 facets wide. Antennal setae fine, dense, shorter than segment width (Fig. 8). Length/width of flagellomere 4 of antenna 1.95–2.33; transition of basal part to neck pronounced; neck lengh/segment width 0.30–0.50, unicolour. Maxillary palpus 3-segmented, basal segment with 1 seta; 2nd segment with 6–8 setae; 3rd segment with 5–7 setae; sensillae present (Fig. 10).

Thorax. Notum brown. Thoracic setae weak, white. Anterior pronotum with 4–6 setae, episternum 1 with 4–8 setae. Wings (Fig. 7). Wing length 1.60–1.80 mm, width/length 0.43–0.46, R1/R 0.64–0.85, c/w 0.49–0.57. Membrane without macrotrichia. Venation weak, with faint stM; M-fork of normal shape; R1 ending clearly before base of M-fork; bM, r-m bare. Halter bright, short. Legs. Foreleg: foretibia (Fig. 10) with dense patch of setae and curved margin; claws untoothed; length of basitarsomere/length of foretibia 0.55–0.60; length of femur/length of metatarsus 0.56–0.86. Length of metatarsus/length of tibia: foreleg 1.17–1.48, hind leg 0.85–0.97. Length of hind tibia/length of thorax 0.95–1.16. Abdomen. Abdominal setae weak, sparse. Tergal setae white. Sternal setae white.

Hypopygium (Figs 9, 12). Hypopygium 0.6–0.8 times as long as wide. Base of gonocoxites with normal, weak hairs; gonocoxites wide and strong, narrowly separated; inner margin of gonocoxites U-shaped; inner membrane of hypopygium bare. Gonostylus elongated, narrowed and curved; 1.0–1.3 times longer than wide; inner margin straight, or concave in ventral view; apex tapered. Apical tooth without internal structure, shorter than subapical megasetae; 1.0–1.3 times longer than broad. Awl-like setae absent. With 6–8 megasetae in the apical half of the gonostylus and the basalmost megasetae



Figures 7–12. *Dichopygina tibetana* Leng, Heller and Huang, **sp. nov.**, male. 7–11. Holotype (Sample ID: SM02765; Process ID: SCILA001-16); 12. Paratype (SM02766; SCILA002-16). 7. Wing, dorsal view. 8. The 4th flagellomere, lateral view. 9. Genitalia, ventral view. 10. Apex of foretibia, prolateral view. 11. Palpus, lateral view. 12. Left gonostylus, ventral view. Scale bars: 7 = 1.0 mm; 8–12 = 0.1 mm.

longer than the others. Position of basalmost megaseta 42–44% from apex. Whiplash-hair absent. Tegmen 0.70–0.75 times as long as broad, variable in shape, from subtrapezoidal to subtriangular, without sclerotized borders, with central process. Aeadeagal with apical structure and teeth.

Distribution. China (Tibet).

Etymology. This species is named after the Chinese province of its type locality, Tibet.

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References

Frey, R. 1948. Entwurf einer neuen Klassifikation der Mückenfamilie Sciaridae. II. Die nordeuropäischen Arten. *Notulae Entomologicae*, 27: 33–92.

Hebert P.D.N., Stoeckle M.Y., Zemlak T.S., Francis C.M. 2004. Identification of birds through DNA barcodes. *PLOS Biology*, 2: 1657–1663.

Heller, K., Rulik, B. 2016. *Ctenosciara alexander-koenigi* sp. n. (Diptera: Sciaridae), an exotic invader in Germany? *Biodiversity Data Journal*, 4: e6460. doi: 10.3897/BDJ.4.e6460.

Heller, K., Vilkamaa, P., Hippa, H. 2009. An annotated check list of Swedish black fungus gnats (Diptera, Sciaridae). *Sahlbergia*, 15: 23–51.

Heller, K., Köhler, A., Menzel, F., Olsen, K.M., Gammelmo, Ø. 2016. Two formerly unrecognized species of Sciaridae (Diptera) revealed by DNA barcoding. *Norwegian Journal of Entomology*, 63: 96–115.

Kimura, M. 1980. A Simple Method for Estimating Evolutionary Rates of Base Substitutions through Comparative Studies of Nucleotide Sequences. *Journal of Molecular Evolution*, 16: 111–120.

Mohrig, W, Heller, K., Hippa, H., Vilkamaa, P., Menzel, F. 2013. Revision of the black fungus gnats (Diptera: Sciaridae) of North America. *Studia Dipterologica*, 19: 141–286.

Mohrig, W., Krivosheina, N., Mamaev, B. 1982. Beiträge zur Kenntnis der Trauermücken (Diptera, Sciaridae) der Sowjetunion. Teil II: Neue Sciariden aus Süd-Primorje. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere*, 109: 170–187.

Pettey, F.W. 1918. A revision of the genus *Sciara* of the family Mycetophilidae (Diptera). *Annals of the Entomologcial Society of America*, 11: 319–341.

Rudzinski, H.-G. 2008. Beiträge zur Trauermückenfauna Taiwans. Teil V: Gattungen *Dichopygina*, *Camptochaeta*,*Corynoptera* und *Keilbachia* (Diptera Nematocera: Sciaridae). *Entomofauna*, 29(23): 321–360.

Saitou, N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molocular Biology and Evolution*, 4: 406–425.

Ševčík, J., Kaspřák, D., Mantič, M., Fitzgerald, S., Ševčíková, T., Tóthová, A., Jaschhof, M. 2016 Molecular phylogeny of the megadiverse insect infraorder Bibionomorpha *sensu lato* (Diptera). *PeerJ*, 4: e2563. doi:10.7717/peerj.2563.

Shin, S., Jung, S., Heller, K., Menzel, F., Hong, T.K., Shin, J.S., Lee, S.H., Lee, H., Lee, S. 2014. DNA barcoding of *Bradysia* (Diptera: Sciaridae) for detection of the immature stages on agricultural crops. *Journal of Applied Entomology*, 139(8): 638–645.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30: 2725–2729.

Vilkamaa, P., Hippa, H., Komarova, L.A. 2004. The genus *Dichopygina* gen. n. (Diptera: Sciaridae). *Insect Systematics and Evolution*, 35(1): 107–120.