The identification of the species of the ‘Spilogona contractifrons’ species-group and the ‘Spilogona nitidicauda’ species-group (Diptera, Muscidae) based on morphological and molecular analysis

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Abstract. Muscid species of the ‘Spilogona contractifrons’ species-group’ (Spilogona alticola (Malloch, 1920), S. arctica (Zetterstedt, 1838), S. contractifrons (Zetterstedt, 1838), S. orthosurstyla Xue & Tian, 1988) and of the ‘Spilogona nitidicauda’ species-group’ (S. nitidicauda (Schnabl, 1911), S. hissarensis Hennig, 1959, S. imitatrix (Malloch, 1921), S. platyfrons Sorokina, 2018) are notoriously difficult to distinguish. In this paper, their morphological features are analysed, images of the male head, frons and abdomen of all the species are given, and the male terminalia are figured. The study of extensive material has shown that all the morphologically recognised species in each of these groups are valid species. An identification key is provided for both groups of species. To confirm the morphological differences, genetic differences in the cytochrome oxidase I gene of flies of the ‘Spilogona contractifrons’ species-group and of the ‘Spilogona nitidicauda’ species-group’ were analysed. It is shown that members of both groups of species have not only distinguishing morphological characters but also fixed substitutions in the DNA sequences. Since a low interspecific polymorphism is known in the Muscidae Latreille, 1802, the revealed genetic distances confirm the existence of separate species or subspecies in each of the groups studied.

Keywords. COI gene, DNA barcoding, flies, identification key, male terminalia.

Introduction

The genus Spilogona Schnabl, 1911 is one of the most speciose genera of the Muscidae Latreille, 1802 and is dominant both in the number of species and in the number of individuals in cold habitats, such
This genus has been most comprehensively studied in the Nearctic Region, in particular in the territory of northern Canada and Alaska (Huckett 1965). The number of arctic species of *Spilogona* known from the Nearctic Region is 88. The tundra zones of the Palearctic Region have not been studied for a long time, and accordingly the number of known species of *Spilogona* for these territories is significantly less (66) (Hennig 1959a, 1959b, 1959c). Recent papers on this genus have increased the number of Palearctic species to 90, whilst the number of Nearctic species has decreased to 86 (Sorokina 2018).

It should be noted that the changes in the number of species of *Spilogona* is due not only to the description of new species, but also to the synonymization of some Nearctic and Palearctic species. The reason for the latter is that most species of *Spilogona* have a circumpolar or arctic-montane distribution, but this fact was not previously taken into account by researchers when describing new species. Moreover, the genus *Spilogona* consists of small to medium-sized, dark-coloured muscids covered in greyish to blackish-brown dusting, frequently revealing paired dark markings, which makes them very similar to each other. Furthermore, the high number of species encountered in arctic situations in combination with an often poor state of preservation of specimens collected in traps, preserved in alcohol, etc., make species identification of samples of *Spilogona* a difficult task even for the specialist. There are therefore still are many problems in clarifying the taxonomic status of the various known species of *Spilogona*.

This paper is dedicated to flies of the ‘*Spilogona contractifrons* species-group’ and the ‘*Spilogona nitidicauda* species-group’. Members of these species groups are some of the most dominant in the tundra zone. Like other species of the genus, adults of these groups of species are predaceous on other small and soft-bodied insects close to running water. One of their important roles in nature is their known activity as predators on the adults and larvae of biting black flies (Simuliidae) and on other small insects (Werner & Pont 2006a, 2006b). However, adults of these species live at the same time and in the same biotopes and have very similar morphological characters, and this causes difficulty with their identification and therefore confusion in the results of field research. Moreover, there are different opinions as to the number of existing species in each of these groups of species.

The ‘*Spilogona contractifrons* species-group’ includes four morphologically very similar species: *S. contractifrons* (Zetterstedt, 1838), *S. alticola* (Malloch, 1920), *S. arctica* (Zetterstedt, 1838) and *S. orthosurstyla* Xue & Tian, 1988. The first three species are widely distributed, and consequently have given rise to discussion for many years. Opinions have varied about the presence of only two, or even one species, but not three. Collin (1930) recorded just two species, *S. contractifrons* and *S. arctica* from Greenland. Hennig (1959a: 288) and Pont (1986: 166) regarded *S. arctica* as a synonym of *S. contractifrons*. *Spilogona alticola* was previously known only from North America. In his monograph, Huckett (1965: 238) included all three as valid species but added, under the entry for *S. contractifrons*, “I am doubtful whether *alticola* Malloch can be regarded specifically as different to *contractifrons*”. Later, Michelsen (2006: 118) reinstated *S. arctica* as a valid species. In a recent paper by the first author of the present paper, four species of this group of species were reported in the Altai Mountains (Sorokina 2018).

The ‘*Spilogona nitidicauda* species-group’ included three morphologically similar species: *S. nitidicauda* (Schnabl, 1911), *S. hissarensis* Hennig, 1959 and *S. imitatrix* (Malloch, 1921). Recently, a fourth species (*S. platyfrons* Sorokina, 2018) of this group has been described (Sorokina 2018). *Spilogona hissarensis* is known only from the type series.

In the genus *Spilogona*, species limits are occasionally ambiguous and species identification based on morphological characters is difficult. In such cases, a molecular-genetic analysis can be applied. Species
identification and species delimitation are more accurate when based not only on morphological but also on molecular-genetic characters. Unfortunately, there are very few papers devoted to the DNA barcoding of the Muscidae and most of them have used the sequence data to perform phylogenetic analyses (Savage et al. 2004; Schuehli et al. 2004, 2007; Kutty et al. 2008, 2014; Haseyama et al. 2015). Nevertheless, there is one paper in which the relevance of DNA barcoding as a taxonomic tool in muscid fly identification has been established (Renaud et al. 2012).

The aim of this paper is to analyse genetic differences in the cytochrome oxidase I gene of flies of the ‘Spilogona contractifrons species-group’ and the ‘Spilogona nitidicauda species-group’, to confirm the morphological differences.

Material and methods

The material of Spilogona used in this study, including type material, is deposited in the Siberian Zoological Museum of the Institute of Systematics and Ecology of Animals, Russian Academy of Sciences, Siberian Branch, Novosibirsk (SZMN), the Zoological Museum of the Moscow State University, Moscow (ZMUM), the Zoological Institute, Saint Petersburg (ZISP), and the Canadian National Collection of Insects, Ottawa (CNC).

Morphological analyses

Specimens were examined using an Altami PSO745-T microscope for external morphological characters. For the dissection of the male and female terminalia, the end of abdomen was removed and boiled in a 10% KOH solution for 15–20 s. After dissection and study, the abdomen and terminalia were washed and then stored in microvials of glycerine pinned directly underneath the specimens. Pictures were made with the camera Canon EOS 600D. Illustrations were made in ink and then edited using Adobe Photoshop CS.

Molecular-genetics analyses

The molecular-genetic analysis was based on the study of the COI gene, the most standardized short genetic marker in an organism’s DNA that is used as a taxonomic DNA barcoding method (Ratnasingham & Hebert 2007). Total DNA was extracted from samples of Spilogona using the DIAton™ DNA Prep kit (Isogen, Russia). Polymerase chain reaction (PCR) of the cytochrome c oxidase subunit I (COI) locus was conducted with an Encyclo PCR kit (Evrogen, Russia) using TY-J-1460 (Simon et al. 1994) and COIR (Shaikevich 2007) primers. The following amplification conditions were used: initial denaturation at 94°C (5 min); 35 cycles: denaturing at 94°C (30 s), annealing at 55°C (30 s), elongation at 72°C (40 s); and final elongation at 72°C (10 min). The PCR fragments were purified from agarose gel with a Clean-Up Extraction Kit (Evrogen, Russia) and were sequenced using the BigDye Termination kit 3.1 (Applied Biosystems, USA). All sequences were deposited in the GenBank under accessions numbers MH413049–MH413064.

Specimens for genetic analysis were taken from different populations in the territories of Russia. The institution code, the voucher and the GenBank accessions number of specimens are indicated within brackets. A total of 16 specimens of 6 species of Spilogona was examined:

Spilogona alticola
**Spilogona arctica**

RUSSIA: 1 ♂, Altai Republic, Kosh-Agach district, Severo-Chuyskyi Range, valley of Aktru River, 50°06′ N, 87°48′ E, 2064 m a.s.l., 21 Jul. 2013, V. Sorokina leg. (SZMN SA9 MH413051); 1 ♂, Krasnoyarsk Krai, Taimyr Peninsula, 12.5 km to south from Dixon, the mouth of the river Lemberova, 73°24′ N, 80°39′ E, 23 Jul. 2012, A. Barkalov leg. (SZMN, SA1 MH413049); 1 ♂, Nenets AO, lower part of the river Pechora, 68°55′ N, 53°50′ E, 11 Jul. 2008, A. Ozerov leg. (SZMN SA4 MH413050).

**Spilogona contractifrons**


**Spilogona imitatrix**


**Spilogona nitidicauda**

RUSSIA: 1 ♂, Altai Republic, Kosh-Agach district, environs of Muzdy-Bulak lake, 49º17′ N, 87º39′ E, 2450 m a.s.l., 2 Jul. 2005, V. Sorokina leg. (SZMN SN38 MH413062); 1 ♂, Chukotka AO, Beringovskii district, 40 km SSW Beringovskii, 62º43′ N, 178º55′ E, 22 Jul. 2012, A. Stekolshchikov leg. (SZMN SN40 MH413063); 1 ♂, Chukotka AO, Beringovskii district, 40 km SSW Beringovskii, 62º43′ N, 178º55′ E, 22 Jul. 2012, A. Stekolshchikov leg. (SZMN SN41 MH413064).

**Spilogona platyfrons**

RUSSIA: 1 ♂, Altai Republic, Ulagan district, Shapshal’skiy mountain range, upper part of Shui River, ~50º33′ N, 89º50′ E, 2550–2900 m a.s.l., 24 Jul. 2007, V. Sorokina leg. (SZMN SP32 MH413058); 1 ♂, Altai Republic, Ust-Kokska district, 47 km W of Ust-Kokska, Krasnaya Mountains, 50º04′ N, 85º13′ E, 2000–2100 m a.s.l., 16 Jul. 2015, V. Sorokina leg. (SZMN SP33 MH413059); 1 ♂, Altai Republic, Ust-Kokska district, 47 km W of Ust-Kokska, Krasnaya Mountain, 50º04′ N, 85º13′ E, 2000–2100 m a.s.l., 16 Jul. 2015, V. Sorokina leg. (SZMN SP34 MH413060).

To investigate the genetic relationships of the isolates of *Spilogona*, Maximum Likelihood phylogenetic trees based on the COI gene nucleotide data were reconstructed; values lower than 50% were eliminated. A matrix of pairwise distances were estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved 38 nucleotide sequences and there was a total of 408 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

The following abbreviations are used in the text: a = anterior; acr = acrostichal; ad = anterodorsal, av = anteroventral; d = dorsal; dc = dorsocentral; p = posterior, pd = posterodorsal; ph = posthumeral, post = postsutural; ps = presutural; pv = posteroventral; v = ventral; AO = Autonomous Okrug; ML = Maximum Likelihood.

The citation of some original labels is given in quotation marks, but interpretations of these data are given in square brackets.
Results

Taxonomic analysis of ‘Spilogona contractifrons species-group’

Flies of this group of species can be distinguished as follows: scutum with 3 post dc setae, mid tibia without pv and with 1 ad seta, fore tibia with 1 p seta, hind femur with a row of pv setae, mid femur with anterior preapical seta, haltere yellow, facial edge not projecting beyond level of profrons, prementum shining, scutellum without downwardly-directed preapical setulae on upper border of declivities, abdomen plump, katepisternal setae arranged in a more or less equilateral triangle like Coenosia Meigen, 1826.

Four species of this group are currently recognised: the widespread S. alticola, S. arctica, S. contractifrons, and the East Asian S. orthosurstyla. Research for the current paper has included a detailed study of extensive material from the territory of Russia, from both tundra zones and mountain areas, and personal study of the bulk of Huckett’s collection deposited in the Canadian National Collection, Ottawa, Canada.

The study of extensive material has confirmed the existence of four species in this group. Despite the difficulties in the recognition of species of this group, there are several helpful diagnostic characters: the shape of male sternite 5, the shape of male terminalia, and the colour of the scutum (Figs 1–2, Table 1). The colour of the scutum can be variable in different populations but the shape of the male sternite 5 is constant and is clearly visible in dry specimens (Fig. 1 G–I).

At present it is impossible to separate females of these species. Males can be distinguished by the following couplets:

1. Scutum completely dark brown (mountain populations) or light grey with indistinct stripes (arctic tundra populations); sternite 5 with a narrow median notch (Fig. 1E, H), with a wavy inner edge; in lateral view hypandrium with a protuberance; surstylius sharply curved at middle (Fig. 1K) ................................................................. S. arctica (Zetterstedt, 1838)

− Scutum grey or brownish dusted with more or less distinct dark stripes; sternite 5 with a rather wide median notch; hypandrium without a distinct protuberance; shape of surstylius not as above .......... 2

2. Sternite 5 with a wide rectangular median notch, inner edge more or less straight (Fig. 1F, I); scutum with distinct dark stripes; surstylius short, gently curved, about as long as cerci (Fig. 1L) .............................................................................................................. S. alticola (Malloch, 1920)

− Sternite 5 not as above; scutum with indistinct dark stripes, often diffuse; surstylius longer than cerci ........................................................................................................................................ 3

3. Sternite 5 with an extended and pointed caudal margin (Fig. 2C); surstylius straight, curved only at apex (Fig. 2A) ................................................................. S. orthosurstyla Xue & Tian, 1988

− Sternite 5 with a rounded caudal margin (Fig. 1D, G); surstylius not straight, weakly curved at middle (Fig. 1J) ........................................................................ S. contractifrons (Zetterstedt, 1838)
Fig. 2. *Spilogona orthosurstyla* Xue & Tian, 1988. A. Terminalia, lateral view. B. Terminalia, dorsal view. C. Sternite 5. Scare bars: 0.25 mm.
**Table 1.** Diagnostic characters of males of ‘*Spilogona contractifrons* species-group’.

<table>
<thead>
<tr>
<th></th>
<th><em>S. alticola</em> (Malloch, 1920)</th>
<th><em>S. arctica</em> (Zetterstedt, 1838)</th>
<th><em>S. contractifrons</em> (Zetterstedt, 1838)</th>
<th><em>S. orthosurstyla</em> Xue &amp; Tian, 1988</th>
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<tbody>
<tr>
<td>Colour of scutum</td>
<td>grey and brown dusted with</td>
<td>completely dark brown (mountains)</td>
<td>grey or brownish-grey dusted with brown stripes, sometimes diffuse (Fig. 1A)</td>
<td>brown dusted with indistinct dark stripes</td>
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<tr>
<td></td>
<td>distinct dark stripes (Fig. 1C)</td>
<td>or grey dusted (arctic tundra) with indistinct dark stripes (Fig. 1B)</td>
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<tr>
<td>Shape of sternite 5</td>
<td>with a wide median notch,</td>
<td>with a narrow median notch, inner edge wavy, with a rounded caudal margin (Fig. 1E, 1H)</td>
<td>with a fairly wide median notch, the inner edge strongly curved, with a rounded caudal margin (Fig. 1D, 1G)</td>
<td>with a fairly wide median notch, the inner edge</td>
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<td></td>
<td>inner edge more or less straight,</td>
<td>a rounded caudal margin (Fig. 1F, 1I)</td>
<td>not curved, with extended and pointed caudal margin (Fig. 2C)</td>
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<tr>
<td></td>
<td>with a rounded caudal margin (Fig. 1F, 1I)</td>
<td></td>
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<tr>
<td>Shape of surstylus</td>
<td>short, gently curved (Fig. 1L)</td>
<td>sharply curved at middle (Fig. 1K)</td>
<td>weakly curved at middle (Fig. 1J)</td>
<td>straight, curved only at apex (Fig. 2A)</td>
</tr>
<tr>
<td>Shape of hypandrium</td>
<td>without processes, or with a small tubercle, narrow</td>
<td>with protuberance (Fig. 1K)</td>
<td>without protuberance</td>
<td>without protuberance</td>
</tr>
</tbody>
</table>

**Spilogona alticola** (Malloch, 1920)  
Fig. 1C, F, I, L  

**Type locality**  
USA: New Hampshire.

**Material examined**  
CANADA: 2 ♂♂, Manitoba, 4 km S Churchill, Beach Bay, 58°45′ N, 94°08′ W, Malaise trap, 17–21 Jul. 2007, A. Renaud leg. (SZMN); 1 ♀, same data as for preceding except: pan traps, 25–29 Jul. 2007 (SZMN); 1 ♀, Manitoba, Goose Creek Rd., Pump House, 58°38′ N, 94°14′ W, Malaise trap, 07–11 Aug. 2007, A. Renaud leg. (SZMN).

RUSSIA: 14 ♂♂, Altai Republic, Kosh-Agach district, Ukok plateau, environs of Muzdy-Bulak lake, 49°17′ N, 87°39′ E, 2400–2900 m a.s.l., sweep in vegetation, 26 Jun. 2005, 1 Jul. 2005, A. Barkalov and V. Sorokina leg. (SZMN); 2 ♂♂, Altai Republic, Kosh-Agach district, Ukok plateau, Rodonovy spring, upper part of Zhumaly river, 49°27′ N, 88°03′ E, 2410 m a.s.l., sweep in vegetation, 24 Jun. 2005, V. Sorokina leg. (SZMN); 13 ♂♂, Altai Republic, Kosh-Agach district, Ukok plateau, environs of
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Kal’dzhin-Kul’-Bas lake, 49°19′ N, 87°26′ E, 2400–2600 m a.s.l., sweep in vegetation, 20 Jul. 2006, V. Sorokina and T. Novgorodova leg. (SZMN); 1 ♂, Chukotka AO, lower part of the Anadyr river, 64°50′ N, 175°58′ E, 5 m a.s.l., 20 Jul. 2013, A. Barkalov leg. (SZMN).

Distribution

Holarctic Region; in the Palearctic, from Southern Siberia to the north of the Russian Far East. Arcto-alpine species.

*Spilogona arctica* (Zetterstedt, 1838)

Fig. 1B, E, H, K

Type locality

GREENLAND.

Material examined

CANADA: 1 ♂, Manitoba, Goose Creek Rd., Pump House, 58°38′ N, 94°14′ W, Malaise trap, 10–14 Jul. 2007, A. Renaud leg. (SZMN).


A. Stekolshchikov leg. (SZMN); 8 ♂♂ 8 ♀♀, Chukotka AO, lower part of the Anadyr river, 64°26′ N, 175°53′ E, 5 m a.s.l., 18, 24–25 Jul. 2013, A. Barkalov leg. (SZMN); 7 ♂♂ 2 ♀♀, Chukotka AO, 30 km NW Krasneno, Anadyr river, 64°46′ N, 174°8′ E, 1 Aug. 1991, V. Pekin leg. (SZMN); 1 ♂, “Chita region” [Zabaykalsky Krai], Sokhondo Nature Reserve, 49°41′ N, 174°8′ E, 1350–1400 m a.s.l., 2 Jun. 1991, V. Pekin and D. Logunov leg. (SZMN); 7 ♂, Krasnoyarsk Kray, environs of Norilsk, Talnakh, 69°21′ N, 87°45′ E, birch forest, 10, 12 Jul. 1967, K. Gorodkov leg. (ZISP); 4 ♂ 1 ♀, same data as for preceding except: in village, 22 Jul. 1973; 1 ♂, Krasnoyarsk Kray, upper part of Pura river [left tributary of Pyasina river], 10 km NW of Berezmu lake [71°51′ N, 87°30′ E], willow in bank of river, 21 Jul. 1967, K. Gorodkov leg. (ZISP); 6 ♂ 1 ♀, Krasnoyarsk Kray, Taymyr Peninsula, 90 km S of Tareya village, Pyasina river, [72°25′ N, 90°56′ E], willow in bank of river, 23 Jul. 1967, K. Gorodkov leg. (ZISP); 4 ♂ 1 ♀, Krasnoyarsk Kray, Taymyr Peninsula, 2.5 km S of Dikson, 73°24′ N, 80°39′ E, 26 Jul. 2012, A. Barkalov leg. (SZMN); 8 ♂ 4 ♀, Krasnoyarsk Kray, Taymyr Peninsula, 20 km NW of Khatanga, Ary-Mas station, 73°03′ N, 101°56′ E, 14 m a.s.l., 10–22 Jul. 2010, A. Barkalov leg. (SZMN); 2 ♂♂, Magadan region, Ust'-Omchug, 61°08′ N, 149°39′ E, 2 Sep. 1987, K. Gorodkov leg. (ZISP); 4 ♂, same data as for preceding except: larch woodlands, 1 Jul. 1971; 2 ♀♀, same data as for preceding except: bog, 1 Aug. 1971, K. Gorodkov and Chelnokov leg. (ZISP); 1 ♂, Magadan region, 20 km S of Gizhiga village, “Chaibukha aerodrome”, [61°50′ N, 160°32′ E], 2 Sep. 1987, K. Gorodkov leg. (ZISP); 1 ♂, Magadan region, environs of Magadan, Marchekan, 59°32′ N, 150°47′ E, 28 Aug. 1969, K. Gorodkov leg. (ZISP); 1 ♂ 1 ♀, Magadan region, the motorway R-504, Yablonevyi Pass, 120 km NNE of Magadan, 60°35′ N, 151°32′ E, 900 m a.s.l., 17 Jul. 2014, N. Vikhrev leg. (ZMUM); 6 ♂, Nenets AO, lower part of the Pechora river, 68°20′ N, 53°18′ E, 11 Jul. 2008, A. Ozerov leg. (2 ♂♂ ZMUM, 4 ♂♂ SZMN); 2 ♀♀, Yamalo-Nenets AO, 73 km NE of Labytnangi, Longot'egan stream, 66°43′ E, 175 m a.s.l., 6, 22 Jul. 2015, A. Barkalov and V. Zinchenko leg. (SZMN).


USA: 1 ♂ 1 ♀, Alaska, Aleutian Islands, Chowiet Island, 56°02′ N, 156°44′ W, 107 m a.s.l., sweep in grass meadow, 10 Jul. 2009, A. Renaud leg. (SZMN).

Distribution

Holarctic Region; in the Palaearctic, from Greenland and Northern Europe to the Russian Far East. Arcto-alpine species.

Spilogona contractifrons (Zetterstedt, 1838)

Fig. 1A, D, G, J

Type locality

SWEDEN: “Lapland”.

Material examined

CANADA: 1 ♂, Manitoba, Goose Creek Rd., Pump House, 58°38′ N, 94°14′ W, Malaise trap, 17–21 Jul. 2007, A. Renaud leg. (SZMN); 1 ♂, Manitoba, 4 km S of Churchill, Beach Bay, 58°45′ N, 94°08′ W, sweep in vegetation, 24 Jul. 2007, A. Renaud leg. (SZMN); 1 ♀, Manitoba, Churchill, 12 km E on Launch road, 58°45′ N, 93°59′ W, Malaise trap, 3–7 Jul. 2007, A. Renaud leg. (SZMN); 1 ♀, Manitoba, 24 km E of Churchill, Ramsay Creek, 58°44′ N, 93°47′ W, Malaise trap, 25–29 Jul. 2007, A. Renaud leg. (SZMN).
RUSSIA: 1 ♂, Altai Republic, Kosh-Agach district, Ukok plateau, environs of Kal’dzhin-Kul’-Bas lake, 49°19′ N, 87°26′ E, 2450 m a.s.l., 21 Jul. 2006. V. Sorokina leg. (SZMN); 2 ♂♂, Altai Republic, Kosh-Agach district, Rodonovy spring, upper part of Zhumaly river, 49°27′ N, 88°03′ E, 2410 m a.s.l., 4 Jul. 2005, 23–24 Jul. 2006. T. Novgorodova and V. Sorokina leg. (SZMN); 1 ♂, Altai Republic, Kosh-Agach district, Severo-Chuysky Ridge, valley of Aktru River, 50°06′ N, 87°48′ E, 2064 m a.s.l., 21 Jul. 2013, V. Sorokina leg. (SZMN); 2 ♀♀, “Chita region” [Zabaykalsky Krai], 6 km N, of Teli Mountain, Toreyskie lakes, 50°06′ N, 115°41′ E, steppe, 6 Jul. 2011. V. Dubatolov leg. (SZMN); 1 ♂, Kurgan region, environs of Kurgan, 55°30′ N, 66°19′ E, sweep in grass meadow, 17 Jun. 2015. V. Sorokina leg. (SZMN); 1 ♂, Magadan region, Ust’-Oschug, 61°08′ N, 149°39′ E, 1 Jul. 1971, Gorodkov leg. (ZISP); 1 ♀ 1 ♂, Magadan region, 20 km S of Gizhiga village, “Chaubukha aerodrome” [−61°50′ N, 160°32′ E], valley of Chaubukha River, 7 km from mouth of river, 2 Sep. 1987, Gorodkov leg. (ZISP); 1 ♂, Magadan region, Aborigen field station, 61°20′ N, 149°23′ E, 500 m a.s.l., 5–10 Aug. 1990, M. Wood leg. (SZMN); 10 ♂♂ 3 ♀♀, Magadan region, environs of Sokol village, 59°07′ N, 150°45′ E, 11–19 Jul. 2014, N. Vikhrev leg. (1 ♀ 1 ♂ in SZMN, rest in ZMUM); 1 ♂, Magadan region, 92 km NE of Magadan, valley of Donyshko river, 60°20′ N, 151°23′ E, 570 m a.s.l., 17 Jul. 2014, N. Vikhrev leg. (ZMUM); 1 ♂, Magadan region, Koni Peninsula, environs of Cape Ploskyi cordon, valley of Khindzha river, 59°09′ N, 151°38′ E, 29 Jun. 2016, N. Tridrikh leg. (SZMN); 2 ♀♀, same data as for preceding except: birch copse on the slope, 22 Aug. 2017; 1 ♀, Magadan region, Koni Peninsula, Cape Skalistyi, valley of Skalistaya river, 59°07′ N, 150°23′ E, yellow plate traps, 23 Aug. 2017, N. Tridrikh leg. (SZMN); 1 ♂ 2 ♀♀, Nenets AO, Khaypudyrskaya Bay, “Chuba”, 68°16′ N, 59°57′ E, yellow plate trap, meadow, 6–16 Aug. 2015, O. Makarova and Bizin leg. (SZMN); 2 ♀♀ 2 ♂♂, Nenets AO, Pakhancheskaya Bay, 68°29′ N, 57°26′ E, yellow plate trap, tundra, 26 Jul.–5 Aug. 2015, O. Makarova and Bizin leg. (SZMN); 1 ♂ 1 ♀, Nenets AO, Bolvanskaya Bay, 68°05′ N, 54°47′ E, yellow plate trap, anthropogenic meadow, 18–25 Jul. 2015, O. Makarova and Bizin leg. (SZMN); 1 ♂ 4 ♀♀, Novosibirsk region, Zdvinsk district, environs of Shirokaya Kurya village [54°34′ N, 78°08′ E], field station, 8 Jul. 2002, Zavarukhina leg. (SZMN).


Distribution
Holarctic Region; in the Palaearctic, from Northern Europe to the Russian Far East. Arcto-boreal-mountain species.

**Spilogona orthosurstyla** Xue & Tian, 1988
Fig. 2A–C

Type locality
CHINA: Hebei.

Material examined
RUSSIA: 1 ♂, Altai Republic, Turochak district, 30 km SW of Iogach, 51°31′ N, 87°23′ E, 1600 m a.s.l., 21 Jun. 2003, V. Sorokina leg. (SZMN).

Distribution
East Palaeartic: China (Hebei, Shanxi), Russia (Altai Mts). East Asian mountain species.

Remark
In view of the distribution of other species of this group, we can assume that this species also lives in the arctic zone.
Molecular-genetic analysis of ‘Spilogona contractifrons species-group’

Since in our collection there is only one specimen of *S. orthosurstyla* and it is not in good condition, the molecular analysis was performed only on the other three species of this group.

Genetic distances between species are shown in the ML tree (Fig. 3) and in Table 2. Regardless of the geographic origin of the specimens, all three studied species in this group have identical *COI* gene sequences within the species. At the same time, at least five nucleotide substitutions (single nucleotide polymorphisms (SNPs)) vary between *S. alticola* and *S. contractifrons*. *Spilogona arctica* differs from *S. alticola* with 13 SNP and from *S. contractifrons* with 12 SNP; one substitution results in amino acid change (M112L).

The genetic distance between *S. alticola* and *S. contractifrons* is 0.6%, and between each of these species and *S. arctica* is 1.8%. All three species formed a distinct cluster on the ML tree with high (98–99%) statistical support in which the associated taxa clustered together (Fig. 3).

The following results were obtained after comparing the sequences of the studied specimens with the annotated sequences in Genbank. *Spilogona arctica* from the Altai, Taimyr and from the Nenets AO is identical with *S. arctica* from Sweden. All of them are distinguished by a single substitution of C438T from *S. arctica* from Canada and Greenland (*Supplementary file*). *Spilogona contractifrons* from the Altai, Nenets AO, Kurgan and Naryan-Mar is identical in the *COI* gene with *S. contractifrons* from Norway and Sweden. But *S. alticola* from the Altai clustered with *S. contractifrons* from Canada (Fig. 3).

### Taxonomic analysis of ‘Spilogona nitidicauda species-group’

Flies of this group of species can be distinguished as follows: scutum with 4 post dc setae, anepisternum with interspatial setae, facial edge not projecting beyond level of profrons, hind femur without pv setae, scutellum without downwardly-directed preapical setulae on upper border of declivities, abdomen flattened with large trapezoidal marks on tergite 3.

Four species of this group are currently recognised: *S. nitidicauda*, *S. hissarensis*, *S. platyfrons* and *S. imitatrix*. *Spilogona hissarensis* was studied only from the original type-series. The other species of this group were studied with material from the territory of Russia and from Hackett’s collection deposited in the Canadian National Collection, Ottawa, Canada.

Despite the difficulty in distinguishing the species of this group, there are several helpful diagnostic characters: the width of frons and parafacial, presence of pollen on the prementum, the colour of the scutum and abdomen, the shape of male terminalia (Figs 4–5). Additional diagnostic characters are given in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. alticola</em> (Malloch, 1920)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>S. contractifrons</em> (Zetterstedt, 1838)</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>S. arctica</em> (Zetterstedt, 1838)</td>
<td>0.018</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>S. imitatrix</em> (Malloch, 1921)</td>
<td>0.046</td>
<td>0.039</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>S. nitidicauda</em> (Schnabl, 1911)</td>
<td>0.057</td>
<td>0.051</td>
<td>0.053</td>
<td>0.016</td>
</tr>
<tr>
<td>6</td>
<td><em>S. platyfrons</em> Sorokina, 2018</td>
<td>0.055</td>
<td>0.049</td>
<td>0.049</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*Table 2.* Estimates of evolutionary divergence between studied species of *Spilogona* Schnabl, 1911. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. The analysis involved 16 nucleotide sequences.
Fig. 3. Maximum Likelihood phylogenetic tree shows evolutionary relationships of species of *Spilogona* Schnabl, 1911.
### Table 3. Diagnostic characters of the ‘Spilogona nitidicauda species-group’.

<table>
<thead>
<tr>
<th>Character</th>
<th>S. nitidicauda (Schnabl, 1911)</th>
<th>S. hissarensis Hennig, 1959</th>
<th>S. imitatrix (Malloch, 1921)</th>
<th>S. platyfrons Sorokina, 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frons</td>
<td>fronto-orbital plates separated by a distinct frontal vitta (Fig. 4E)</td>
<td>fronto-orbital plates separated by a very narrow frontal vitta</td>
<td>fronto-orbital plates touching in upper part or separated by a very narrow frontal vitta (Fig. 4D)</td>
<td>fronto-orbital plates touching for greater part of length, frons very narrow (Fig. 4F)</td>
</tr>
<tr>
<td>Distance between eye margins</td>
<td>as wide as distance across posterior ocelli shining</td>
<td>narrower than distance across posterior ocelli shining</td>
<td>narrower than distance across posterior ocelli shining</td>
<td>as wide as diameter of anterior ocellus shining, with a patch of light dust centrally on underside</td>
</tr>
<tr>
<td>Prementum</td>
<td>shining</td>
<td>shining</td>
<td>shining</td>
<td>shining</td>
</tr>
<tr>
<td>Notopleuron</td>
<td>bare</td>
<td>bare</td>
<td>with some hairs in upper part, but not around posterior setae</td>
<td>bare</td>
</tr>
<tr>
<td>Interspatial setae</td>
<td>one</td>
<td>two</td>
<td>one, two or three</td>
<td>one or two</td>
</tr>
<tr>
<td>Scutum (in frontal view)</td>
<td>anterior half of presutural region with grey dust, sometimes without distinct dust</td>
<td>without grey dust</td>
<td>presutural region with grey dust, sometimes without distinct dust</td>
<td>whole presutural region with whitish-grey dust</td>
</tr>
<tr>
<td>Halteres</td>
<td>yellow</td>
<td>dark brown</td>
<td>yellow</td>
<td>yellow, sometimes brownish-yellow</td>
</tr>
<tr>
<td>Mid tibia</td>
<td>1 ad and sometimes v present</td>
<td>without ad and v</td>
<td>without ad and v</td>
<td>without ad and v</td>
</tr>
<tr>
<td>Mid femora</td>
<td>2–4 stout pv-v</td>
<td>5–6 stout pv-v</td>
<td>2–4 stout pv-v</td>
<td>5–6 stout pv-v</td>
</tr>
<tr>
<td>Hind femora (apical part)</td>
<td>3–4 av</td>
<td>5–6 av</td>
<td>usually 3–4 av</td>
<td>5–6 av</td>
</tr>
<tr>
<td>Abdomen</td>
<td>marks separated (Fig. 4H)</td>
<td>marks narrowly separated</td>
<td>usually marks of tergite 3 connected, sometimes narrowly separated (Fig. 4G)</td>
<td>marks narrowly separated (Fig. 4I)</td>
</tr>
<tr>
<td>Sternite 5</td>
<td>processes without dense setulae (Fig. 5H)</td>
<td>processes without dense setulae (Fig. 5G)</td>
<td>processes with dense setulae (Fig. 5G)</td>
<td>processes without dense setulae (Fig. 5I)</td>
</tr>
<tr>
<td>Cercal plate</td>
<td>oval in shape (Fig. 5E)</td>
<td>triangular in shape (Fig. 5G)</td>
<td>Trapezoid in shape (Fig. 5D)</td>
<td>triangular in shape (Fig. 5F)</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prementum</td>
<td>shining</td>
<td>unknown</td>
<td>shining</td>
<td>shining, with patch of light dust centrally on underside</td>
</tr>
<tr>
<td>Notopleuron</td>
<td>bare</td>
<td>unknown</td>
<td>with some hairs in upper part, but not around posterior setae</td>
<td>usually bare, sometimes with several hairs in upper part</td>
</tr>
<tr>
<td>Interspatial setae</td>
<td>one</td>
<td>usually two or one</td>
<td>usually one or two</td>
<td>usually one or two</td>
</tr>
<tr>
<td>Mid femora</td>
<td>1 stout pv-v in basal ¼ and 1 extra hair-like seta directly at base</td>
<td>1 long stout pv-v in basal ¼ and 1–2 extra stout or hair-like setae directly at base</td>
<td>row of 3–5 hair-like pv-v in basal 1/2 (one seta stouter)</td>
<td></td>
</tr>
</tbody>
</table>
The species of this group can be separated by the following couples:

**Males**

1. In frontal view, presutural region of scutum without grey dust; haltere dark brown ......................
   ..................................................................................................................................................\textit{S. hissarensis} Hennig, 1959
   – In frontal view, presutural region of scutum with grey dust; haltere yellow or brownish-yellow .... 2

2. Fronto-orbital plates touching along the greater part of their length, narrower than diameter of anterior ocellus; distance between eye margins on upper part of frons about as wide as diameter of anterior ocellus; prementum shining but with a patch of light dust centrally on underside; presutural region mostly with whitish-grey dust; mid femur with 5–6 long and stout $pv-v$ in basal half; hind femur with 5–6 $av$ at apex ..........................\textit{S. platyfrons} Sorokina, 2018
   – Fronto-orbital plates touching only in upper part or separated by a narrow frontal vitta; distance between eye margins on upper part of frons as wide as or narrower than the distance between posterior ocelli; prementum completely shining, without any dust; extreme anterior half of presutural region with grey dust; mid femur with 2–4 stout $pv-v$ in basal half; hind femur with 3–4 $av$ at apex ....... 3

3. Notopleuron with some hairs in upper part, but not around posterior seta; fronto-orbital plates touching in upper part or separated by a very narrow frontal vitta; mid tibia without $ad$ and $v$; dark marks on tergite 3 usually connected or narrowly separated; caudal margin of sternite 5 with dense setulae .................................................................\textit{S. imitatrix} (Malloch, 1921)
   – Notopleuron completely bare; fronto-orbital plates usually separated by a distinct frontal vitta, sometimes touching; mid tibia with 1 $ad$ and sometimes a short $v$ present; tergites with well separated marks; caudal margin of sternite 5 without dense setulae ..............\textit{S. nitidicauda} (Schnabl, 1911)

**Females**

1. Prementum of proboscis shining but with a patch of light dust centrally on underside; mid femur with a row of 3–5 hair-like $pv-v$ in basal $\frac{1}{2}$, sometimes one of them stouter ................................\textit{S. platyfrons} Sorokina, 2018
   – Prementum of proboscis completely shining; mid femur with 1 stout $pv-v$ in basal $\frac{1}{4}$ and 1–2 additional stout or hair-like setae directly at base ......................................................... 2

2. Notopleuron completely bare; anepisternum with 1 interspatial seta; mid tibia usually with $ad$; mid femur with 1 stout $pv-v$ in basal $\frac{1}{4}$ and 1 additional hair-like seta .......\textit{S. nitidicauda} (Schnabl, 1911)
   – Notopleuron with some hairs in upper part; usually anepisternum with 2 interspatial setae, rarely with one; mid tibia without $ad$; mid femur with 1 long stout $pv-v$ in basal $\frac{1}{4}$ and 1–2 additional stout or hair-like setae .........................................................\textit{S. imitatrix} (Malloch, 1921)

**\textit{Spilogona hissarensis}** Hennig, 1959

**Type locality**

TAJIKISTAN: Varzob.

**Material examined**

**Holotype**

Paratype

Distribution
Central Palaearctic: Tajikistan.

Remark
Mountain species, but probably living in the arctic tundra too.

Spilogona imitatrix (Malloch, 1921)
Figs 4A, D, G, 5A, D, G

Type locality
CANADA: Labrador, Nain.

Material examined
CANADA: 2 ♀♀, Manitoba, 24 km E of Churchill, Pistol range, 58°45′ N, 93°56′ W, sweep in vegetation, 12 Jul. 2007, A. Renaud leg. (SZMN).

RUSSIA: 1 ♂, Altai Republic, Kosh-Agach district, upper part of Naryn-Gol River, 49°49′ N, 89°32′ E, 2520 m, 15–18 Jul. 2009, T. Novgorodova leg. (SZMN); 10 ♂♂ 6 ♀♀, Altai Republic, Kosh-Agach district, 7 km NW of Kuray, south slope of Kurayskii mountain ridge, 50°18′ N, 87°51′ E, 2588 m a.s.l., 17, 19 Jul. 2013, V. Sorokina leg. (SZMN); 5 ♂♂ 1 ♀, Altai Republic, Kosh-Agach district, Ukok plateau, Rodonovy spring, upper part of Zhumal River, 49°27′ N, 88°03′ E, 2410 m a.s.l., 24 Jun. 2005, V. Sorokina leg. (SZMN); 6 ♂♂ 2 ♀♀, Altai Republic, Kosh-Agach district, 4 km NW of Kal’dzhin-Kul’-Bas lake, 49°19′ N, 87°26′ E, 2600 m a.s.l., 12 Jul. 2007, A. Barkalov leg. (SZMN); 1 ♂ 1 ♀, Altai Republic, Kosh-Agach district, 6–8 km NW of Maitobe Mountain, 49°34′ N, 87°43′ E, 2400–2700 m a.s.l., 8–10 Jul. 2006, T. Novgorodova leg. (SZMN); 6 ♂♂ 1 ♀, Altai Republic, Ongudai district, Terektsinskiy range, upper part of Bolshoi Yaloman River, 50°28′ N, 89°48′ E, 2550–2878 m a.s.l., 23–25 Jul. 2007, V. Sorokina and A. Barkalov leg. (SZMN); 44 ♂♂ 23 ♀♀, Altai Republic, Ulagan district, Shapshal’skiy mountain range, 50°32′ N, 89°48′ E, 2550–2878 m a.s.l., on flowers of Schultzia crinita Spreng., 23–25 Jul. 2007, V. Sorokina and A. Barkalov leg. (SZMN); 1 ♂, Altai Republic, Kurayskiy range, 50°20′ N, 87°45′ E, 2500–2700 m a.s.l., 3 Jul. 2008, A. Barkalov leg. (SZMN).

Distribution
Holarctic Region; in the Palaearctic only from southern Siberia. Arcto-alpine species.

Spilogona nitidicauda (Schnabl, 1911)
Figs 4B, E, H, 5B, E, H

Type locality
RUSSIA: Yamalo-Nenetsk AO.
Material examined

Syntype
RUSSIA: 5 ♂ ♂ 5 ♀ ♀, “Karskaja tundra, s. Tobol’s. gub., 21.vii.09, Zaizev” [Yamalo-Nenetskiy AO, east slope of Polar Ural, mouth of the River Kara, ~69°02′N, 64°35′E, 21 Jul. 1909, Zaizev leg.]. 1 ♂, holotype of Limnophora (Spilogona) nitidicauda var. depressa Schnabl, same data as for preceding (ZISP).

Other material examined
RUSSIA: 9 ♂ ♂ 2 ♀ ♀, Altai Republic, Kosh-Agach district, Ukok plateau, environs of Muzdy-Bulak lake, 49°17′ N, 87°39′ E, 2450 m a.s.l., 26 Jun. 2005, 1–2 Jul. 2005, A. Barkalov and V. Sorokina leg. (SZMN); 24 ♂ ♂ 8 ♀ ♀, Altai Republic, Kosh-Agach district, Ukok plateau, environs of Kal’dzhin-Kul’-Bas lake, 49°19′ N, 87°26′ E, 2400–2600 m a.s.l., 17–21 Jul. 2006, V. Sorokina and T. Novgorodova leg. (SZMN); 4 ♂ ♂ 1 ♀, Altai Republic, Kosh-Agach district, Ukok plateau, 8 km NW of Maitobe Mountain, 49°34′ N, 87°43′ E, 2400–2500 m a.s.l., 8–10 Jul. 2006, V. Sorokina leg. (SZMN); 12 ♂ ♂, Altai Republic, Kosh-Agach district, Ukok plateau, Rodonovy spring, upper part of Zhumaly River, 49°27′ N, 88°03′ E, 2410 m a.s.l., 24 Jun. 2005, 4 Jul. 2005, 23–24 Jul. 2006, V. Sorokina and T. Novgorodova leg. (SZMN); 1 ♂ ♂, Altai Republic, Kosh-Agach district, Severo-Chuyskiy Ridge, valley of Aktru River, 50°06′ N, 87°48′ E, 1865–2064 m a.s.l., 21 Jul. 2013, T. Novgorodova leg. (SZMN); 2 ♀ ♀, Altai Republic, Ongudai district, Terekinskiy range, upper part of Bolshoi Yaloman River, 50°28′ N, 86°19′ E, 2200–2300 m a.s.l., 3 Jul. 2007, V. Sorokina leg. (SZMN); 1 ♂, Altai Republic, Ulagan district, Kurayskiy range, 50°20′ N, 87°45′ E, 2500–2800 m a.s.l., 29–30 Jun. 2008, A. Barkalov leg. (SZMN); 5 ♂ ♂ 16 ♀ ♀, Altai Republic, Ulagan district, environs of Dzhulukul’ lake, 50°28′ N, 89°46′ E, 2200 m a.s.l., 21 Jul. 2007, V. Sorokina leg. (SZMN); 2 ♂ ♂ 5 ♀ ♀, Altai Republic, Ulagan district, Shapshal’skiy mountain range, upper part of Shuy River, 50°32′ N, 89°48′ E, 2550–2878 m a.s.l., 23–25 Jul. 2007, V. Sorokina leg. (SZMN); 1 ♂ ♂, Altai Republic, Ust-Koksa district, 47 km W Ust-Kokska, Krasnaya Mountain, 50°04′ N, 85°13′ E, 2000–2100 m a.s.l., 16 Jul. 2015, V. Sorokina leg. (SZMN); 15 ♂ ♂ 3 ♀ ♀, Chukotka AO, Beringovskiy district, 40 km SSW of Beringovskiy, 62°43′ N, 178°55′ E, yellow plate trap, 22–23, 26 Jul. 2012, A. Stekolshchikov leg. (SZMN); 1 ♀, Chukotka AO, 30 km NW of Krasneno, Anadyr river, 64°46′ N, 174°8′ E, 15 Jul. 2014, A. Barkalov leg. (SZMN); 1 ♂, Krasnoyarsk Kray, Taymyr Peninsula, Kuria village, upper part of Pyasina river [70°16′ N, 88°30′ E], alder forest near village, 13 Jul. 1967, K. Gorodkov leg. (ZISP); 1 ♀, Krasnoyarsk Kray, Taymyr Peninsula, 114 km S of Khatanga, Kotuy river, 71°02′ N, 103°00′ E, 2–5 Jul. 2010, A. Barkalov leg. (SZMN); 1 ♂ ♂, Nenets AO, Khaypudyrskaya Bay, “Chuba”, 68°16′ N, 59°57′ E, yellow plate trap, meadow, 6–16 Aug. 2015, O. Makarova and Bizin leg. (SZMN); 1 ♂ ♂, Nenets AO, Bolvanskaya Bay, 68°05′ N, 54°47′ E, yellow plate trap, anthropogenic meadow, 18–25 Jul. 2015, O. Makarova and Bizin leg. (SZMN); 1 ♀, Nenets AO, Pakhancheskaya Bay, 68°29′ N, 57°26′ E, 26 Jul.–5 Aug. 2015, O. Makarova and Bizin leg. (SZMN); 2 ♂ ♂, Yamalo-Nenets AO, 73 km NE of Labytnangi, Longot’egan river, 67°19′ N, 66°43′ E, 175 m a.s.l., 2, 10 Jul. 2015, A. Barkalov leg. (SZMN).


Distribution
Holarctic Region; in the Palaearctic, from North Scandinavia, Russia and China. Arcto-alpine species.
**Spilogona platyfrons** Sorokina, 2018
Figs 4C, F, I, 5C, F, 1

**Type locality**
RUSSIA: Altai Mountains.

**Material examined**

**Holotype**
RUSSIA: 1 ♂, Altai Republic, Ulagan district, Shapshal’skiy mountain range, upper part of Shui River, ~50º33′ N, 89º50′ E, 2550–2900 m a.s.l., 25 Jul. 2007, V. Sorokina leg. (SZMN).

**Paratypes**

**Distribution**
Palaeartic Region: southern Siberia.

**Remark**
Mountain species, but probably living in the arctic tundra too.

**Molecular-genetic analysis of ‘Spilogona nitidicauda species-group’**

Since only the old type specimens of *S. his sarensis* are known, the molecular analysis was performed on the other three species of this group.

DNA variability is higher within taxa of the ‘Spilogona nitidicauda species-group’ in comparison with taxa of the ‘Spilogona contractifrons species-group’. Intraspecies DNA variability was found in *S. nitidicauda* and *S. platyfrons* from different localities. The isolate SN38 of *Spilogona nitidicauda* from the Altai (Muzdy-Bulak lake) differs from the isolates SN40 and SN41 of *S. nitidicauda* from
Chukotka AO (Beringovskii) by a synonymous substitution of C186T. Both isolates from Chukotka have identical DNA. The SP32 of *S. platyfrons* from the Shapshalsky ridge (Altai) differs from the SP33 of *S. platyfrons* from the Krasnaya Mountain (Altai) by substitutions of T636C ([Supplementary file](#)). *Spilogona nitidicauda* can be distinguished from *S. platyfrons* by 3–4 SNPs. *Spilogona imitatrix* differs from *S. platyfrons* with 6 SNP, and from *S. nitidicauda* with 7 SNP.

The genetic distance between *S. nitidicauda* and *S. platyfrons* is 0.4%, between *S. platyfrons* and *S. imitatrix* is 1.2%, and between *S. nitidicauda* and *S. imitatrix* is 1.6%. All three species formed distinct clusters on the ML tree, but the differences between *S. nitidicauda* and *S. platyfrons* are insignificant statistically.

The following results were obtained after comparing the sequences of the studied specimens with the annotated sequences in GenBank. Unfortunately, there were no DNA sequences of *S. nitidicauda* and *S. platyfrons* in GenBank nor in the BOLD database, so comparison with our results was impossible. But there were many DNA sequences of *S. imitatrix* from Canada.

*Spilogona imitatrix* from the Altai is identical with several individuals of *S. imitatrix* from Canada (KR615453 and KR622696). However, several individuals from GenBank identified as *S. imitatrix* have a very variable DNA, as can be seen on the ML tree (Fig. 3). Such a high polymorphism may be due to errors in morphological identification.

**Discussion**

The analysis of genetic distances based on the differences in the cytochrome oxidase I gene in members of the ‘*Spilogona contractifrons* species-group’ and the ‘*Spilogona nitidicauda* species-group’ confirmed that there are not only morphological differences but also genetic differences between closely related species.

Our results with the DNA analysis of the COI gene have demonstrated that *S. alticola*, *S. contractifrons* and *S. arctica* are not synonymous because the genetic differences between individuals of these species persist in geographically distant locations (Fig. 6). The DNA of *S. arctica* is identical in individuals from different regions of Russia, Sweden, Greenland and Canada. Individuals of *S. contractifrons* from Russia are identical with *S. contractifrons* from Sweden and Norway, and all of them form a distinct cluster of “European” or “Palaearctic” *S. contractifrons*. It should be emphasised that the specimens of *S. arctica* and *S. contractifrons* from Russia were identical with specimens from the type localities (Sweden and Greenland) but show differences from specimens of these species from Canada.

In turn, specimens from Canada designated in GenBank as *S. contractifrons* were identical with specimens from the Altai identified by us as *S. alticola*. The latter species was described from specimens from USA (New Hampshire) and was later found in different areas of Northern Canada (Huckett 1965). The reasons for this result could be either the incorrect identification of the specimens deposited in GenBank, or a rejection of the existence of *S. alticola* species by Canadian researchers and a designation of such specimens as *S. contractifrons*.

In the ‘*Spilogona nitidicauda* species-group’ there are stable nucleotide differences between *S. nitidicauda* and *S. platyfrons* from different places and years of collection despite the morphological similarity. Although the genetic distance between *S. nitidicauda* and *S. platyfrons* is only 0.4%, *S. imitatrix* differs from them significantly. The DNA distance between *S. imitatrix* and *S. nitidicauda* is 1.6%, and that between *S. imitatrix* and *S. platyfrons* is 1.2% which demonstrates the separate species status of *S. imitatrix*. In Muscidae, ranges of 0.18% and 3.01% have been reported as averages for the means and maxima of COI intraspecific distances, respectively (Renaud *et al.* 2012). Limits of species with a
distance to the nearest neighbour of < 2% in 137 species of Muscidae were supported by morphological characters and confirmed that the minimum interspecific distance in Muscidae ranges well below 2% for many species (Renaud et al. 2012).

*Spilogona imitatrix* was described from specimens from Labrador (Canada) and was later found in different regions of Canada and USA (Alaska). Recently this species was found in the Palaearctic Region for the first time, here known only in the Altai Mountains (Sorokina 2018). The DNA of *S. imitatrix* from the Altai (Terektinsky ridge) was identical with the DNA of several individuals of *S. imitatrix* from the Northwest Territories (Canada). There is a rather large number of the sequences of this species

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**Fig. 6.** Localities of the species of *Spilogona* Schnabl, 1911 used in the DNA analysis. Symbols denote the species, whilst the colour shows the same DNA sequences. **A.** ‘*S. contractifrons* species-group’. **B.** ‘*S. nitidicauda* species-group’.
from Canada in GenBank. Several DNA sequences of *S. imitatrix* from Canada were randomly taken for genetic matching with the specimen of this species from the Altai. It transpired that one sequence (KR628150) clustered with *S. platyfrons* from the Altai, whilst the other sequences of Canadian specimens (# HM389123, # HM389124) differed from all of them by six nucleotide substitutions (Fig. 3). Such differences between DNA sequences of one species can be explained by errors in the initial morphological identification. This has been proved by the work of the first author of this paper (VSS) with the Muscidae collection in the CNC, where the following species were found under the name *S. imitatrix*, and *vice versa*: *S. alticola*, *S. arctica*, *S. deflorata* (Holmgren, 1872), *S. incauta* (Huckett, 1932), *S. nitidicauda*, *S. trigonifera* (Zetterstedt, 1838), *S. sororcula* (Zetterstedt, 1845), *S. trilineata* (Huckett, 1932) and another *Spilogona* sp.

These misidentifications are not surprising, since most members of the genus *Spilogona* are difficult to distinguish, even for muscid specialists. Therefore, in addition to a genetic analysis it is important to have an idea of the most stable morphological characters in each group. According to our results with this genus, the following can be considered as the most stable morphological characters in *Spilogona*: presence of *pv/v* setae on mid tibia, presence of *pv* setae on hind femur, presence of dust on the prementum, shape of the sternite 5, presence interspatial seta(e) on anepisternum, number of *post dc* setae on the scutum (3 or 4), presence on the scutellum of downwardly-directed preapical setulae on upper border of declivities. The last character was proposed and used only by Huckett (1965) for the separation of species into groups in his key to the species of *Spilogona*, in addition to the other well-known characters. Other researchers (Hennig 1959a, 1959b, 1959c; Gregor et al. 2002) used well-established characters for identification, specifically the number of *post dc* setae on the scutum (3 or 4), the elongation of the face, and the presence of *pv* setae on hind femur. The character suggested by Huckett (downwardly-directed preapical setulae on the upper border of declivities of scutellum) is quite difficult to see, which is probably why it has not been generally used by the authors of the keys. However, in our opinion, this is one of the important characters for the identification of the species of *Spilogona*. To demonstrate this, several outgroup species (*S. aerea* (Fallén, 1825), *S. bifimbriata* Huckett, 1965, *S. baltica* (Ringdahl, 1918) and *S. tornensis* (Ringdahl, 1926)) were included in the genetic analysis. These species have the same characters (3 and 4 *post dc* setae, mid tibia without *v* seta, hind femur with and without *pv* setae, face not projecting) as in the species groups analysed above, but, unlike them, these species have scutellum with downwardly-directed preapical setulae on the upper border of declivities. The results of the genetic analysis showed a distinct distance between the cluster of the ‘*Spilogona contractifrons* species-group’ and the ‘*Spilogona nitidicauda* species-group’ on the one hand, the members of which have scutellum without downwardly-directed preapical setulae on the upper border of declivities, and on the other hand the cluster of species having these setulae on the scutellum (Fig. 3). The members of the ‘*Spilogona contractifrons* species-group’ and the ‘*Spilogona nitidicauda* species-group’ thus have not only distinguishing morphological characters but also fixed substitutions in the DNA sequences. Since a low interspecific polymorphism is known in the Muscidae, the revealed genetic distances confirm the existence of separate species or subspecies in each of the groups studied.

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