Defying the northern limit: New records and DNA barcodes of *Symmerus* Walker, 1848 (Diptera, Ditomyiidae) from Northern Norway

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What is believed to be the northernmost records of the family Ditomyiidae in the world are presented from south of and north of the arctic circle in Nordland county in Norway. A population of *Symmerus annulatus* (Meigen, 1830) is recorded from an elm (*Ulmus minor*) forest at Teisdalen Nature Reserve at 65.35°N, while another population of *Symmerus nobilis* Lackschewitz, 1937 is recorded from a mixed deciduous forest at the border of Fiskvågflåget Nature Reserve at 67.09°N. Specimens of both species, including associated females, were successfully DNA barcoded and assigned widely separated (16%) Barcode Index Numbers (BINs) on The Barcode of Life Data System (BOLD). While males of the two European *Symmerus* Walker, 1848 species are adequately described, illustrated and photographed in the literature, the females have gained much less attention. Hence, photographic plates of the female of both species are presented accompanied with a discussion of the subtle differences between the two. Their distribution and habitat requirements in Norway indicate a more northern and less thermophilic forest depending range for *S. nobilis* than for *S. annulatus*.

Key words: Diptera, Ditomyiidae, *Symmerus*, northernmost distribution, female description, DNA barcoding, NorBOL.

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Introduction

Gammelmo & Rindal (2006) reported both European species of *Symmerus* Walker, 1848 and summarized the scarce knowledge of the family Ditomyiidae in Norway. At the time, known records of the two species were confined to southeastern parts of Norway, giving the impression of a southern, near nemoral distribution pattern confined to thermophilic broadleaved forests, in line with distribution data provided in the world revision of *Symmerus* by Munroe (1974). However, soon after Kjærandsen & Jordal (2007) reported both species much further north amongst the very rich fauna of fungus gnats of the superfamily Sciaroidea recorded in Møre og Romsdal county. They suggested that, when more of northern Norway will be better investigated, another distribution pattern for fungus gnats in Norway would emerge. With substantial insect collecting surveys throughout northern Norway, undertaken by the author in the period 2014–2020 and supported by the Norwegian Biodiversity Information Centre, this has now indeed proved to be the case (see Kjærandsen 2020), even for the more southernly distributed family Ditomyiidae.

Here, new records of both *Symmerus* species are recorded from Nordland county, DNA barcode results are reported and characters for identifying both males and females of the two species are presented. Their distribution and habitat requirements in Norway are then discussed in light of the new records.

Materials and methods

The examined materials originate from ongoing insect collecting surveys throughout Northern Norway, supported by UiT – The Arctic University of Norway and The Norwegian Biodiversity Information Centre. Most examined specimens were dried from the ethanol samples by use of HMDS baths (see Brown 1993), pinned (i.e. glued to minutens) and lodged in the entomological collections at Tromsø University Museum, UiT – The Arctic University of Norway (TMU). Some specimens of both species are kept in 95% ethanol in freezer at TMU as source for further genetic study.

One leg each from fresh specimens were sent to the Canadian Centre for DNA barcoding, BIO (Guelph, Ontario, Canada), for DNA extraction and bi-directional Sanger sequencing as a part of the Norwegian Barcode of Life (NorBOL) initiative (see Kjærandsen 2017), itself a branch of the International Barcode of Life project (iBOL). A Leica MC170HD microscope camera mounted on a Leica M205C stereomicroscope was used to capture images of pinned females and of detached female terminalia macerated in hot lactic acid and stored in glycerine. Stacked images, merged for extended focus applying the Helicon Focus software, were subsequently moderately photo-shopped into illustrative plates. Terminology for the interpretation of female terminalia follows Söli (1997).

The species

***Symmerus annulatus* (Meigen, 1830)**
(Figures 1A, 2A, 3A&B)

New records: **NORWAY**: **NSY** (Nordland), Sømna, Teisdalen Nature Reserve (elm forest), 65.36028°N, 12.38861°E, 1 June–3 August 2018, Malaise trap, Leg. J. Kjærandsen, J. P. Lindemann & P. Dominiak, **7 males** (TSZD-JKJ-104740, TSZD-JKJ-104895, TSZD-JKJ-104896, TSZD-JKJ-104897, TSZD-JKJ-105982, TSZD-JKJ-105983, TSZD-JKJ-105984), **1 female** (TSZD-JKJ-105981).

Barcodes: Four specimens were successfully DNA-barcoded (639(5n)–658(0n) BP) on The Barcode of Life Data System (BOLD) and assigned to the Barcode Index Number (BIN) **BOLD:AAZ0315** (TSZD-JKJ-104740, TSZD-JKJ-104895, TSZD-JKJ-104896 and TSZD-JKJ-104897). Currently this BIN has 11 additional vouchers from continental Europe (Germany, France, Austria, Romania). The reciprocal mean distance to *Symmerus nobilis* in BIN **BOLD:ACB1822** is 15.90 % [SE 0,01].

***Symmerus nobilis* Lackschewitz, 1937**
(Figures 1B, 2B, 3C&D)

New records: **NORWAY**: **NSI** (Nordland), Saltdal, Rognan, Fiskvågmo just outside Fiskvågflåget Nature Reserve (mixed deciduous forest), 67.09244°N 15.36075°E, 28 May–22 July 2019, window trap, Leg. J. Kjærandsen, J. P. Lindemann & P. Dominiak **68 males** (TSZD-JKJ-107547,TSZD-JKJ-107548, TSZD-JKJ-107549, TSZD-JKJ-107550,TSZD-JKJ-107374,TSZD-JKJ-107375,TSZD-JKJ-107376,TSZD-JKJ-107377,TSZD-JKJ-107378, TSZD-JKJ-107379,TSZD-JKJ-107380,TSZD-JKJ-107381,TSZD-JKJ-107382,TSZD-JKJ-107383, TSZD-JKJ-107394), **12 females** (TSZD-JKJ-107551,TSZD-JKJ-107384,TSZD-JKJ-107385,TSZD-JKJ-107386,TSZD-JKJ-107387,TSZD-JKJ-107388,TSZD-JKJ-107389,TSZD-JKJ-107390,TSZD-JKJ-107391,-TSZD-JKJ-107392,TSZD-JKJ-107393,TSZD-JKJ-107395).

Barcodes: Five specimens were successfully DNA-barcoded (466–514(0n) BP) on The Barcode of Life Data System (BOLD) and assigned to the Barcode Index Number (BIN) **BOLD:ACB1822** (TSZD-JKJ-107547,TSZD-JKJ-107548, TSZD-JKJ-107549,TSZD-JKJ-107550,TSZD-JKJ-107551). Currently this BIN has only 1 additional, female voucher from Buskerud in Norway. The reciprocal mean distance to *Symmerus*

FIGURE 1. Female habitus of European *Symmerus* Walker, 1848 species (Ditomyiidae). **A**. *Symmerus annulatus* (Meigen, 1830). **B**. *Symmerus nobilis* Lackschewitz, 1937.

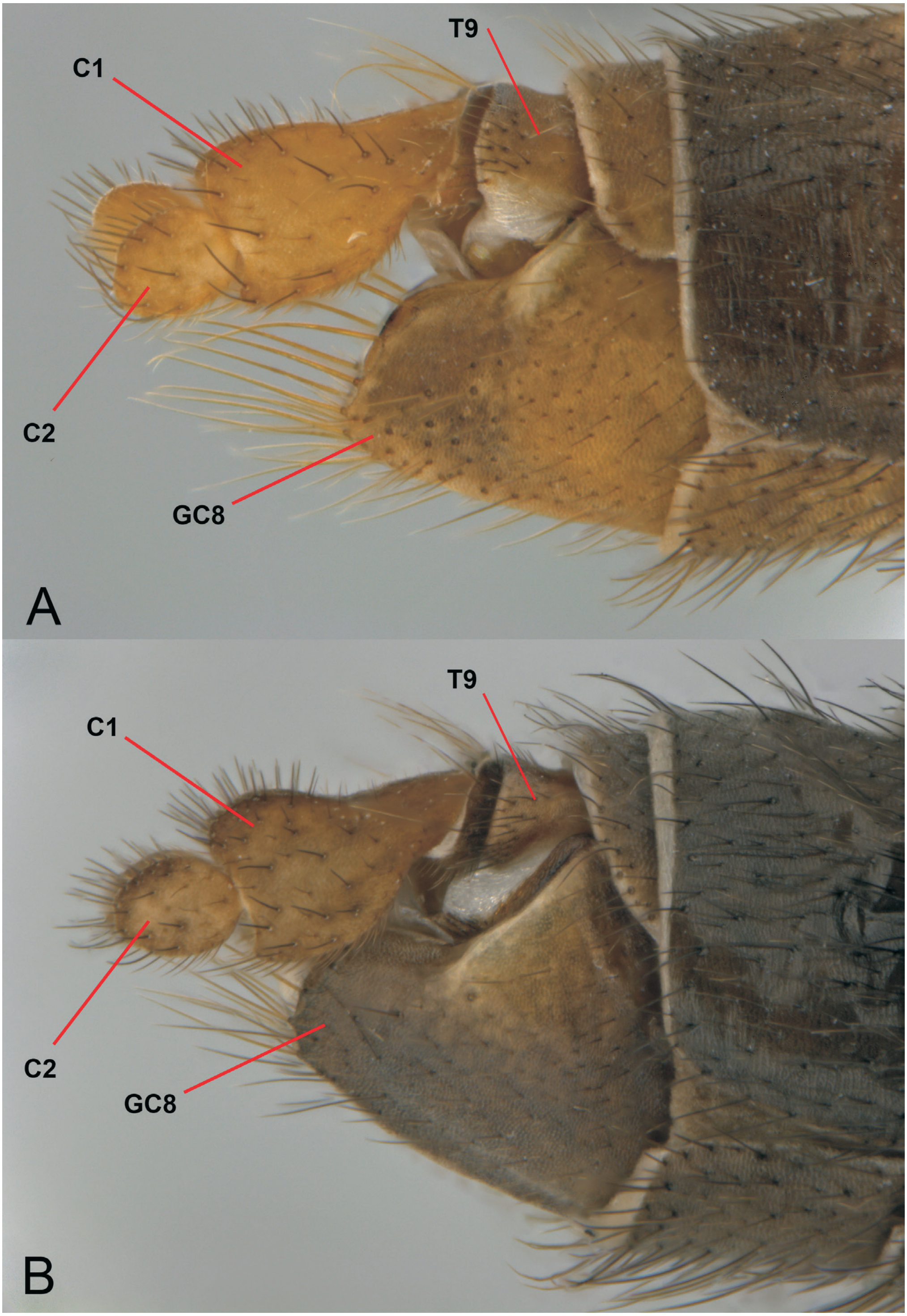

FIGURE 2. Female terminalia of European *Symmerus* Walker, 1848 species (Ditomyiidae). **A**. *Symmerus annulatus* (Meigen, 1830). **B**. *Symmerus nobilis* Lackschewitz, 1937. Abbreviations as in Figure 3.

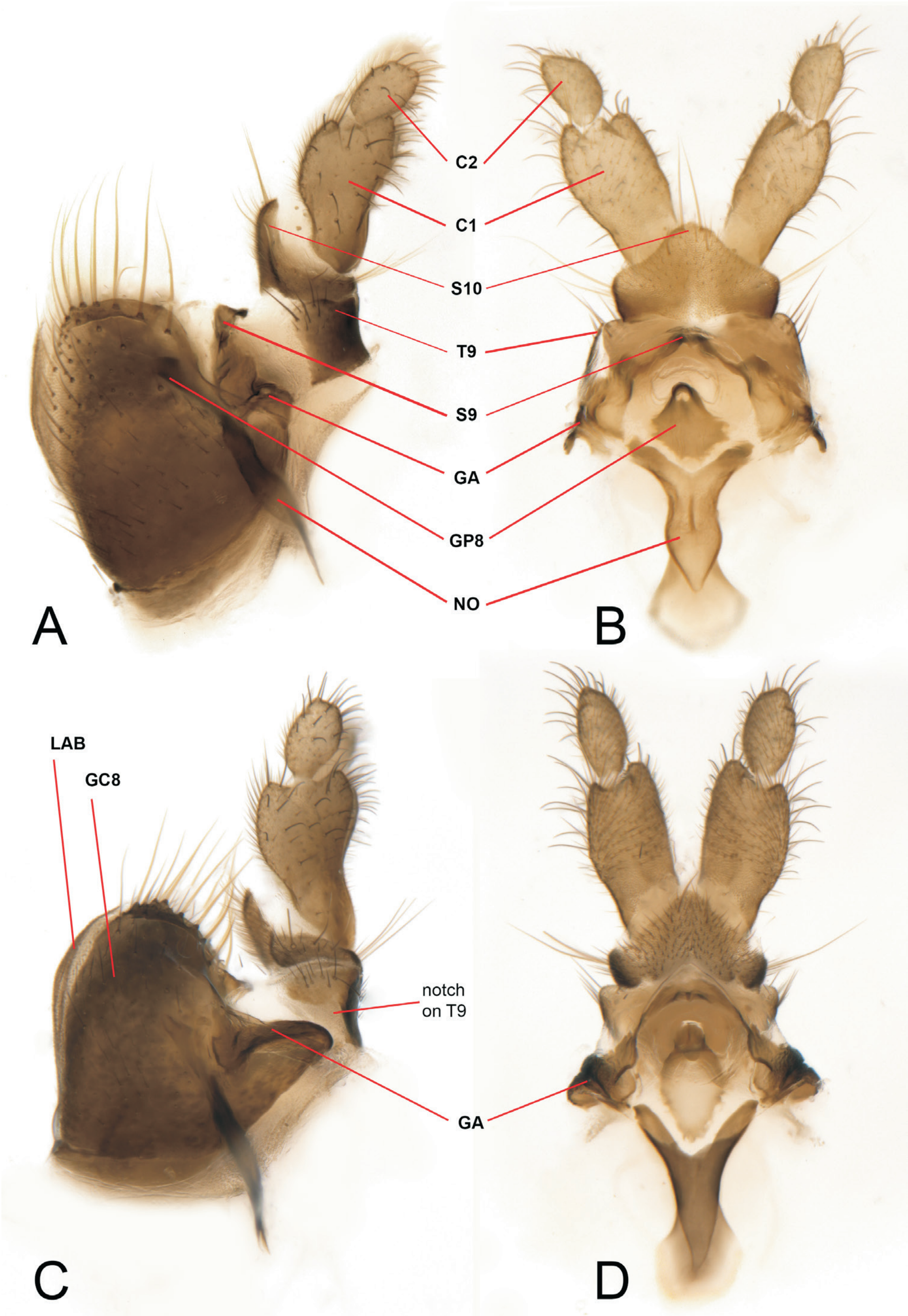

FIGURE 3. Dissected and cleared female terminalia of European *Symmerus* Walker, 1848 species (Ditomyiidae). **A–B**. *Symmerus annulatus* (Meigen, 1830). **C–D**. *Symmerus nobilis* Lackschewitz, 1937. Abbreviations: C1 = Cercus 1; C2 = Cercus 2; GA = Gonocoxal apodeme; GC8 = Gonocoxite 8; GP8 = Gonapophysis 8; LAB = Labia; NO = Notum; S10 = Sternite 10; S9 = Sternite 9; T9 = Tergite 9.

annulatus in BIN **BOLD:AAZ0315** is 15.90 % [SE 0,01].

Discussion

Colour patterns in fungus gnats of the superfamily Sciaroidea are, like in many insects, mainly created through different biochemical pathways of the sclerotization process during hatching, involving the amino acid thyrosine being produced into four colour variants of melanine (black, brown, ochraceous and yellow). This well known genetic pathway is controlled by a few genes, largely "yellow", "ebony" and "tan", but it is further regulated by so called cis-regulatory, genetic switches (see eg. Massey & Wittkopp 2016). Colour patterns produced in this way tend to be rather plastic and generally must be used with great care for species identification. Variation, including seasonal (generational) variation and dark melanism induced by sun exposure (eg. in mountain habitats) is commonplace. Still, many species of fungus gnats appear to produce largely invariant and characteristic patterns of pale yellow to ochraceous, contrasted with dark brown to black.

Identifying males of the two European *Symmerus* species is fairly easy. Their male and female terminalia were illustrated by Zaitzev (1978), and Gammelmo & Rindal (2006) provided high quality habitus images of the males, indicating that their identification can with reasonable certainty rest on their colouration patterns alone. The male of *Symmerus annulatus* is rather pale yellow with darker brown abdominal tergites always with contrasting pale yellow stripes anterior on each segment. The male of *Symmerus nobilis* is darker on thorax and has abdominal tergites all dark brown, forming a continuous dark tergum contrasting against the yellow pleurites.

In the females, however, the abdomen is largely uniformly dark in both species (Figure 1), this making the differences in colouration less obvious. Still, the female of *Symmerus annulatus* appears to have a paler thorax compared with *S. nobilis*, and the latter even has dark coxae. Another notable difference is found in the antenna where the pedicel and basal 4–5 flagellar segments in *S. annulatus* are pale with a gradual darkening towards the dark apical two thirds. In *S. nobilis*, on the other hand, only the pedicel and basal ⅓ of the first flagellar is pale.

Without maceration the dry terminalia of females appears quite similar between the two *Symmerus* species (Figure 2). The generally paler terminalia of *S. annulatus* has a quite edged outline of gonocoxite 8, the subrectangular dilated apical half of cercus 1 is slightly longer than its narrow base, and cercus 2 is also somewhat edgy in outline. In *S. nobilis* the outline of gonocoxite 8 is more smoothly rounded, the smoothly dilated apical half of cercus 1 is hardly longer than its base, and cercus 2 appears a bit smaller and more smoothly egg-shaped. When the terminalia is dissected, cleared in hot lactic acid and viewed in glycerine, some more distinct differences emerge (Figure 3). Gonocoxite 8 in *S. nobilis* has a distinctly pronounced dorsal expansion aligning with a heavily sclerotized goncoxal apodeme (Figure 3C). The latter is produced into a ventral lip representing sternite 9. Tergite 9 has a corresponding notch laterally on the sclerotized tergal plate (Figure 3C). The same elements are present in *S. annulatus*, but much less produced dorsally, and the sclerotized tergal plate of tergite 9 has a distinct, angular lateral corner. The ventral surfaces of sternite 10 and the cerci appear to be more densely covered with darker setulae in *S. nobilis* than in *S. annulatus*. Finally, the inner gonapophysis 8 and its y-shaped notum are similarly built but with slight allometric differences between the two species.

The large genetic distance found between the two *Symmerus* species match with the presumed ancestral position of *Symmerus* and the family Ditomyiidae among the Sciaroidea (see Ševčík *et al.* 2016). The DNA barcodes on The Barcode of Life Data System (BOLD) were assigned to distinct Barcode Index Numbers (BINs) at some 16 % distance from each other, making the BOLD reference library highly reliable for molecular identification of the European *Symmerus* species based on these vouchers.

Both *Symmerus* species are widespread in Europe (Chandler 2005). *S. nobilis*, not found south of France, appears to have a slightly more northern range than *S. annulatus*. Previously, the northern limit for *S. annulatus* was at Øvre Vike-Vikesetra (62.55°N 8.19°E), where both species were recorded, and for *S. nobilis* at Jordalsgrenda (62.77°N 8.32°E). Both these localities are situated in Møre og Romsdal county (Kjærandsen & Jordal 2007). The new records in Nordland move the distribution limit 375 km northeast for *S. annulatus* and 585 km northeast for *S. nobilis*.

Nearly all records of adult *Symmerus* are from broadleaved forests, with the exception of a Russian Karelian site (62.30°N 35.09°E) for *S. nobilis* in spruce (*Picea abies*) dominated forests with a high proportion of aspen (*Populus tremula*) (Jakovlev *et al.* 2014). Their larvae develop in decaying wood of deciduous trees only (Jakovlev *et al.* 2014). Records from Norway fall into the same category although their larvae have never been documented. Gammelmo & Rindal (2006) reported both *Symmerus* species from south facing, thermophilic broadleaved forests. The site at Øvre Vike-Vikesetra (Miljødirektoratet 2018a) is also a large, thermophilic, west-faced broadleaved forest dominated by common hazel (*Corylus avellana*), with some stands of elm (*Ulmus minor*), aspen and goat willow (*Salix caprea*). The site at Jordalsgrenda (Miljødirektoratet 2018b) is an old, mixed deciduous forest, dominated by birch (*Betula pubescens*) and aspen, but also with some hazel, goat willow and bird cherry (*Prunus padus*). The new site at Teisdalen Nature Reserve (Miljødirektoratet 2018c) is a reserve protected for its northern location of a rich broadleaved forest dominated by elm, while the site at the border of Fiskvågflåget Nature Reserve (Miljødirektoratet 2018d) has broadleaved forest dominated by goat willow and birch. At the latter site, in all 80 specimens of *S. nobilis* were collected in a window trap placed next to a decaying log of goat willow, this very likely being the larval habitat at the site. A piece of the log was dissected in search for larvae in ultimo September 2019, without result.

The emerging distribution pattern indicates that *S. annulatus* is restricted to broadleaved thermophilic tree species like beech (*Fagus sylvatica*), elm and lime (*Tilia cordata*), while *S. nobilis* extends its range northwards by being able to live on goat willow and possibly aspen. While *S. annulatus* is considered rather common many places in the south, *S. nobilis* is considered everywhere a rare species (Jakovlev *et al.* 2014). The vital population documented at Fiskvågflåget Nature Reserve may indicate that *S. nobilis* has been overlooked and is more common in the northern range of its distribution. Still, this rather warm hillside remains the only locality, among hundreds of insect samples collected throughout Northern Norway, where the species has been found. Both species are currently red-listed as near threatened (NT) in Norway (Gammelmo *et al.* 2015).

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