

***Holocentropus varangensis* Mey, 1987, a probable hybrid between *Holocentropus insignis* Martynov, 1924 and *H. picicornis* (Stephens, 1836) (Trichoptera, Polycentropodidae)**

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Wiberg-Larsen, P., Andersen, T., Elven H. & Salokannel, J. 2024. *Holocentropus varangensis* Mey, 1987, a probable hybrid between *Holocentropus insignis* Martynov, 1924 and *H. picicornis* (Stephens, 1836) (Trichoptera, Polycentropodidae). *Norwegian Journal of Entomology* 71, 1–7.

Holocentropus varangensis Mey, 1987 was described based on a single male collected by Wolfgang Tobias at Lake Gjøkvatn in Sør-Varanger municipality, northern Norway in 1969. It is very similar to *H. insignis* Martynov, 1924 and *H. picicornis* (Stephens, 1836), and the species can only be separated on relatively small differences in the male genitalia. The holotype of *H. varangensis* is the only specimen known. The species has never been found again neither in northern Norway, nor in neighboring countries. *Holocentropus insignis* and *H. picicornis*, on the other hand, are common and widespread in the northernmost parts of Fennoscandia. Based on this fact, and on detailed comparison of the male genitalia of all three taxa, we regard the holotype of *H. varangensis* as a hybrid specimen between *H. insignis* and *H. picicornis*. Thus *H. varangensis* is a name not valid under the International Code of Zoological Nomenclature.

Key words: Trichoptera, Caddis flies, Polycentropodidae, *Holocentropus*, hybrid, northern Norway.

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Introduction

The genus *Holocentropus* McLachlan, 1878 belongs in the family Polycentropodidae. Five species of *Holocentropus* are found in Europe, all of which occur in Norway (Solem & Andersen 1996). Of these, *Holocentropus dubius* (Rambur, 1842) and *Holocentropus picicornis* (Stephens, 1836) are common in the southern parts of Norway, Sweden and Finland, but are also

found in the northern parts of these countries. *Holocentropus insignis* Martynov, 1924 is also distributed in most parts of all three countries but is more common in their northern parts. A fourth species, *Holocentropus stagnalis* (Albarda, 1874) occurs scattered in all three countries, but is absent in their northernmost parts. In Norway it occurs in the Oslofjord area and further along the southern coast westwards to Rogaland. Finally, *Holocentropus varangensis* Mey, 1987 was

described by Mey (1987) based on a single male collected by Wolfgang Tobias at Lake Gjøkvatn in Sør-Varanger municipality in 1969.

When describing *H. varangensis* Mey (1978) only compared the new species with *H. picicornis* and stated that they are very similar and can only be separated with certainty on structures in the male genitalia. The species has been regarded as endemic to northernmost Norway as it has not been recorded anywhere else outside this area. The fact that the type specimen is the only known representative of the species is indeed surprising due to the comparatively high sampling effort in northernmost Fennoscandia during the last decades. In this study, we therefore evaluate if *H. varangensis* should be regarded as a valid species or an aberration. Specifically, we explore the possibility that it might represent a hybrid between *H. picicornis* and *H. insignis*.

Material and methods

We carried out morphological studies of *H. insignis*, *H. picicornis* and *H. varangensis*. The two first species were included as they are very similar to *H. varangensis* morphologically based on the male genitalia. We also included a specimen of *H. picicornis* from north America as the species has a Holarctic distribution and might show regional morphological differences.

The posterior parts of the abdomen of Danish specimens of *H. insignis* (2 specimens) and *H. picicornis* (1 specimen), including the genitalia, were cleared in 10% KOH, washed, and transferred to glycerol before examination.

The genital terminology is according to Holzenthal *et al.* (2007).

Specimens studied

Holocentropus picicornis (Stephens, 1836)

DENMARK, Fyn: Plovskær Mose (small acid pool), 15 May 1986, 4♂♂, leg. P. Wiberg-Larsen (the genitalia of one of these are shown in Figure 1A–C); Sorte Sø, Gerup Skov (small acid lake), 31 May 1981, 3♂♂, leg. P. Wiberg-Larsen; Sjælland, Bøllemosen (acid pond), 13 June 1976,

6♂♂, leg. P. Wiberg-Larsen; **U.S.A.**, Minnesota: Lake Co., Baptism River, Hwy1, 22 June 1994, 1♂, leg. R. Holzenthal *et al.*, det. R. Blahnik 1994.

Notes: Roger Blahnik identified the specimen from Baptism River as “*Polycentropus picicornis* (Stephens) or near”. The specimen is a voucher for “Barcode of Life”, sample ID09MNKK0333, and was made available for our study by the University of Minnesota Insect Collection, St. Paul, where it is housed.

Holocentropus insignis Martynov, 1924

DENMARK, South Jutland: Stensbæk Plantage, 8 June 1987, 25♂♂, leg. P. Wiberg-Larsen (the genitalia of one of these are shown in Figure 2A–C).

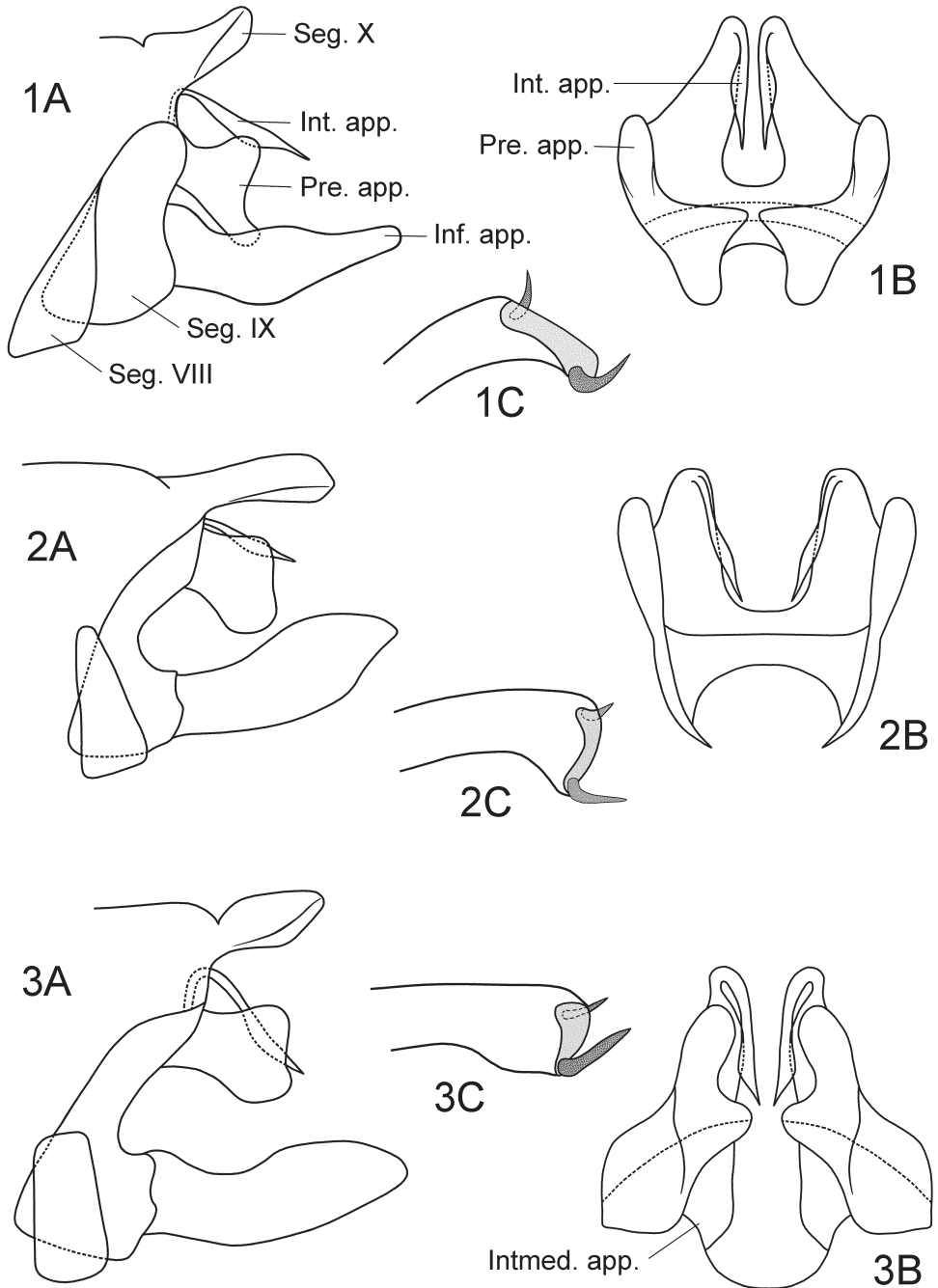
Holocentropus varangensis Mey, 1987

NORWAY, **FI**, Sør-Varanger: Varanger Peninsula, lake inlet at tributary 3 of Gjøkvatn, 10 July 1969, 1♂ (holotype), leg. W. Tobias (the genitalia of the holotype are shown in Figure 3A–C).

Notes: The holotype is housed in the Senckenberg Museum of Natural History, Görlitz, Germany - not in the Museum of Natural History, Berlin, as stated in Mey (1987). The specimen was originally labelled “*H. insignis*” by Wolfgang Tobias. The abdomen including the genitalia was already cleared in KOH by Mey. The holotype was made available for our study by the Senckenberg Museum of Natural History, Görlitz.

DNA sequencing

With permission from Senckenberg Museum, we attempted to obtain a DNA barcode from one leg of the holotype of *H. varangensis*. The leg was processed non-destructively, i.e. securing conservation of the exoskeleton. The aim was to obtain a barcode sequence of the mitochondrial cytochrome oxidase subunit I gene (COI) to be compared with similar available sequences for *H. insignis* and *H. picicornis* (see Boonstra *et al.* 2018). As the mitochondrial DNA is maternally inherited, the barcode of *H. varangensis* - if this is a hybrid - should be identical to that of one of the potential mothers, either *H. insignis* or



FIGURES 1–3. Male genitalia. **1.** *Holocentropus insignis* Martynov, 1924. **2.** *H. picicornis* (Stephens, 1836). **3.** *H. varangensis* Mey, 1987. **A.** Lateral view. **B.** Intermediate appendage and preanal appendages, ventral view. **C.** Aedeagus, lateral view. *Abbreviations:* Seg. VIII–X (abdominal segments VIII–X). Int. app. (internal appendage). Pre. app. (preanal appendage). Inf. app. (inferior appendage). Intmed. app. (intermediate appendage).

H. picicornis. This would strongly support the hybridization hypothesis.

The extraction was carried out by courtesy of the Ecology and Genetics Research Unit, Oulu. The sample was then analyzed (28 April 2022) at the Canadian Centre for DNA Barcoding (CCDB) (Plate: CCDB-40752; Process-id: LEFIJ28025-22). It was then subjected to the “NGSFT” protocol as explained in Prosser et al. (2016).

Results

Overall, our morphological studies of the genitalia belonging to *H. picicornis* (material from both North America and Denmark) and *H. varangensis* (Figures 2–3) closely confirm the descriptions of the two species in Mey (1987). Further, we did not find any significant variation in *H. picicornis* when comparing our material from Denmark, the specimen from Minnesota, and the description of the species by Mey (1987). However, we did find that the preanal appendages in lateral view (Figures 2A, 3A) look the same in both species, not being extended ventrally as drawn by Mey (1987: fig. 2). Further, although the intermediate appendage in *H. varangensis* has a very deep incision; it is fused at the base (Figure 3B), not totally separated as stated by Mey (1987: fig. 4).

In lateral view, *H. insignis* and *H. varangensis* show significant differences (Figures 1A, 3A). The preanal appendage is much more extended ventrally in *H. insignis* than in *H. varangensis*. Further, in *H. insignis* the distal half of the inferior appendage is tapering significantly distally, whereas such tapering is not present in *H. varangensis* (or in *H. picicornis*, Figure 2A). In ventral view, the preanal appendage has overall a similar form in *H. insignis* and *H. varangensis*, both having a distinct process pointing medially (Figures 1B, 3B). However, the incision in the intermediate appendage is relatively shallow in *H. insignis* (and in *H. picicornis*) compared to a very deep incision in *H. varangensis*.

The aedeagus of all three species bears two pairs of dark spines apically, situated dorsally and ventrally (Figures 1C, 2C, 3C). In *H. varangensis*

the apex of the aedeagus is only slightly dilated, whereas it is markedly dilated in the two other species. Consequently, the pairs of spines are situated closer to each other in *H. varangensis* than in the two other species. Both pairs of spines are of approximately the same length in *H. insignis* and *H. varangensis*, whereas in *H. picicornis* the dorsal spines are smaller than the ventral.

Holocentropus insignis and *H. picicornis* are easily separated in their habitus (personal observations; see also Salokannel & Mattila 2018). *Holocentropus insignis* has uniformly yellowish forewings (Figure 4), whereas they are light and dark speckled in *H. picicornis* (Figure 5). Mey (1987) did not mention the wing color in *H. varangensis*, and as the specimen has been stored in alcohol for 54 years, we are not able to describe the forewing color either.

Unfortunately, our attempt to obtain a COI barcode from *H. varangensis* failed. None of the reads produced Trichoptera sequences, but instead sequences belonging to other very different taxonomic groups. The most likely explanation is that the DNA in the sample itself was strongly fragmented, probably due to age and suboptimal preservation. In such cases, even the slightest amount of contaminant DNA (from dust etc.) may easily be amplified. This is supported by the fact that individual reads mostly match very/quite precisely with unrelated taxa.

Discussion

When describing *H. varangensis*, Mey (1987) only compared the new species with *H. picicornis* and not with *H. insignis*. This is surprising especially as Wolfgang Tobias wrote "*H. insignis*" on a label deposited with the holotype of *H. varangensis*. All three species occur in the same geographical area and the male genitalia of the three species are overall very similar. Mey (1987) stated that *H. varangensis* and *H. picicornis* only can be distinguished by modest differences in the genitalia and provided the following traits for separating the two: In lateral view, the preanal appendage is heart shaped in both species, but more rounded in *H. varangensis* than in *H. picicornis* (Mey 1987:



FIGURE 4. Male of *Holocentropus insignis* Martynov, 1924. The species is rather small with a forewing length of 4.9–6.8 mm and almost uniformly yellowish forewings. Photo: Keijo Mattila.



FIGURE 5. Male of *Holocentropus picicornis* (Stephens, 1836). The species is rather small with a forewing length of 4.9–7.7 mm and dark and light speckled forewings. Photo: Keijo Mattila.

figs 1, 2). In ventral view, the preanal appendage of *H. varangensis* has a very broad base, an almost right-angled median incision, and a rounded dorsal section. Only the dorsal section carries hairs. In contrast, the preanal appendage of *H. picicornis* is

narrow from base to dorsal apex, lacks the median incision, and has more widely distributed hairs (Mey 1987: figs 3, 4). The intermediate appendage is narrowly but completely divided in two parts in *H. varangensis*, whereas in *H. picicornis* its two

halves are fused medially to form a broad plate (Mey 1987: figs 3, 4). In *H. varangensis*, the apex of the aedeagus bears two pairs of relatively long, dorsally pointed spines of sub-equal length. In *H. picicornis* the dorsal pair is significantly shorter than in *H. varangensis*, while the ventral pair is somewhat shorter than in *H. varangensis* and does not point distinctly dorsally (Mey 1987: figs 5, 6).

Indeed, the preanal appendage of *H. insignis* and *H. varangensis* show striking similarities, i.e. both having a lateral process projecting mesally. Further, *H. insignis* and *H. varangensis* share two pairs of dark spines on the apex of the aedeagus, being approximately of the same size, whereas in *H. picicornis* the upper pair is smaller than the ventral one. However, there is a distinct difference between *H. insignis* and *H. varangensis* in the shape of the intermediate appendage as the median cleft is much deeper in *H. varangensis* than in *H. insignis*. On the other hand, the inferior appendage of *H. picicornis* and *H. varangensis* are more similar than they are to *H. insignis*, in the latter species being significantly tapered towards apex.

Overall, the male genitalia of *H. varangensis* share morphological characteristics with both *H. insignis* and *H. picicornis*. In most respects, it either takes an intermediate position between the two other species, or it resembles one of the species in particular. Only in the very deep cleft between the two halves of the intermediate appendage does it differ significantly from both the other species. This lends overall support to the hypothesis that *H. varangensis* represents a hybrid between *H. insignis* and *H. picicornis*. These two species are also genetically (COI) very close when compared to *H. dubius* and *H. stagnalis*, indicating that hybridization might occur, as they differ by a distance of about 7% only compared to distances of more than 15% to *H. dubius* or *H. stagnalis* (Boonstra et al. 2018). Also, the larvae of the two species are very similar compared to the larvae of *H. dubius* and *H. stagnalis* (Boonstra et al. 2018).

The hypothesis is further supported by the fact that additional specimens of *H. varangensis* have not been encountered elsewhere for half a century. In this context, it is important to point out that

the type locality is not situated on the Varanger Peninsula as stated on the label of the holotype. Lake Gjøkvatn is situated in the Pasvik Valley very close both to the Norwegian-Finnish and the Norwegian-Russian border. The Trichoptera fauna in this area is well studied (Solem 1970, Andersen & Hagenlund 2012), as is the Trichoptera fauna in northern Finland relatively close to the type locality (e.g. Salmela et al. 2015, Salokannel & Mattila 2018). According to Vladimir Ivanov (*in litt.* 2021), there are also no records of *H. varangensis* from Russia, and he suspects that the species might represent an aberrant form of *H. picicornis*. Finally, there is no indication that *H. varangensis* is found in Northern America.

Whereas *H. varangensis* has only been recorded once, both *H. insignis* and *H. picicornis* are abundant in northern Fennoscandia including areas close to the type locality of *H. varangensis* (Andersen & Hagenlund 2012, Gullefors 2015, Rinne & Wiberg-Larsen 2017, Boonstra et al. 2018, Neu et al. 2018, Salokannel & Mattila 2018). Further, they are both found in Siberia (Yakutia) (Ivanov 2011, Ivanov *in litt.* 2021). Their flight periods are identical (Salokannel & Mattila 2018), whereas larval habitats might differ. In northern areas, the larva of *H. insignis* mainly occurs in oligotrophic, acid pools or ponds (so-called “palsa-mires”, sometimes being less than 5 m² in size), where the larvae live in mats of *Sphagnum* mosses, but it can also be found in more calciferous situations (Boonstra et al. 2018). According to Boonstra et al. (2018), *H. picicornis* may occur in both acid and neutral ponds and lakes, mostly larger than those inhabited by *H. insignis*. Therefore, as the habitats of the two species often occur within short distance of each other, the flight periods overlap, and they probably share the same type of swarming behavior (see Boonstra et al. 2018), they may easily come in contact making interbreeding possible.

There might of course be another possibility why there are no records of *H. varangensis* since its’ original description, namely that the supposed population at Lake Gjøkvatn has gone extinct. However, we find this explanation highly unlikely. Suitable habitats should be widespread in northern Fennoscandia and northwestern Russia and the

region is near pristine with no obvious factors threatening potential habitats.

To conclude, the holotype of *H. varangensis* might represent an aberrant specimen of either *H. insignis* or *H. picicornis*. However, we do not find strong enough support to reject our hypothesis and regard the holotype of *H. varangensis* as a hybrid specimen between the two species. Consequently, *H. varangensis* should be treated as a name not valid under the International Code of Zoological Nomenclature.

Acknowledgements. We are most grateful to Axel Christian, curator at the Senckenberg Museum of Natural History, Görlitz, Germany for giving us the opportunity to study the holotype of *H. varangensis*, and for giving permission to make non-destructive extraction of DNA from one of its legs. Further, we are indebted to Robin E. Thomson, curator at the University of Minnesota's Insect Collection, St. Paul, U.S.A. for giving us the possibility to study a Nearctic specimen of *H. picicornis*. Finally, we thank Marko Mutanen, Ecology and Genetics Research Unit, University of Oulu, Finland for carrying out extraction of DNA from the holotype of *H. varangensis* and taking care of the following analyses at CCDB and Keijo Mattila, ELINT/ESM systems at Patria, Finland to allow us to use his photos of *H. insignis* and *H. picicornis*.

References

- Andersen, T. & Hagenlund, L.K. 2012. Caddisflies (Trichoptera) from Finnmark, northern Norway. *Norwegian Journal of Entomology* 59, 133–154.
- Boonstra, H., Rinne, A., Kubiak, M. & Wiberg-Larsen, P. 2018. Description of the larva of *Holocentropus insignis* Martynov 1924 (Trichoptera: Polycentropodidae) with notes on biology and distribution. *Zootaxa* 4532, 231–247.
- Gullefors, B. 2015. Sveriges nattsländor (Trichoptera), utbredning, vanlighetsgrad, habitat och flygtider. [The Swedish caddisflies (Trichoptera), distribution, frequency, habitats and flight times]. *Entomologisk Tidskrift* 136, 45–161.
- Holzenthal, R.W., Blahnik, R.J., Prather, A.L. & Kjer, K.M. 2007. Order Trichoptera Kirby, 1813 (Insecta), Caddisflies. *Zootaxa* 1668, 639–698.
- Ivanov, V.D. 2011. Caddisflies of Russia: Fauna and biodiversity. *Zoosymposia* 5, 171–209.
- Mey, W. 1987. *Holocentropus varangensis* n. sp. - eine neue Köcherfliege aus Skandinavien (Trichoptera, Polycentropodidae). *Entomologische Nachrichten und Berichte* 31, 255–258.
- Neu, P.J., Malicky, H., Graf, W. & Schmidt-Kloiber, A. 2018. *Distribution Atlas of European Trichoptera*. Die Tierwelt Deutschlands, Teil 84. 891 pp. ConchBooks, Harxheim.
- Prosser, S.W.J., deWaard, J.R., Miller, S.E. & Hebert, P.D.N. 2016. DNA barcodes from century-old type specimens using next-generation sequencing. *Molecular Ecology Resources* 16, 487–497.
- Rinne, A. & Wiberg-Larsen, P. 2017. *Trichoptera larvae of Finland: A key to the caddis larvae of Finland and nearby countries*. 151 pp. Trificon, Finland.
- Salmela, J., Siivonen, S., Dominiak, P., Haarto, A., Heller, K., Kanervo, J., Martikainen, P., Mäkilä, M., Paasivirta, L., Rinne, A., Salokannel, J., Söderman, G. & Vilkkamaa, P. 2015. Insektfångst med Malaise-fälla på naturskyddsområden i Lappland 2012–2014. *Forststyrelsen naturskyddspublicationer, Series A* 221, 9–141. [in Finnish].
- Salokannel, J. & Mattila, K. 2018. *Suomen vesiperhoset. Trichoptera of Finland*. 445 pp. Hyönteistarvike Tibiale Oy, Helsinki.
- Solem, J.O. 1970. Trichoptera from South Varanger, North Norway. *Rhizocrinus* 1(4), 1–8.
- Solem, J.O. & Andersen, T. 1996. *Trichoptera Vårfluer*. Pp. 172–180 in Aagaard, K. & Dolmen, D. (Eds), *Limnofauna Norvegica. Katalog over norsk ferskvannsf fauna*. Tapir, Trondheim.

Received: 8 April 2024

Accepted: 2 May 2024