Hefte zur Tierwelt Deutschlands **5**, 23-31 (2026)
ISSN 2195-4992

Phylogenetic studies in *Trichocera*

(Diptera: Trichoceridae)

Nikola-Michael Prpic-Schäper[1]

I performed a phylogenetic analysis using a selection of publicly available sequence fragments (so-called "DNA barcodes") of the mitochondrial gene Mt-CO1 (this gene encodes subunit I of cytochrome c oxidase) from animals of the genus *Trichocera* (Fig. 1). The analysis reveals two major branches:

Group 1: the taxon *hiemalis*
Group 2: all other taxa

This division, as well as the further branching pattern within Group 2, does not correspond to the currently used internal classification of the genus into the subgenera *Trichocera*, *Metatrichocera*, *Saltrichocera*, and *Staryia* (see Krzeminska 2021).

Group 1: this monophyletic group is formed exclusively by individuals identified as *Trichocera hiemalis*. The group displays a considerable amount of substructuring in northern America.

Fig. 1 (on next page): Phylogenetic tree of selected individuals of the genus *Trichocera*. The analysis is based on the available sequence fragments of the mitochondrial gene *Mt-CO1*. The information content of the sequence fragment (usually less than 700 bp, known as 'DNA barcode') is severely limited due to its short length. However, in closely related species (as in this case), a phylogenetic signal can often still be recovered. For the phylogenetic tree, the genetic distance between the sequence fragments was calculated (the scale indicates the extent of genetic change). Distance-based methods are only meaningful for DNA sequences that evolve according to the molecular clock, which applies to only a few genes, but is approximately the case for the *Mt-CO1* gene. The distance calculations shown are based on a sequence alignment performed using Clustal Omega (Sievers & Higgins 2021; Madeira et al. 2024). The phylogenetic tree was rooted using the species *Tipula oleracea*. The letter-number combinations shown in the phylogenetic tree are the accession numbers of the respective sequences in GenBank (Sayers et al. 2022) and the BOLDsystems database (Ratnasingham & Hebert 2007), respectively.

[1] Author contact: Nikola-Michael Prpic-Schäper, Institut für Zoologie, Justus-Liebig-Universität Gießen, Heinrich-Buff-Ring 38, 35392 Gießen.
E-mail: nprpi@zo.jlug.de

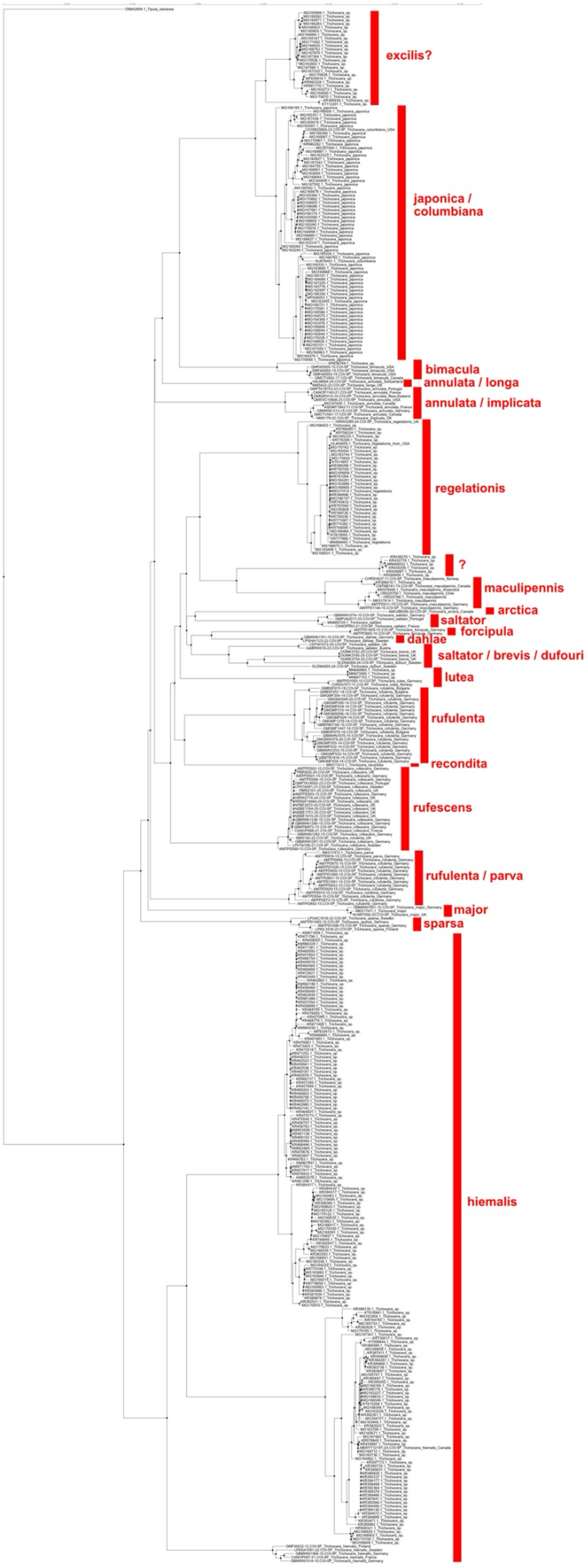


Fig. 1 (legend on previous page)

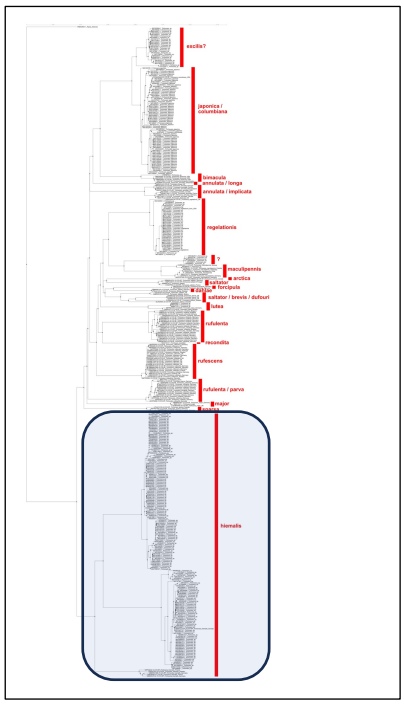


Fig. 2 (legend on next page)

Group 2: At the base of this monophyletic group are the taxa *sparsa* and *major*, each forming a monophyletic group. This is followed by a monophyletic group comprising individuals identified as either "parva" or "rufulenta". It is interesting to note that the taxon *rufulenta* forms two completely separate groups in the analysis. I therefore suspect that there are inaccuracies in the identification of this taxon, and that as a result, individuals of the taxon *parva* are often misidentified as belonging to the taxon *rufulenta*. It is possible that the "rufulenta" individuals in the "rufulenta/parva" monophyletic group are misidentified *Trichocera parva*, and the second monophyletic "rufulenta" group could then represent the actual taxon *Trichocera rufulenta*.

The remaining members of Group 2 are subdivided into two large monophyletic clades in the tree:

> Subgroup A: this subgroup comprises the taxa *regelationis*, *maculipennis*, *arctica*, *saltator*, *forcipula*, *dahlae*, *brevis*, *dufouri*, *lutea*, *rufulenta*, *recondita* and *rufescens*.
>
> Subgroup B: this subgroup comprises the taxa *excilis* (presumably; see below), *japonica*, *columbiana*, *bimacula*, *annulata*, *longa* and *implicata*.

In Subgroup A, the taxon *rufescens* (with the exception of the likely misidentified specimen with accession number NMS182-22.COI-5P, which was identified as *Trichocera rufulenta*) forms a monophyletic group. The taxon *recondita* is also distinct. It forms the sister group to a monophyletic clade comprised of specimens identified as "*rufulenta*". As explained above, this group may represent the actual *Trichocera rufulenta*.

The next distinct branch is the taxon *lutea* as a monophyletic group. Marginally distinct from the other groups is a monophyletic group comprising the taxa *arctica*, *saltator*, *forcipula*, *dahlae*, *brevis*, and *dufouri*. Internally, however, this grouping is problematic: while the taxa *arctica*, *forcipula*, and *dahlae* are better defined and appear as monophyla, the taxon *saltator* is polyphyletic, and the taxon *dufouri* is paraphyletic with respect to the taxon *brevis*.

Fig. 2 (on previous page): Magnified view of Group 1 in the phylogenetic tree in Fig. 1. The magnified portion is indicated by the box in the inset.

Fig. 3 (on next page): Magnified view of Group 2 in the phylogenetic tree in Fig. 1. The magnified portion is indicated by the box in the inset.

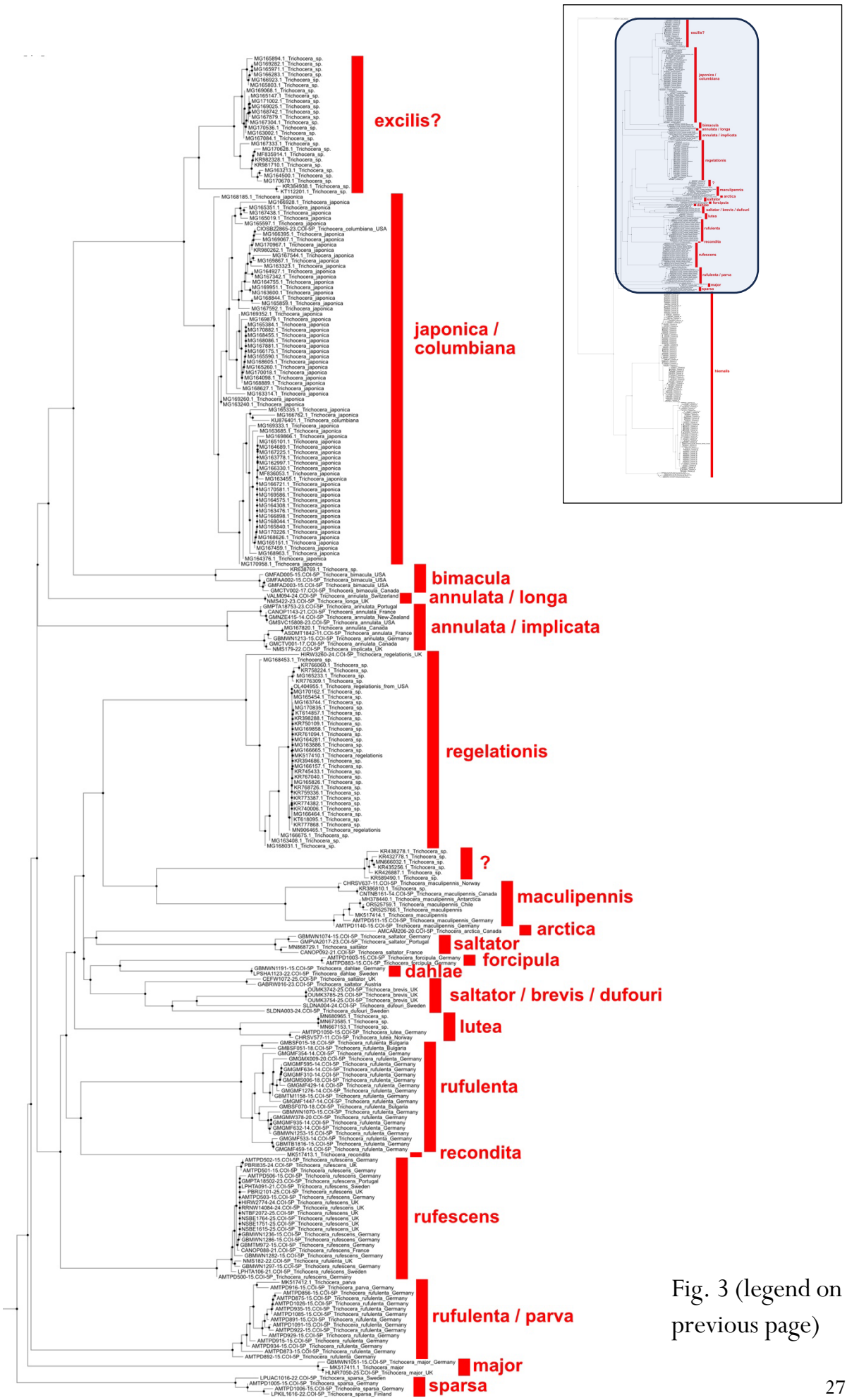


Fig. 3 (legend on previous page)

Interestingly, these issues reflect current taxonomic difficulties: Krzeminska (2021) has already pointed out that the taxon *saltator* appears to consist of two morphologically distinct groups: one group (the "true" *Trichocera saltator*) whose females possess long ovipositors, and a second group whose females possess short ovipositors and therefore resemble the females of *Trichocera brevis*. This could explain the results of the phylogenetic analysis, since two "saltator" groups are also present there: a clearly distinct and monophyletic "saltator" group and a second "saltator" group that clusters together with individuals identified as "brevis". The two sequences from specimens identified as "dufouri" also fall into this second group. However, these form a monophyletic group only when combined with the specimens identified as "brevis". The "dufouri" specimens could therefore be misidentified *brevis* specimens. Alternatively, the taxa *brevis* and *dufouri* may be synonyms. Another possibility to consider, however, is that the taxon *brevis* evolved from the taxon *dufouri*, since in such analyses at the individual level, species do not have to appear monophyletic as long as they behave biologically as two distinct species (i.e., they do not interbreed). It is evident, therefore, that there is a considerable need for further research on this topic.

Finally, in Subgroup A, the taxa *regelationis* and *maculipennis* form clearly defined monophyletic groups. A distinct monophyletic group comprising individuals from North America appears to be the sister group of *maculipennis*; I am unable to assign a name to this group and have therefore marked it with a question mark in Fig. 1 and Fig. 3.

In Subgroup B, certain animals identified as "annulata" and "implicata" form a monophyletic group. Both taxa, *annulata* and *implicata* are very similar in the structure of both the male and female genitalia, making misidentifications quite likely. Synonymy between the two taxa is also a possibility. However, the taxon *annulata* is not monophyletic in the analysis: an "annulata" specimen from Switzerland forms another monophyletic group together with an animal identified as "longa" from the United Kingdom of Great Britain and Northern Ireland. Further research is therefore needed on this topic as well.

The taxon *bimacula* forms another distinct monophyletic group within Subgroup B. The next monophyletic group mainly consists of individuals identified as "japonica". This group includes two individuals identified as "columbiana", which may be misidentifications, however, as Krzeminska (2021) points out that *Trichocera columbiana* is poorly characterized in the existing identification literature, leading to frequent misidentifications. Finally, there is another monophyletic group consisting

of North American specimens that represents the sister group to the "japonica"/"columbiana" specimens. Since this apparently represents a "japonica"-related taxon from North America, this could be the taxon *excilis*, which was long considered synonymous with *Trichocera japonica*, but is now regarded as a separate taxon (see discussion in Krzeminska (2021)). However, since this is uncertain, I have marked the taxon name *excilis* with a question mark in Fig. 1 and Fig. 3.

In this analysis, it is immediately apparent that the assessment of the internal structure of the two main groups (Group 1 and Group 2) differs significantly. In Group 1, despite obvious internal structuring and holarctic distribution, only a single species – namely *Trichocera hiemalis* – is recognized. In Group 2, however, a very similar internal structure and similar worldwide distribution has attracted considerably more attention from taxonomists, and each lineage is recognized as a separate species. One might question whether such unequal treatment is appropriate. Two approaches are conceivable:

(a) The lineages in Group 1 are also recognized as separate species and formally described accordingly.

(b) As in Group 1, no taxonomic significance is attached to the internal substructure within Group 2, and the entire Group 2 is regarded as a single species bearing the oldest available name *Trichocera regelationis* Linnaeus, 1758.

Salokannel et al. (2026) have recently investigated phylogenetic relationships based on new DNA barcoding sequence data obtained from Finnish specimens. They report a good congruence of morphologically delimited species (mainly based on genitalia morphology) with phylogeny based on barcode sequences. Unfortunately, the phylogenetic analysis by Salokannel et al. (2026) does not specify the root that has been used in the analysis. Because rooting has significant impact on the branching pattern and evolutionary trajectory reconstruction, and therefore contains crucial information for topology comparisons between independent phylogenetic analyses, it is difficult to compare their results with the results presented in the present study. There is therefore still a considerable need for further research in *Trichocera* phylogenetics and evolution, which ideally should include not only additional genetic data (e.g., sequences of nuclear genes or entire genomes), but also the often

unknown biology of the taxa (e.g. gene flow analyses or studies on reproductive biology).

Sources and references

Krzeminska E. 2021. Key and atlas to the genus Trichocera Meigen in Europe (Diptera, Trichoceridae). Acta Zoologica Cracoviensia 64, 1-157.

Madeira F, Madhusoodanan N, Lee J, Eusebi A, Niewielska A, Tivey ARN, Lopez R, Butcher S. 2024. The EMBL-EBI Job Dispatcher sequence analysis tools framework in 2024. Nucleic Acids Research 52(W1):W521-W525.

Ratnasingham S, Hebert PDN. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7, 355-364.

Salokannel J, Viitanen E, Kakko A, Nyström H, Elo R. 2026. Finnish winter gnat (Diptera, Trichoceridae) fauna in the light of morphology and DNA barcodes. Memoranda Societatis pro Fauna et Flora Fennica 102, 60-73.

Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, Connor R, Funk K, Kelly C, Kim S, Madej T, Marchler-Bauer A, Lanczycki C, Lathrop S, Lu Z, Thibaud-Nissen F, Murphy T, Phan L, Skripchenko Y, Tse T, Wang J, Williams R, Trawick BW, Pruitt KD, Sherry ST. 2022. Database resources of the national center for biotechnology information. Nucleic Acids Research 50(D1):D20-D26.

Sievers F, Higgins DG. 2021. The Clustal Omega Multiple Alignment Package. Methods in Molecular Biology 2231, 3-16.

Hefte zur Tierwelt Deutschlands **5**, 23-31 (2026)
ISSN 2195-4992

Supplementary information

The phylogenetic tree shown in Fig. 1 and the sequence dataset used in its reconstruction are available for download from the Zoographia Germaniae website:

File #1: Not annotated phylogenetic tree with high resolution
File name: SI_HTD_5_23-31_Trichocera_phylogeny.pdf
File size: 458 kb

File #2: List of the DNA barcode sequences used in the phylogenetic analysis
File name: SI_HTD_5_23-31_Trichocera_barcodes_data_set.fasta
File size: 300 kb

Publication date of this article: November 5, 2026

© The Author 2026. All rights reserved.
The Author does not warrant or assume any legal liability or responsibility for the accuracy, currency, completeness, or usefulness of any information, apparatus, product, or process disclosed. The Author acknowledges the rights of others to protect their copyrights, patent and trademark rights. The mentioning or display of registered trademarks or items protected by copyright or other rights in this publication does not mean that the Author holds any rights in it or that rights in it are free or available. The Author does not endorse or recommend any commercial products, processes, or services.