Limits of classical molecular markers in resolving the *Cheilosia crassiseta* species complex (Diptera: Syrphidae)

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Introduction

- The hoverfly genus *Cheilosia* Meigen, 1822 (Eristalinae: Rhingiini) is one of the largest genera of hoverflies
- Thirteen subgenera, one of which is *Teniochilosia*
- Some taxa remain poorly resolved, with persistent taxonomic uncertainties

- This study focuses on the *Cheilosia crassiseta* species complex on the Balkan Peninsula
- Presence of three potential taxa (*Cheilosia crassiseta*, *C.* aff. *crassiseta*, and *C*. aff. *vangaveri*)
- Aim - to achieve a more comprehensive understanding of the diversity within this group and to resolve ambiguous species boundaries, using molecular data, and by covering populations from both the Balkans and the Alps

Material and Methods

- In total 34 specimens belonging to the *Cheilosia crassiseta* species complex
- Genomic DNA - NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany)
- Amplification and sequencing:
  - 3′ and 5′ regions of the mitochondrial COI gene
  - nuclear 28S rRNA gene
  - ITS2 region
- The 3′ and 5′ sequence fragments of COI gene were aligned manually
- The D2–3 domain of the 28S rRNA gene was aligned using the G-INS-i strategy implemented in MAFFT version 7
- The nuclear ITS2 sequences were aligned using the E-INS-i alignment strategy implemented in MAFFT version 7
- Combined into a single sequence matrix
- Bayesian and Maximum Likelihood trees - both trees rooted on *Cheilosia derasa*.

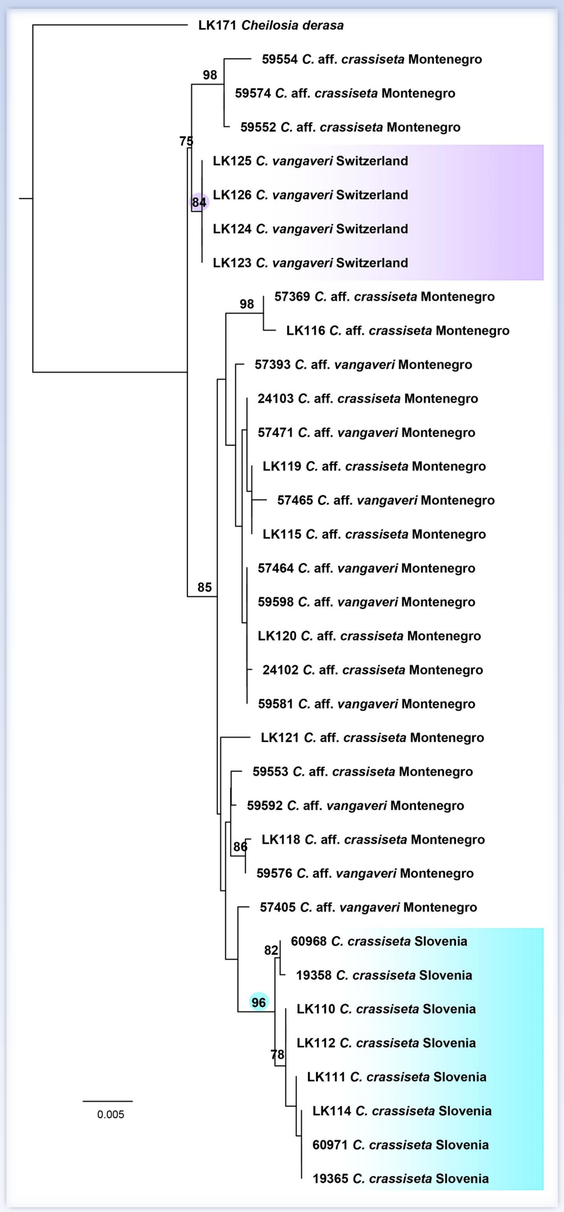


Figure 1. Maximum Likelihood tree of the Cheilosia crassiseta species complex based on combined 3′ and 5′ COI, 28S rRNA and ITS2 sequences. Bootstrap values are indicated near nodes

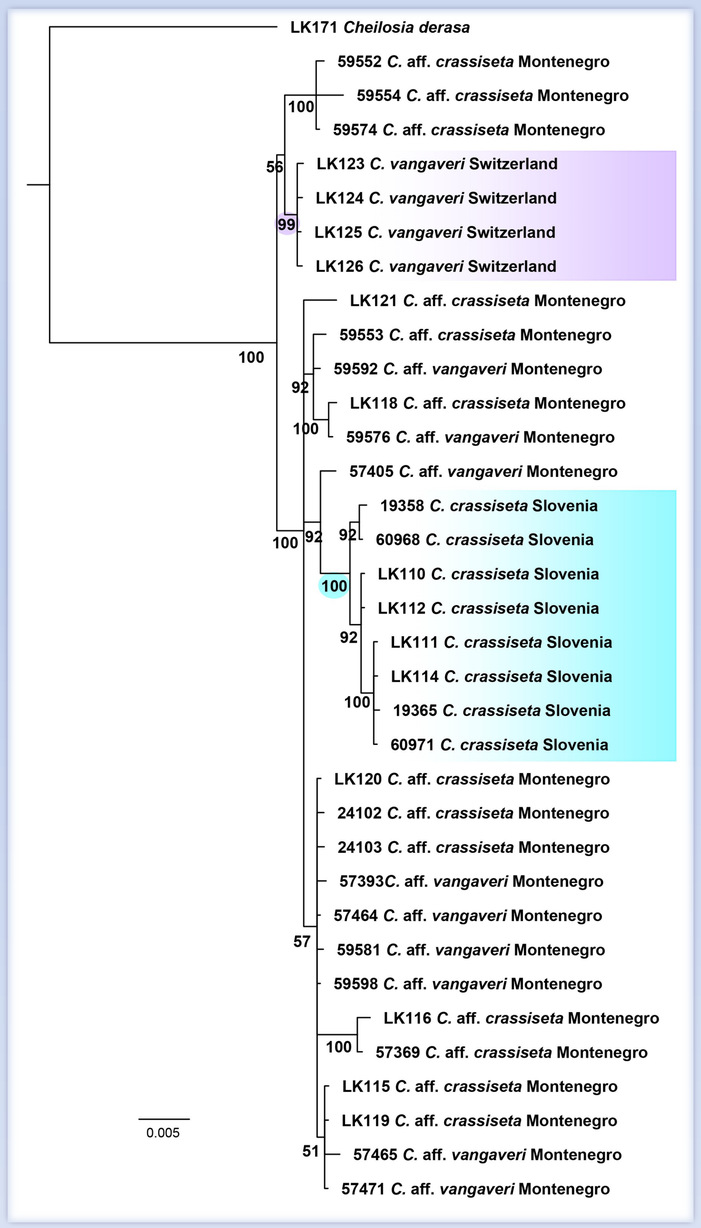


Figure 2. Bayesian tree of the Cheilosia crassiseta species complex based on combined 3′ and 5′ COI, 28S rRNA and ITS2 sequences. Bayesian posterior probabilities are indicated near nodes

Results

- Molecular data supported the presence of two taxa in Alpine region:
- *C. crassiseta* and *C. vangaveri*

- No sufficient resolution reached to delimit the morphologically well-defined taxa from the Balkans

- Two of them from Montenegro: *C.* aff. *crassiseta* and *C*. aff. *vangaveri* show clear morphological and molecular differences in comparison to taxa occurring in the Alpine region, but could not be mutually delineated

- The necessity of introducing novel approaches

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