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Cheilosia vernalis (Diptera, Syrphidae) complex: molecular and morphological variability

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Genus *Cheilosia* (Diptera, Syrphidae) with more than 300 Palaearctic species is the most speciose group of hoverflies in Europe. One of the most widespread taxa with unclear taxonomic status is *Cheilosia vernalis* (Fallen, 1817). This species shows great morphological variation in external morphological characters. A subdivision of the taxon has been suggested, but the male terminalia of the different morphological forms appear identical. The aim of this study was to integrate the information from mitochondrial COI sequence data with re-evaluated morphological characters for exploring species boundaries and for revealing phylogenetic relationships of *C. vernalis* and closely related species. We sequenced a 698 bp fragment of the 3´-end of the mitochondrial gene cytochrome oxidase subunit I of 43 specimens agreeing with traditional morphological interpretation of *C. vernalis* (Diptera, Syrphidae), sampled from a broad geographic range in Europe and single representatives of 10 species of the *vernalis–melanura* complex. Parsimony analysis of the molecular data showed *C. vernalis* specimens grouped in three different lineages. The *C. vernalis* lineage presents 8 different mitochondrial haplotypes. We found no congruence between these haplotypes and diagnosable morphological characteristics of the adult fly specimens supporting species subdivision. Based on re-examination of type material two new synonyms are proposed: *C. rotundiventris* Becker, 1894 and *C. ruficollis* Becker, 1894 are junior synonyms of *C. vernalis* (Fallen, 1817). New diagnostic morphological character for *C. reniformis* Hellen, 1930 is added and first distribution data of this species in Europe are given. Taxonomic status of *C. longifila* Becker, 1894 remains unresolved.

Introduction

The genus *Cheilosia* (Diptera, Syrphidae) is a genus with Holarctic distribution, comprising more than 300 Palaearctic species. It is the most speciose group of hoverflies in Europe. The species of *Cheilosia* are typically dark with shiny thorax and abdomen, with their pilosity ranging from black to yellow and red. Adults are commonly encountered visiting flowers, beginning in early spring on flowers of *Salix* spp. and during the summer on yellow (e.g. *Ranunculus*

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spp.) or white (Compositae) flowers. Flowers are visited for ingestion of nectar and pollen. The immature stages are known for about thirty species, the feeding modes comprise saprophagy, phytophagy, fungivory and sap feeding of coniferous trees (Rotheray 1993, Stuke 2000, Stuke & Carstensen 2000, 2002). Barkalov's (2002) subgeneric classification of the genus *Cheilosia* comprised ten subgenera, of which subgenus *Hiatomyia* is exclusively Nearctic and sg. *Endoiasimyia* is Oriental in distribution.

One of the most widespread taxa with unclear taxonomic status is *C. vernalis* (Fallen, 1817). *Cheilosia vernalis* belongs to the *melanura*-group of species in *Cheilosia s. str*. According to Vujić (1992), the *melanura*-group contained > 11 European species with the least differentiated male genitalia of the genus. Presently, this group comprises 13 taxa: *C. bergenstammi* Becker, 1894; *C. brachysoma* Egger, 1860; *C. bracusi* Vujić & Claussen, 1994; *C. carbonaria* Egger, 1860; *C. chloris* (Meigen, 1822); *C. cynocephala* Loew, 1840; *C. fraterna* (Meigen, 1830); *C. lenis* Becker, 1894; *C. lenta* Becker, 1894; *C. melanura* Becker, 1894; *C. rhynchops* Egger, 1860; *C. rufimana* Becker, 1894; *C. sootryeni* Nielsen, 1970; and *C. vernalis* (Fallen, 1817). Distributions and ecological data of all species are presented in Speight (2006). An identification key based on allozyme markers of eight species occurring on the Balkan Peninsula was recently published by Milankov *et al*. (2002b).

Cheilosia vernalis is a widespread taxon known from most European countries and from Siberia (Russia), and has also been registered from the Oriental region (Peck 1988). It is a small robust black species, with the tibiae having strong orange bases and tips; sternites 2–4 are brightly shining and entirely undusted; the eye pilosity is very dark (Stubbs & Falk 2002). This species shows great morphological variation in such characters as coloration and length of body pilosity (hairs), shape of facial profile, size and colour of antennae and structure of arista. The immature stages remain undescribed, but the taxon is known to be an internal feeder in a variety of plants, the stems of *Achillea millefolium, Cirsium salisburgense, Matricaria chamomilla* and *Sonchus oleraceus* and in the involucre of *Tragopogon* (Bankowska 1980, Torp 1984, Stuke 2000). In the Palaearctic catalogue (Peck 1988) six synonyms of *C. vernalis* were listed. Additionally, Barkalov (1993) synonymized *C. rotundicornis* (Hellen, 1914) with *C. vernalis*. In addition to *C. rotundicornis*, a few names for morphologically similar taxa with unresolved taxonomic status are listed in the Palaearctic catalogue (Peck 1988) but recently some were included in identification keys and checklists (e.g. Ssymank 1999, van Veen 2004), namely *C. longifila* Becker, 1894; *C. rotundiventris* Becker, 1894 and *C. ruficollis* Becker, 1894.

Based on the variable morphology some authors have suggested that *C. vernalis* constitute a species complex of morphologically cryptic species (Speight & Lucas 1992, Vujić 1996, Speight 2006). The taxon exhibits seasonal dimorphism, the spring brood being entirely or predominantly brown haired, frequently with an orange basoflagellomere, while the summer brood is often predominantly dark haired, with a dark basoflagellomere. Milankov *et al*. (2002a) studied the geographical variation of genetic variability in four populations of *C. vernalis* from different habitats from the Balkan Peninsula using allozymes. They found no correlation between morphological character patterns and particular genotypes or alleles. Specimens presenting distinct morphological traits did not have unusual genotypes, and specimens with unique genotypes had no particular morphological differences when compared with specimens with the most frequent genotypes.

The aim of this study was to explore the informativeness of a fragment of mitochondrial COI 3´-region (cox 1) sequences in conjunction with morphological characters for potential species delimitation, and to use the DNA characters for exploring phylogenetic relationships of *Cheilosia vernalis* and closely related species. Fast evolving mitochondrial DNA (mtDNA), and particularly the cytochrome-*c* oxidase subunit I (COI), is an extensively used molecular marker for insects. It has proven useful for reconstructing phylogenetic relationships among closely related species, for studying population history and has proven informative for species delimitation in many insect groups (e.g. Simon *et al*. 1994, Zhang & Hewitt 2003, Avise 2004). Genome information should be an active com-

ponent of modern taxonomy, but DNA should not be the sole source of information retrieval. Integrative taxonomy, making use of both morphological and molecular characters, results in potentially better understood and supported species hypotheses.

To resolve the taxonomic and nomenclatural problems of above listed taxa with unclear taxonomic status, the available type material of these taxa was studied.

Material and methods

Specimen sampling

A total of 43 specimens (Table 1; for type material *see* Appendix) were used for COI-3´ sequencing. Male genitalia were preserved separately for the purpose of morphological studies and DNA voucher specimens (body parts that remain after allozyme and DNA analysis) are deposited in the Zoological Museum (MZH) of the Finnish Museum of Natural History (Helsinki, Finland).

DNA was extracted from legs or other parts of single frozen individuals (–20 °C) using the Nucleospin Tissue DNA extraction kit (Machery-Nagel) following manufacturer's protocols and re-suspended in 50 μl of ultra-pure water. Amplification was attempted for both COI and the nuclear Internal Transcribed Spacer 2 region, but the PCR amplifications of the latter one were unsuccessful.

DNA sequencing

PCR reactions were carried out in 25 μl reactions containing 2 μl DNA extract, 1 μl of each primer (at 10 pmol μl^{-1}), 0.25 μl of DNA polymerase (5U μl^{-1}), 2 μl 2.5 mM $MgCl_2$, 2.5 μl 10X Buffer II (MBI Fermentas) and 4 μl 200 mM dNTP (GeneAmp) and ultrapure water. Thermocycler conditions were initial denaturing at 95 °C 2 min, 29 cycles of 30 s denaturing at 94 °C, 30 s annealing at 49 °C, 2 min extension at 72 °C, followed by a final extension of 8 min at 72 °C. The universally conserved primers used for amplifying and sequencing the COI fragment (738-bp) were the forward primer C1-J-2183 (5´-CAA CAT TTA TTT TGA TTT TTT GG-3´) (alias JERRY) and the reverse primer TL2-N-3014 (5´-TCC AAT GCA CTA ATC TGC CAT ATT A-3´) (alias PAT) (Simon *et al*. 1994). PCR products were purified using the GFX PCR Purification Kit (Amersham Biotech) and then sequenced (with the PCR primers) in both directions using the Big Dye Terminator Cycle Sequencing Kit ver. 1.1 (Applied Biosystems) at one-fourth of the recommended volumes on an ABI PRISM 377 (Applied Biosystems) semi-automated DNA sequencer. The sequences were edited for base-calling errors and assembled using Sequence Navigator (ver. 1.01).

Parsimony analysis

Nucleotide divergences within and between species were calculated using uncorrected *p* distances. Parsimony analysis was performed using NONA (Goloboff 1999) and spawn with the aid of Winclada (Nixon 2002), using heuristic search algorithm with 1000 random addition replicates (mult × 1000), holding 100 trees per round (hold/100), maxtrees set to 100 000 and applying TBR branch swapping. All base positions were treated as equally weighted characters.

Morphological studies

The morphological analysis included 20 characters. We used characters of the head (facial profile, presence and distribution of eye pilosity), of the antennae (colour of basoflagellomere, presence and distribution of aristal hairs), the wing vein patters, the shape of sternites and tergites and the presence or absence of pollinosity on tergites (dusting), coloration of different parts of legs, colour of body pilosity.

All specimens (or remnants of specimens) used for DNA sequencing were morphologically analyzed. Additionally, more than 800 specimens from the European part of the distributional range of *Cheilosia vernalis* were studied, including samples from Finland to Balkan Peninsula; from UK to Romania (Museum, MZH, collection of Department of Biology, University of Novi Sad).

Table 1. List of included specimens. DNA voucher numbers, collecting locality and GenBank accession numbers.

DNA voucher no.	Taxon	Locality and date	GenBank accessions COI
VM177	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Kopaonik, 6.VI.1998	EF091448
VM221	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 23.VIII.1999	EF091461
VM124	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 24.V.1996	EF091445
VM207	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 24.V.1996	EF091455
VM524	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Järfälla, 26.IV.2002, H. Bartsch leg.	EF091465
VM526	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Nynäshamn, 6.VI.2003, H. Bartsch leg.	EF091466
VM222	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 23.VIII.1999	EF091462
VM521	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Upplands-Bro, 9.V.2002, H. Bartsch leg.	EF091463
VM522	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Järfälla, 26.IV.2002, H. Bartsch leg.	EF091464
VM523	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Järfälla, 26.IV.2002, H. Bartsch leg.	EF091470
Y157	*Cheilosia vernalis* (Fallen, 1817)	Russia, Siberia, 2000, A. Barkalov	EF091468
VM176	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 8.V.1998	EF091447
VM215	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Morinj, 16.III.1998	EF091456
VM216	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Morinj, 16.III.1998	EF091457
VM217	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Morinj, 16.III.1998	EF091458
VM218	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Morinj, 16.III.1998	EF091459
VM179	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Morinj, 16.III.1998	EF091449
VM220	*Cheilosia vernalis* (Fallen, 1817)	Montenegro, Durmitor, 23.VIII.1999	EF091460
Y137	*Cheilosia vernalis* (Fallen, 1817)	Finland, Helsinki, Vuosaari, 2.V.2004, S. Kerppola	EF091469
Y138	*Cheilosia vernalis* (Fallen, 1817)	Finland Helsinki, Vuosaari, 2.V.2004, S. Kerppola	EF091467
VM182	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091450
VM186	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091451
VM188	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091452
VM198	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091454
VM230	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091446
VM193	*Cheilosia vernalis* (Fallen, 1817)	Serbia, Fruška Gora, 31.III.2000	EF091453
VM669	*Cheilosia vernalis* (Fallen, 1817)	Finland, Aland Islands, Södersundav, 10.V.2005	EF091472
VM671	*Cheilosia vernalis* (Fallen, 1817)	Finland, Aland Islands, Södersundav, 10.V.2005	EF091473
VM673	*Cheilosia vernalis* (Fallen, 1817)	Finland, Aland Islands, Jomala, 10.V.2005	EF091474
VM674	*Cheilosia vernalis* (Fallen, 1817)	Finland, Li: Utsjoki, 6.VII.2004, J. Kahanpää	EF091475
VM675	*Cheilosia vernalis* (Fallen, 1817)	Finland, Li: Utsjoki, 6.VII.2004, J. Kahanpää	EF091476
VM676	*Cheilosia vernalis* (Fallen, 1817)	Finland, LKW: Kittilä, 8.VII.2004, J. Kahanpää	EF091477
VM679	*Cheilosia vernalis* (Fallen, 1817)	The Netherlands Doorn Postweg, 5.V.2005, 153.3 + 449.3 K. Goudsmits leg.	EF091478
VM680	*Cheilosia vernalis* (Fallen, 1817)	The Netherlands, Doorn Postweg, 5.V.2005, 153.3 + 449.3 K. Goudsmits leg.	EF091479
VM681	*Cheilosia vernalis* (Fallen, 1817)	The Netherlands, Remmerden, Uiterwaarden 11.IV.2005, 164.6 + 444.7 K. Goudsmits leg.	EF091480
VM804	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Uppland, Järfälla, 13–17.5.2005, H. Bartsch leg.	EF100773
VM805	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Uppland, Järfälla, 13–17.V.2005, H. Bartsch leg.	EF100774
VM806	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Uppland, Järfälla, 13–17.V.2005, H. Bartsch leg.	EF091481
VM807	*Cheilosia vernalis* (Fallen, 1817)	Sweden, Uppland, Järfälla, 13–17.V.2005, H. Bartsch leg.	EF091482
VM813	*Cheilosia vernalis* (Fallen, 1817)	Finland, N: Helsinki, Vuosaari, 27.V.2005, S. Kerppola leg.	EF091484
VM814	*Cheilosia vernalis* (Fallen, 1817)	Finland, N: Helsinki, Vuosaari, 27.V.2005, S. Kerppola leg.	EF091485
VM815	*Cheilosia vernalis* (Fallen, 1817)	Finland, N: Helsinki, Vuosaari, 27.V.2005, S. Kerppola leg.	EF091486

continued

Table 1. Continued.

DNA voucher no.	Taxon	Locality and date	GenBank accessions COI
S527	*Cheilosia vernalis* (Fallen, 1817)	Estonia Tartu, Moisavahe 42-46 (ME87), 9.IX.2004, T. Keskula leg.	EF100775
Y293	*Cheilosia vernalis* (Fallen, 1817)	Germany, Hessen, Utphe, Norloff-an GK: 34929-55891, 5.V.2005, A. v. Eck.	EF100772
Y297	*Cheilosia vernalis* (Fallen, 1817)	Finland, KiL: Kittila Rauhala, 75369: 3831. 15.VII.2005, leg. A. Haarto	EF100771
Y217	*Cheilosia vernalis* (Fallen, 1817)	Finland, Om: Raahe, 17.V.2004, K. Varpenius	EF091471
Y48	*Cheilosia vernalis* (Fallen, 1817)	Finland, N: Vantaa, Keimola	EF091441
Y50	*Cheilosia vernalis* (Fallen, 1817)	Finland, N: Vantaa, Keimola	EF091442
VM5	*Cheilosia melanura* Becker, 1894	Montenegro, Durmitor, 20.VI.1998	EF091434
VM2	*Cheilosia rhynchops* Egger, 1860	Montenegro, Durmitor, 23.VI.1998	NS
VM19	*Cheilosia rhynchops* Egger, 1860	Serbia, Kopaonik, 19.VI.1996	EF091439
VM10	*Cheilosia bergenstammi* Becker, 1894	Montenegro, Durmitor, 20.VI.1998	EF091433
VM4	*Cheilosia bracusi* Vujic & Claussen, 1994	Montenegro, Durmitor, 20.VI.1998	EF091440
VM8	*Cheilosia carbonaria* Egger, 1860	Montenegro, Durmitor, 25.VI.1997	EF091438
VM94	*Cheilosia lenta* Becker, 1894	Montenegro, Durmitor, 8.V.1998	EF091437
VM39	*Cheilosia lenis* Becker, 1894	Serbia, Kopaonik, 6.VI.1998	EF091435
VM93	*Cheilosia lenis* Becker, 1894	Montenegro, Durmitor, 3.VI.1998	EF091436
VM525	*Cheilosia fraterna* (Meigen, 1830)	Sweden, Järfälla, 26.IV.2002, H. Bartsch leg.	EF091444
VM155	*Cheilosia vujici* Claussen & Doczkal, 1996	Serbia, Kopaonik, 1996	EF091432

Results

COI variation

As compared with *Drosophila yakuba* mitochondrial DNA sequence (Clary & Wolstenholme 1985), we obtained a fragment 698 bp of COI spanning nucleotide positions 2232 to 2930 (for GenBank accessions numbers *see* Table 1).

Uncorrected pairwise interspecific divergences varied between 6.2% to 5.7% between the outgroup and ingroup taxa, between 0.6%–6.3% between ingroup taxa, while the intraspecific values for *Cheilosia vernalis s.l.* ranged from 0.1% to 3.2%.

COI sequences and haplotypes

A total of nine haplotypes were registered for the 46 sequenced specimens (Table 2). The haplotype A1 is represented by two female specimens from two high mountains on the Balkan Peninsula. The differences from typical *Cheilosia vernalis* are a more or less straight wing vein M1 and shorter hairs on lower part of eyes and mesonotum. The presence and distribution of eye pilosity is similar to *C. brachysoma* Egger, 1860 described from central Europe, but other characters are more related to *C. vernalis*. Morphological analysis of specimens from same localities and date, that are not used for COI-3´ sequencing, showing possible diagnostic morphological characters of this taxon: eye hairs relatively shorter in both sexes in comparison with *C. vernalis*; males with predominantly black-haired scutum and presence of black hairs on the lateral margins of tergites, at least on posterior corner of tergite 2; abdomen more slender than in *C. vernalis*, relation between length and width of sternite 3 from 1:1.6 to 1:1.7 in males and from 1:2 to 1:2.3 in females; frons in female shiny, without microtrichia.

We conclude that the identity of this taxon remains unclear without more data (molecular data for additional specimens, especially males).

Based on molecular data this is clearly supported as separate species. If mentioned diagnostic characters show consistency in additional specimens, none of the analysed type specimens correspond with this taxon (*C. rotundiventris*, *C. rotundicornis* and *C. ruficollis*). Also the original

Table 2. Mitochondrial COI haplotype variation of 29 non-continuous sites of obtained *Cheilosia vernalis* sequences.

			22222222222222222222222222222 22444445555556666667777778888 79456792588990145993467992339 19571263669257918459832061283 * * ** * *
VM177	Serbia, Kopaonik	A1	TTCCTCATCATCTTTGTATTGTTATGCAA
VM221	Montenegro, Durmitor	A1	.....
VM193	Serbia, Fruška Gora	B1a	CATTC..ATTAT..A.CG..AACTCATGC
VM230	Serbia, Fruška Gora	B1a	CATTC..ATTAT..A.CG..AACTCATGC
VM182	Serbia, Fruška Gora	B1b	CATTC..ATTAT..A.CG..AACTCAT.C
VM186	Serbia, Fruška Gora	B1b	CATTC..ATTAT..A.CG..AACTCAT.C
VM188	Serbia, Fruška Gora	B1b	CATTC..ATTAT..A.CG..AACTCAT.C
VM198	Serbia, Fruška Gora	B1b	CATTC..ATTAT..A.CG..AACTCAT.C
Y138	Finland, Helsinki	B2	CATTC..ATTAT..A..G..AA.TCAT.C
Y137	Finland, Helsinki	B2	CATTC..ATTAT..A..G..AA.TCAT.C
VM215	Montenegro, Morinj	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM216	Montenegro, Morinj	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM217	Montenegro, Morinj	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM218	Montenegro, Morinj	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM179	Montenegro, Morinj	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM176	Montenegro, Durmitor	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM679	Netherlands	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM673	Finland, Aland	C1	CATT...ATTATCAA.C.C.AA..CAT.C
Y293	Germany, Hessen	C1	CATT...ATTATCAA.C.C.AA..CAT.C
VM521	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM522	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM523	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM804	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM806	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM807	Sweden, Järfälla	C2	CATT...ATTATCAA...C.AA..CAT.C
VM669	Finland, Aland	C2	CATT...ATTATCAA...C.AA..CAT.C
VM671	Finland, Aland	C2	CATT...ATTATCAA...C.AA..CAT.C
VM675	Finland, Utsjoki	C2	CATT...ATTATCAA...C.AA..CAT.C
VM674	Finland, Utsjoki	C2	CATT...ATTATCAA...C.AA..CAT.C
VM813	Finland, Helsinki	C2	CATT...ATTATCAA...C.AA..CAT.C
VM814	Finland, Helsinki	C2	CATT...ATTATCAA...C.AA..CAT.C
VM815	Finland, Helsinki	C2	CATT...ATTATCAA...C.AA..CAT.C
Y157	Russia, Siberia	C2	CATT...ATTATCAA...C.AA..CAT.C
S527	Estonia, Tartu	C3	CATT...ATTATCAA...CAAA..CAT.C
Y217	Finland, Raahe	C4	CATT...ATTATCAA.C...AA..CAT.C
VM220	Montenegro, Durmitor	C5	CATT...ATTATCAAAC.C.AA..CAT.C
VM222	Montenegro, Durmitor	C6	CATT..GATTATCAA...C.AA..CAT.C
VM680	Netherlands	C7	CATT...GTTATCAA...C.AA..CAT.C
VM805	Sweden, Järfälla	C7	CATT...GTTATCAA...C.AA..CAT.C
VM124	Montenegro, Durmitor	C7	CATT...GTTATCAA...C.AA.------
VM207	Montenegro, Durmitor	C8	CATT..GGTTATCAA...C.AA..CAT.C
VM524	Sweden, Järfälla	C8	CATT..GGTTATCAA...C.AA..CAT.C
VM526	Sweden, Järfälla	C8	CATT..GGTTATCAA...C.AA..CAT.C
VM676	Finland, Kittilä	C8	CATT..GGTTATCAA...C.AA..CAT.C
Y297	Finland, Kittilä	C8	CATT..GGTTATCAA...C.AA..CAT.C
VM681	Netherlands	C9	CATT.T.ATTATCAA...C.AA..CAT.C

description of *C. longifila* (Becker, 1894) does not fit this diagnosis. The only indication about a possible name of this taxon is the analysed material from Becker collection under the name *C. obscura* Loew, 1840. That species was described based on an unknown number of female specimens (Loew, 1840). Becker (1894) studied this type material and concluded that the taxon is a synonym of *C. vernalis*. This synonymy was cited by all following authors. The type material can not been traced in collection, but remaining specimens determined by Becker as *C. obscura*, and collected from type area (Poland, Poznań) fit the presented concept. If additional investigations agree with our presumption about diagnostic characters of this taxon, it will be necessary to designate a neotype, redescribe the species and revalidate this name.

The present study showed that — based on comparison with type material — specimens possessing haplotype B2 are conspecific with *C. reniformis* Hellen, 1930. Clear morphological diagnostic characters (shape of dusted spots on abdominal tergite 3 which is small and distant from lateral margins) separated this species from all other *C. vernalis* specimens. We compared analysed specimens with the holotype and a female specimen of this taxon from Siberia, Novosibirsk, and we conclude that the revalidation of this taxon made by Barkalov (1993) is supported by presented morphological and molecular data (possessing a unique haplotype). This is the first record of the species for Europe. In many respects this species is not easy to recognise. It is very similar to typical *C. vernalis* and characters mentioned by Barkalov (1993) can be variable, especially the absence of marginal bristles, which can be present in some specimens. But the shape of pollinose spot on tergite 3 is a reliable diagnostic character. This taxon is until now known from only one locality in Helsinki, Finland. The taxon was on wing during the first week of May, earlier than other *C. vernalis* complex taxa.

The haplotype B1 was registered only from a population from the Pannonian low mountain Fruska Gora geographically isolated from other regions (Table 2). Specimens possessing this COI haplotype exhibit a combination between typical *C. vernalis* and *C. reniformis* morphological characters. In molecular characters it is more similar to *C. reniformis* (differs by two nucleotide changes). We conclude that this haplotype might represent one population of *C. reniformis*, or it is a good species, separated from ancestor during last interglacial period, based on the geographically isolated distribution (allopatric speciation).

The specimens representing haplotypes C1–C9 show extensive morphological variability. We undertook a morphological analysis of more than 500 specimens from different parts of Europe possessing same character combinations as the haplotype specimens C1–C9. The analysis included 20 regions and more than 30 populations. Almost all diagnostic character states are present in many of studied populations, especially on the Balkan Peninsula. Pairwise comparisons of haplotypes C1–C9 revealed differences ranging between 0.1%–0.6% (1–4 nucleotide changes).

Geographical distributions of haplotypes

The mountain Durmitor (Montenegro) possessed the largest number of haplotypes, a total of six, while for the other sampling sites in Serbia and Montenegro 1–2 haplotypes were registered (Table 3 and Fig. 1). Private haplotypes, B2, C3 and C9, were found in Finland, Estonia and The Netherlands, respectively. Haplotypes C1, C8 and C2 presented widest distribution patterns (Fig. 1).

Table 3. Registered numbers of *Cheilosia vernalis* mtDNA COI-3´ fragment haplotypes.

Locality	Haplotype
DU Montenegro: Durmitor	A1,C1, C4, C5, C6, C7
KO Serbia: Kopaonik	A1
MO Montenegro: Morinj	C1
FG Serbia: Fruska Gora	B1a, B1b
SW Sweden, Skåne	C2, C6, C7
RU Russia, Siberia	C2
FI Finland, Helsinki	B2, C2
FI Finland, Aland	C2
FI Finland, Utsjoki	C1, C2, C7
Estonia	C3
The Netherlands	C1, C7, C9

Fig. 1. Distribution of *Cheilosia vernalis* recorded haplotypes (mtDNA COI 3´ fragment, *n* = 50) in Europe.

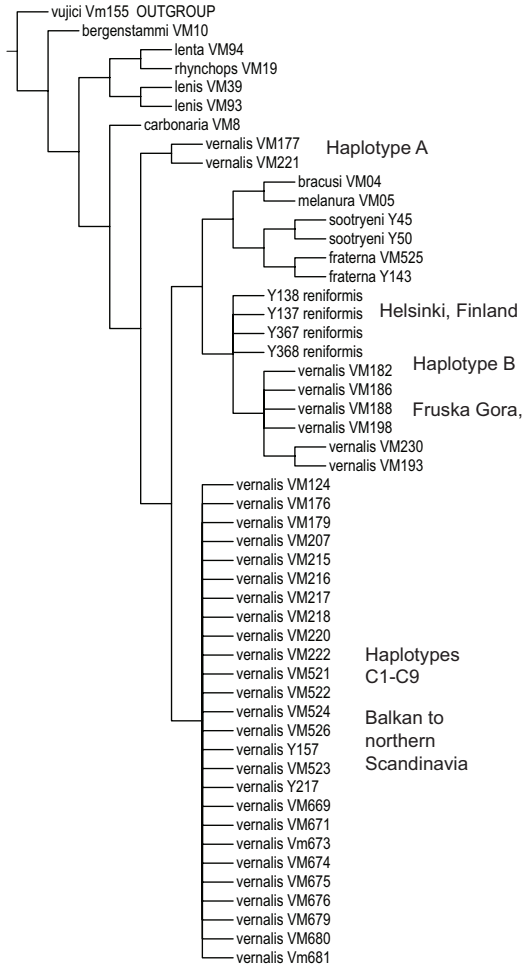

Fig. 2. Strict consensus of 24 equally parsimonious trees resulting from parsimony analysis of *Cheilosia vernalis–melanura* group taxa (CI = 0.77, RI = 0.88).

Parsimony analysis

Alignment of COI sequences was trivial due to the lack of indels and was done by eye. The parsimony analysis included 53 terminals, using *Cheilosia vujići* Claussen & Doczkal, 1998 as outgroup. The data matrix consisted of a 698 bp fragment of the 3´-end of the COI gene. The parsimony analysis resulted in 24 equally parsimonious trees of 169 steps length, CI = 0.77, RI = 0.88. The strict consensus tree is shown in Fig. 2.

Morphological analysis

Morphological characters and their observed variation in the analyzed specimens of *Cheilosia vernalis* are described in Table 4.

Discussion

Morphological characters

This taxon, currently recognized as *Cheilosia vernalis,* is a morphologically highly variable species (Table 4). Also larval host plant usage is variable, as larvae have been found to develop in a variety of plant species. One of the most variable characters of adult morphology is the relation between presence of yellow and black hairs on different parts of body. The pale-haired male specimens have black hairs restricted to a central patch in the hind part of scutum, and some restricted to the sides of tergites 2 and 3, or completely lacking black pilosity. Other specimens (especially summer generation) express an almost entirely black-haired scutum, and show the black hairs on tergites 2–4 extending to the posterior lateral corners. The morphological variability also includes the shape of facial tubercle and basoflagellomere, and the length, number and colour of scutellar marginal bristles. Also coloration of tibiae and tarsi can vary, except

Table. 4. Description of studied morphological characters of *Cheilosia vernalis*.

Character	Sex	Range of variation
1. Length of arista	male	arista long, relation between basoflagellomere and arista varies from 1:2.8 to 1:4
	female	from 1:2 to 1:2.5
2. Pile on arista	male/female	from surface covered with very short hairs to specimens with pile long as diameter of arista in most broader part
3. Shape of basoflagellomere	male/female	basoflagellomere small; variation from oval slightly broader than long; through rounded, to 1.2 times longer than broad with antero-dorsal angle
4. Colour of basoflagellomere	male/female	from dark, through dark with pale basal corner, to almost completely reddish
5. Hairs on eyes	male	generally long and black; the longest ones can be from almost as long as basoflagellomere, to half of its length; lower fifth of eyes usually also covered with hairs, but in some specimens almost bare
	female	hairs are shorter and pale, except black ones on upper part; lower part also with sparse white hairs (absent in some specimens)
6. Eye contiguity	male	relation between height of frontal triangle and eye contiguity from 1:1 to 0.8:1
7. Shape of facial tubercle and oral margin in profile	male/female	variable, showing a rather abruptly produced upper edge, whilst it is distinctly sloping in others; generally oral margin more protruded than facial tubercle, but can be opposite in some specimens
8. Width of frons	female	occupies in it's widest part from 1/3 to 1/2 of head width
9. Pollinosity of frons	female	lateral sulci usually covered with microtrichia, but reduced in some specimens
10. Colour of hairs on scutum	male	from completely pale haired to scutum covered with many black hairs, especially in posterior part or almost entirely black haired; in posterior half of scutum frequently a central patch of very short black hairs is also present
	female	usually with pale haired scutum, but many specimens have some adpressed black hairs on posterior half
11. Length of hairs on scutum	male	usually longer than basoflagellomere, but in shorter in some specimens and population
	female	shorter than in males, about half of length of basoflagellomere, sometimes very short and adpressed
12. Color of hairs on scutellum	male/female	from all completely pale (including bristles), to pale with many black hairs present
13. Presence of bristles on scutellum	male/female	usually present, but some specimens without distinct marginal bristles (the length of scutellar marginal bristles varies from equal to the length of the scutellum to only half of its length)
14. Presence and colour of long hairs on tergites	male	from completely pale long hairs on tergites to presence of black hairs especially on lateral sides of tergites
15. Colour of hairs on central parts of tergites 2–4	male	this part of tergites is covered with shorter and adpressed hairs, all pale to almost completely black
16. Shape of sternite 3	male	abdomen is stocky, can be more or less rounded, relation between length and width of sternite 3 varies from 1:1.8 to 1:2.5
	female	relation between length and width of sternite 3 varies from 1:2.4 to 1:3.2

continued

Table. 4. Continued.

Character	Sex	Range of variation
17. Colour of tibia	male/female	tibia pale with dark ring that can be broad, two times longer than pale parts, or narrow, occupying less than 1/5 of it's length
18. Colour of basal tarsal segments of fore and mid legs	male/female	varies from brown to clear yellow
19. Colour of hairs on legs	male/female	from predominately pale to presence of many black ones
20. Wing veins	male/female	the meeting point of veins M1 and R 4 + 5 can be very variable; the angle of junction can be from acute to blunt

the presence of strong orange bases and tips of all tibiae. Further variation is the hair length (present in both sexes) including those on eyes and scutum.

These observations were discussed in some papers (e.g., Vujić 1992, Speight & Lucas 1992, Speight 2006), and some authors suggested that *C. vernalis* constitutes a species complex of morphologically cryptic species.

Status of *Cheilosia vernalis* haplotypes: integrating molecular and morphological characters

Based on the evidence presently at hand we could not find consistent morphological character patterns to support the observed different mtDNA haplotypes of the C lineage. The Balkans constitutes one of the important European ice-age refugia. We hypothesise that the multiple haplotypes of *Cheilosia vernalis* represents the result of presence of multiple refugia in the Balkan Peninsula during Pleistocene glaciations, and the subsequent spreading of these populations across Europe. Congruence between datasets that provides the strongest support for hypotheses of species delimitation is not evident in this case. Thus, the observed variability of haplotypes and morphology is included within the present broad concept of *C. vernalis*. The haplotype variability observed in this study, however, is congruent with intraspecific uncorrected divergence ranges of 0%–0.89% and 0.14%–1.13% reported in studies of *Merodon* hoverfly species (Mengual *et al*. 2006, Milankov *et al*. 2008). However, we stress that the present conclusions do not exclude the possibility that based on further molecular and morphological studies of an enhanced dataset additional species could be recognised within the presented *C. vernalis* concept, especially on the borders of its range.

Taxonomical changes

Based on this conclusion and the re-examination of type material two new synonyms are established: *Chilosia rotundiventris* Becker, 1894 and *C. ruficollis* Becker, 1894 are junior synonyms of *C. vernalis* (Fallen, 1817). Because of the presumably lost type material in the depositary museum, the taxonomic status of *C. longifila* Becker, 1894 remains unresolved.

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Appendix: Type material studied

ZMHU = Zoologisches Museum, Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany; MZH = Zoological Museum of the Finnish Museum of Natural History, Helsinki, Finland.

Cheilosia longifila Becker, 1894. — ZMHU: material from Duda collection (males); the holotype mentioned by Becker in the original description ("Ein Männchen aus Schlesien, Wölfelsgrund, In meiner Sammlung") was not found in Becker collection.

Cheilosia obscura Loew, 1840. — ZMHU: material from Becker collection; the type material mentioned by Loew in the original description ("Einige Male das Weibchen") was not found in collection.

Cheilosia reniformis Hellén, 1930. — MZH: holotype, male, labelled: (1) Jeniseisk, (2) Wuorentaus, (3) 350 [handwritten, black ink], (4) reniformis Hellén det., Mus. Zool. H:fors Spec. typ. No. 4733 Ch. reniformis Hellén.

Cheilosia rotundicornis Hellén, 1914. — MZH: syntype, female [wings missing], labelled: (1) Hiitola, (2) L. v. Essen, (3) 2313 [printed], (4) Mus. Zool. H:fors, Spec. typ No. 4590, *Chilosia rotundicornis* Hell., (5) *Cheilosia vernalis* (Fall.), Barkalov det. 1988.

Cheilosia rotundiventris Becker, 1894. — ZMHU: syntype, male, labelled: (1) type v. Rondani *Cheilosia vernalis* Fall., det. K. Malski, (2) 26525, (3) *rotundiventris* type v. Rondani, (4) 143. Syntype, female designated by Malski as allotype, labelled (1) *Cheilosia rotundiventris,* (2) 27866. Syntype, female, labelled (1) *Cheilosia rotundiventris*, (2) 26768.

Cheilosia ruficollis Becker, 1894. — ZMHU: three females syntypes (26873; 26784; 27734 *Cheilosia ruficollis* Beck. female prof. Mik, bei Wien).

Cheilosia vernalis (Fallen, 1817). — ZMHU: male, *Cheilosia vernalis* Fall. * Zett.; Silesia; 3805 [in Zoologisches Museum, Berlin, a * sign on the label indicates a type designation]. Supposed syntype.