Cryptotendipes usmaensis (Pagast, 1931) – a cryptic species of Chironomidae (Insecta, Diptera)

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Abstract

We investigated the taxonomic status of *Cryptotendipes usmaensis* (Pagast, 1931), a cryptic species of Chironomidae (Insecta, Diptera), focusing on its morphological and molecular characteristics. We found discrepancies in the descriptions of adult males and pupal exuviae, suggesting the possibility of multiple species or significant polymorphism within *C. usmaensis*. Studies of recent collections from Finland revealed two distinct pupal morphotypes of *C. usmaensis* coexisting in the same habitat. DNA barcoding of adult males from Finland and other countries suggest the existence of two genetically distinct groups of the species. The findings emphasize the need for a comprehensive taxonomic review of *C. usmaensis* and suggest that species identification in some Chironomidae may require a different approach, i.e. combination of pupal morphology and molecular data, rather than relying solely on adult male morphology.

Introduction

Eight species of *Cryptotendipes* are known to occur in Europe. Two of these, *C. usmaensis* (Pagast, 1931) and *C. nigronitens* (Edwards, 1929) have males with a strongly humped tergite IX elevated far above the anal point. The males can be separated by the length of the anal point of the hypopygium using the key in Langton and Pinder (2007).

Studies of adult males and pupal exuviae assigned to *C. usmaensis* have opened a question whether it is a polymorphic species or might involve two different species (Langton and Visser 2003), further justified attention due to barcoding results from Finland present in the Barcode of Life Data Systems (BOLD, Ratnasingham et al. 2024).

Felix Pagast collected the type material of *C. usmaensis* from the Lake Usma, Latvia, where he worked during the early 1930s. Pagast's own collection may have been lost in the second World War, including the *C. usmaensis* material. Dr. Martin Spies kindly went through the large volume of *Cryptotendipes* material that is deposited in the ZMS Munich, Germany, but the collection apparently did not contain any specimen that could be regarded as a type specimen of *C. usmaensis*. There is nothing in the published literature on *Cryptotendipes* to suggest that type material of *C. usmaensis* could be stored elsewhere.

Adult males and pupae of *C. usmaensis* have been described several times since Pagast's publication in 1931, but in the absence of the type material the authors have relied on specimens from other geographic locations and habitats. As a result, descriptions differ considerably and are frequently not congruent with that of Pagast (1931).

In the following, we summarize taxonomic work on different morphotypes of *C. usmaensis* in an attempt to clarify whether there are one or more species involved. Specimens from Finland, recently collected and partly barcoded, were an important basis for the work.

Material and Methods

The data for this study were collected from various lakes and rivers in southern Finland, during different years and occasions (e.g., Raunio and Paasivirta 2008). Pupal exuviae of recently eclosed Chironomidae were collected by scooping floating debris from river and lake margins. The samples were collected with a hand net (mesh size 250 µm), especially from accumulation areas indicated by a foam and floating material, and behind obstacles (Wilson and Ruse 2005). Such samples are considered to represent chironomid taxa emerged within the past 48 hours, upstream or upwind of the sampling site (Wilson and Ruse 2005). Samples of chironomid pupal exuviae often include pharate specimens, which are useful for taxonomic studies

as they allow linking pupal exuviae to adults.

Pupal exuviae of both *Cryptotendipes* forms found in this study and a pharate male of *C.* sp. 2 have been deposited in Zoologische Statssammlung München, Munich, Germany.

Results

Pupal exuviae of Cryptotendipes usmaensis

Pagast (1931) described the pupa of *C. usmaensis* and mentioned that it was different from that of other known *Cryptotendipes* species as described by Lenz (1926). Lenz (1959) also noted the difference in the pupae. Langton and Visser (2003) separated two pupal morphotypes, one as *Cryptotendipes usmaensis* Pagast and the other one as a form called *Cryptotendipes* pe1c. The latter form was considered as an extreme variation of *C. usmaensis*. However, in Pagast's (1931) description of pupal exuviae of *C. usmaensis* the number of lateral taeniae of abdominal segments V-VIII is: 2, 3, 4, 4. Langton and Visser (2003) gave different numbers: 4, 4, 4, (3)4. Sæther (2010) separated the form *Cryptotendipes* near *usmaensis* from Pagast's (1931) species, based on different numbers of lateral taeniae on segments V-VIII and by the larger number of taeniae in the fringe of the anal lobe (17-40 vs. 13-14 that of *C. usmaensis*).

Adult male of Cryptotendipes usmaensis

As well as for the pupal exuviae, there is variation in characters in the descriptions of what is believed to be the adult male of *C. usmaensis.* The illustration of a dorsal view of the hypopygium in Goetghebuer and Lenz (1937) is very similar to Pagast's original illustration of *C. usmaensis*. The illustration in lateral view in Albu (1980) is also very similar but differs in the dorsal view particularly as the anal point is not apically expanded. Langton and Pinder (2007) adapted their illustration of the male hypopygium in a dorsal view from Pagast (1931) who showed that the adult male tergite IX is strongly humped, and that the tergite on each side of the anal point bears a low hump with three bristles (Fig. 1).

More recently, a worldwide key to adult males of *Cryptotendipes* was published by Pal and Hazra (2018) and later modified by Mukherjee et al. (2020). In couplet 6 of the key by Mukherjee et al. *C. usmaensis* is apparently considered NOT to have a high dorsal ridge. The illustration of the hypopygium in the key shows a different species to Pagast's *C. usmaensis* also by the much thinner gonostyli and the lack of lobes on tergite IX at each side of the anal point. Furthermore, in the following couplet 8, *C. usmaensis* is identified by the presence of setae on basal half of the anal point, as already mentioned in the key by Sæther (1977). However, Pagast (1931) did not mention this character. His drawing (Fig. 1) in a lateral view shows no seta on the anal point, although the drawing in a dorsal view leaves some room for different interpretations. A possible explanation is that due to the high ridge of tergite IX, lateral setae on the tergite may appear in slide mounts as if the setae are flanking the anal point. None of the hypopygia illustrations of the world's species of *Cryptotendipes* in Mukherjee et al. (2020) agrees well with Pagast's *C. usmaensis*. *Cryptotendipes lyalichi* (Zorina, 2006) is rather similar but lacks the tergite IX lobes at each side of the anal point.

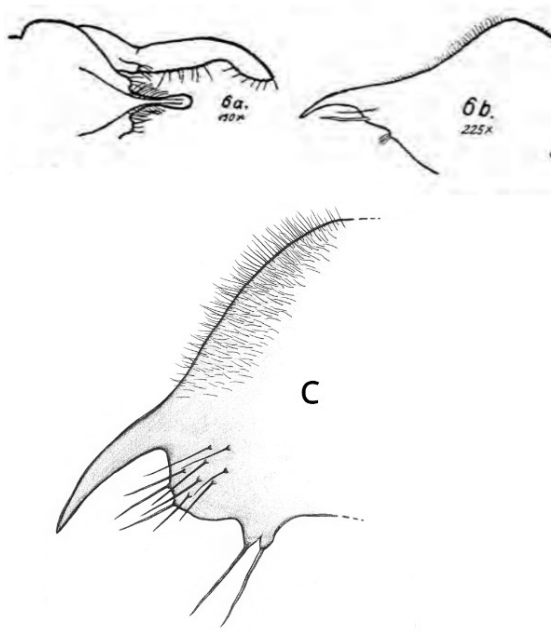

Figure 1. Hypopygium of *Cryptotendipes usmaensis* by Pagast (1931) in a dorsal view (6a) and lateral view (6b), together with our illustration in lateral view (C).

Cryptotendipes usmaensis in Finland

There are four species of *Cryptotendipes* recorded from Finland (Paasivirta 2014); *C. usmaensis* is by far the most common with larvae inhabiting mainly oligotrophic lakes. Two distinct pupal morphotypes of *C. usmaensis* have been identified among the Finnish specimens, yet adult males associated with the two pupal morphotypes could not be morphologically separated. We refer to these pupal

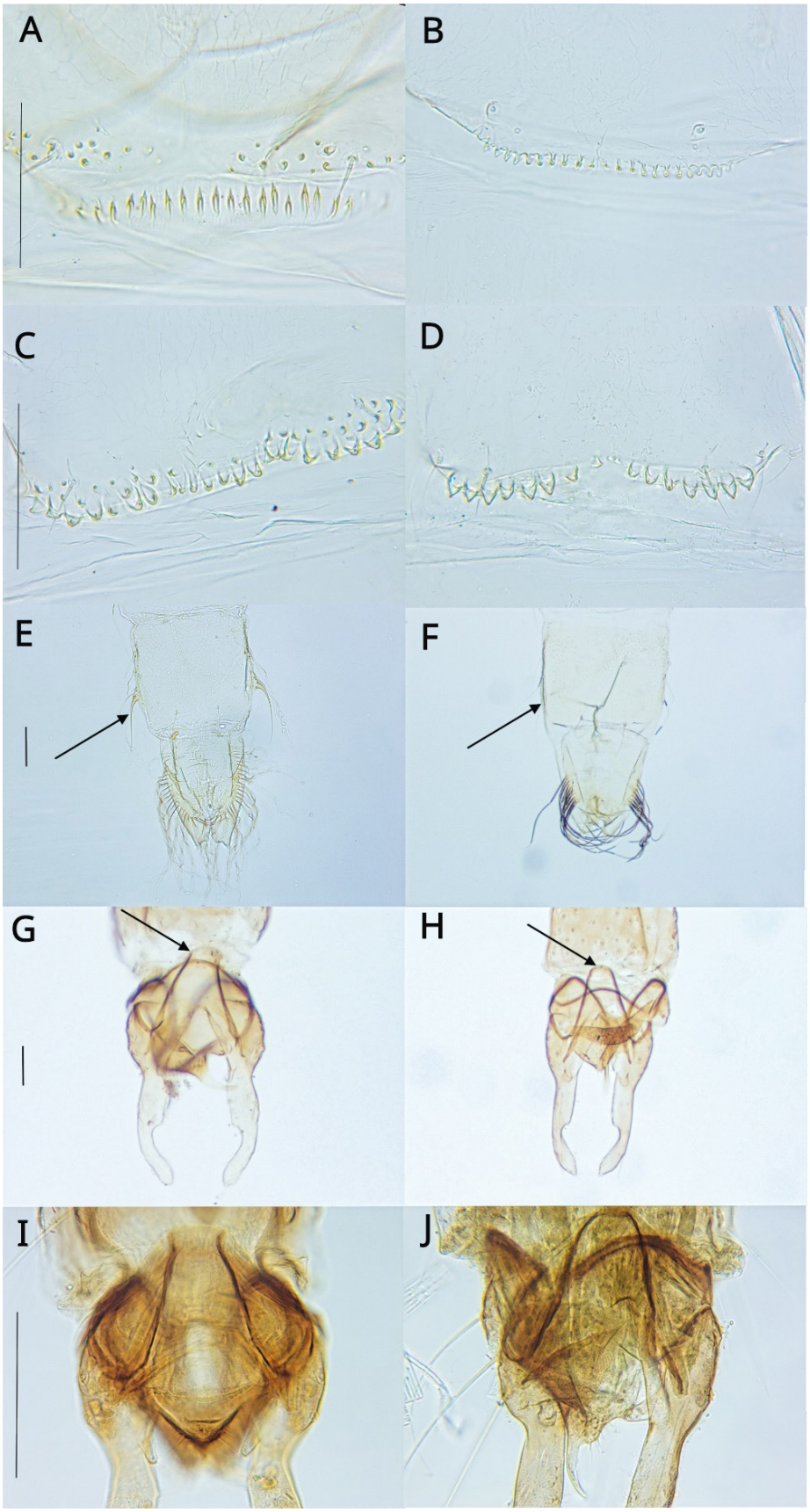

Figure 2. *Cryptotendipes* sp. 1 (A, C, E, G, I) and *C.* sp. 2 (B, D, F, H, J). Caudal hooklets of abdominal segments II present (A), absent (B); pupal exuviae tergite III (C, D); pupal exuviae tergites VIII-IX (E, F); adult male hypopygium (G-J). Arrows points to the presence (E) and absence (F) of posterolateral comb on pupal tergite VIII, and different shapes of transverse sternapodeme (G-H) of the two forms. Scale bars = 100 µm.

morphotypes as *Cryptotendipes* sp. 1 (Fig. 2A, C and E) and *Cryptotendipes* sp. 2 (Fig. 2B, D and F). Using the key by Langton and Visser 2003, *C*. sp. 1 runs to *C. usmaensis*. However, *C*. sp. 1 differs from *C. usmaensis* sensu Langton and Visser (2003) by having paired anterolateral spine patches on sternite I. It also bears larger number of taeniae in the fringe of the anal lobe than Pagast's *C. usmaensis* (Table 1). *Cryptotendipes* sp. 2, on the other hand, shows some resemblance to *C. pseudotener*, as the dorsal toothed mounds (sensu Langton and Visser 2003) or caudal hooklets (sensu Epler 2018) of abdominal tergites III-V (or III/IV alone) may be medially interrupted (see Fig. 2D). However, most specimens examined show continuous caudal hooklets. The numbers of teeth on the dorsal toothed mounds also seem to differ between the two forms (Fig. 3).

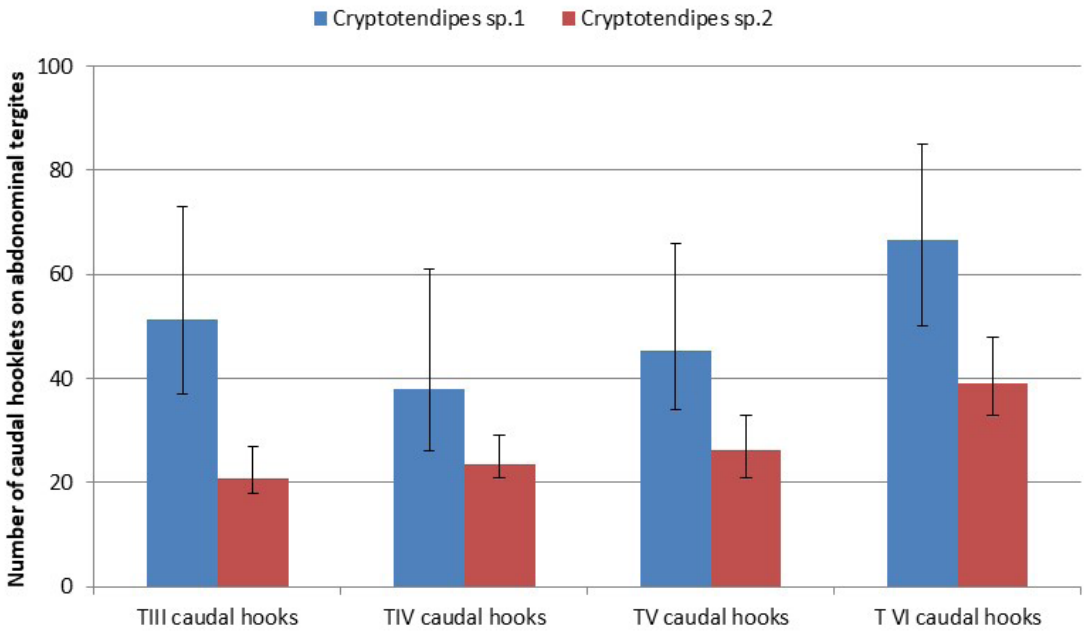

Figure 3. Numbers of hooklets on dorsal toothed mounds of abdominal tergites III-VI of pupal exuviae of the *Cryptotendipes* sp. 1 and *C*. sp. 2. Bars show the averages (n = 4 in each) and whiskers show variation (min-max).

The pupal form *Cryptotendipes* sp. 2 is recorded only from three oligotrophic lakes in SE Finland (lakes Suolajärvi, Niskajärvi and Simpeleenjärvi), while the form *Cryptotendipes* sp. 1 is more common and has been found from various locations in Finland, including rivers and lakes. There appears to be no differences in emergence periods, as both forms eclose from early June to early August. In Simpeleenjärvi, the two forms coexist.

Pagast (1931) mentioned that the pupa of *C. usmaensis* has hump-like small hooks dorsally on III-V and ventrally on III-IV in a single row, dorsally on II and VI in two rows, nowhere interrupted in the middle. This indicates that the form *C*. sp. 2 is different from Pagast's (1931) species (Figs 2B and D). In addition, paired anterolateral spine patches on sternite I of *C*. sp. 1 are absent in the form *C*. sp. 2. Perhaps the most striking difference between the two forms is the absence of posterolateral comb of VIII in *C*. sp. 2 (Table 1, Fig. 2F), which is unique among known *Cryptotendipes* species. However, the numbers of lateral taeniae on segments V-VIII appear to vary and may not be a useful character for species identification, as used e.g. by Sæther (2010) (Table 1).

We were not able to find any obvious differences in the hypopygium of the two forms, keeping in mind that the anal point of most specimens could been seen only in a lateral view due to slide mounting procedures. However, we noted that the transverse sternapodeme of *C.* sp. 1 is less sclerotized (Fig. 2G), whereas it is more distinct in *C.* sp. 2 (Fig. 2H).

Molecular data of Cryptotendipes usmaensis

In BOLD (Ratnasingham et al. 2024), there are currently only a handful of specimens identified as *C. usmaensis*. There are three specimens from Finland, three from Germany and one from Austria. Analysis of the barcode gene CO1 data of *C. usmaensis* in BOLD indicates that the specimens form two genetically distinct groups, so called BINs, separated by 3.3% which is a distance often long enough to separate species

Table 1. Key characteristics of pupal exuviae of the Finnish *Cryptotendipes* sp. 1 and *C.* sp. 2.

Taxon	St. I spine patches	T II dorsal toothed mounds	T III-V dorsal toothed mounds	Comb of VIII	Numbers of IX taeniae	T IX dorsal seta	Lateral taeniae of V-VIII
Cryptotendipes sp. 1	Present	Present (usually paired)	Continuous	Present	24.4 (20-27)	Present or absent	3-4, 3-4, 3-4, 3-5
Cryptotendipes sp. 2	Absent	Absent	Interrupted or continuous	Absent	14.5 (10-20)	Present or absent	3-4, 3-4, 3-4, 3

of Chironomidae (Song et al. 2016, Brodin 2025). There is only one specimen in BIN BOLD:AFT5715 (*Cryptotendipes* sp. 2 from Finland), while six in BIN BOLD:ADA4237. Interestingly, both groups of *C. usmaensis* are closer (among-group distances of 2.3% and 2.6%, respectively) to a group of 22 specimens from Manitoba, Canada (BIN BOLD:ACR8808), which are identified only as *Cryptotendipes* sp. (all females).

Discussion

In summary, there are several forms of *Cryptotendipes usmaensis* described as pupal exuviae and adult males. Some of the discrepancies seen in literature are likely derived from the absence of type specimens, and the fact that *C. usmaensis* appears to have many morphotypes at least regarding pupae. Data from Finland indicates that morphologically apparently very similar adult males may have distinctly different pupal exuviae, and that the differences seen in pupae are supported by the molecular data. The two forms have been found to coexist in the same lake and emerge simultaneously.

Our analyses of morphological and molecular data assigned to *C. usmaensis* did not provide enough information to conclude if there are two species involved or a single one with at least two distinct pupal exuviae forms and a long barcode distance of 3.3%. There are species of Chironomidae with as much as 10-12% intraspecific barcode distance with adult males not possible to separate morphologically (Lin et al. 2018). A more thorough taxonomic study on *Cryptotendipes usmaensi*s involving more pupal exuviae and barcoded males might provide knowledge to arrive at another conclusion. It could even challenge the fact that the current morphological concept for practically all scientifically named chironomid species is mainly tied to adult male specimens. *Cryptotendipes usmaensis* might be among the species in which the male morphology does not appear to differ sufficiently for the purpose of species identification. Thus, we may need to acknowledge that for such chironomid taxa, species identification could rely mainly on pupal exuviae and molecular data instead. Less than ten species of Chironomidae present in Europe are based originally on the pupal stage, in one or two cases with so far unknown males or females (Lehmann 1972). But above all, a review of *Cryptotendipes* in general, and particularly a re-description of *C. usmaensis* is required.

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