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IDENTIFICATION OF SEX PHEROMONES OF CLEARWING MOTHS *SYNANTHEDON CEPHIFORMIS* (OCHSENHEIMER, 1808), *S. LORANTHI* (KRÁLÍČEK, 1966) AND *S. SPULERI* (FUCHS, 1908) (LEPIDOPTERA: SESIIDAE) IN SLOVENIA

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Abstract – The results are presented of faunistic research of the morphologically similar and related clearwing moth species *Synanthedon cephiformis* (Ochsenheimer, 1808), *S. loranthi* (Králíček, 1966) and *S. spuleri* (Fuchs, 1908) in Slovenia using the method of pheromone traps. New sex attractants for males of the species *S. cephiformis* and *S. loranthi* were discovered, which are also the first known effective pheromones for *S. cephiformis* in general. The distribution and biology of the mentioned species in Slovenia are briefly described. External morphological characters for the determination of males caught in pheromone traps of species of the *S. tipuliformis* group are given, as well as the composition of the effective pheromones and a phylogenetic tree.

KEY WORDS: Sesiidae, *Synanthedon*, pheromones, Slovenia.

Izvleček – IDENTIFIKACIJA SPOLNIH FEROMONOV STEKLOKRILCEV *SYNANTHEDON CEPHIFORMIS* (OCHSENHEIMER, 1808), *S. LORANTHI* (KRÁLÍČEK, 1966) IN *S. SPULERI* (FUCHS, 1908) (LEPIDOPTERA: SESIIDAE) V SLOVENIJI

Prikazani so rezultati favnističnih raziskav morfološko podobnih in sorodnih vrst *Synanthedon cephiformis* (Ochsenheimer, 1808), *S. loranthi* (Králíček, 1966) in *S. spuleri* (Fuchs, 1908) z uporabo metode feromonskih pasti v Sloveniji. Odkrite so bile nove seksualne vabe za samčke vrst *S. cephiformis* in *S. loranthi*, ki so tudi prvi znani učinkoviti feromoni za *S. cephiformis* na splošno. Na kratko sta opisani razširjenost in biologija navedenih vrst v Sloveniji. Podani so zunanji morfološki

znaki za določevanje v feromonske pasti ujetih samčkov vrst iz skupine *tipuliformis*, kot tudi sestavine učinkovitih feromonov in filogenetsko drevo.

KLJUČNE BESEDE: Sesiidae, *Synanthedon*, feromoni, Slovenija.

Introduction

New pheromone compositions for *S. cephiformis* and *S. loranthi* were tested in Slovenia, resulting in additional information about pheromone trapping of males of some species of the *S. tipuliformis* group. As reported in the literature (Bartsch et al., 1997; Lepidopterologen-Arbeitsgruppe, 2000; Pühringer & Ryrholm, 2000; Bąkowski, 2002, 2013a, 2013b), no pheromone response from males of *S. cephiformis* to known attractants has so far been observed. This work investigated how effective the use of pheromone traps is in faunistic research of the local clearwing fauna and also how the composition of the pheromones can be useful in identifying the males caught in these pheromone traps. Four closely related species from the *S. tipuliformis* group occur in Slovenia and neighbouring countries. This group, described as the subgenus *Tipulia* Králíček & Povolný, 1977 (Laštůvka & Laštůvka, 2001), is represented by the following species: *Synanthedon tipuliformis* (Clerck, 1759), *S. spuleri* (Fuchs, 1908), *S. cephiformis* (Ochsenheimer, 1808), *S. loranthi* (Králíček, 1966) and *S. conopiformis* (Ochsenheimer, 1808). All these species have a well-developed proboscis and visit various flowers (*Ligustrum* spp., *Sambucus ebulus* L., *Rubus* spp., *Mellilotus* spp. etc.) on sunny days. Except for *S. conopiformis*, even for specialists specimens of this group are sometimes difficult to distinguish by their external and genital morphology, especially when the specimens are damaged. For all these reasons, the old literature contains a lot of erroneous determinations of imagos and the host plants of their caterpillars. *S. tipuliformis* and *S. conopiformis*, widespread in Slovenia, are not discussed in detail in this work.

Methods

The author has used synthetic pheromones in pheromone traps as a very successful method in faunistic studies of many clearwing species. A large number of pheromone traps were hung at selected localities in different habitats in Slovenia. The traps were baited with various types of synthetic pheromones obtained from Pherobank (PRI-Plant Research International, Wageningen, The Netherlands). Some pheromone compositions were custom made at the author's request and have been in use for several seasons. Each pheromone (or lures of double loaded rubber pheromone dispensers) was placed separately in a transparent plastic delta trap (RAG-Trap) with exchangeable bottom coated with sticky material, or in funnel traps (VARs+ -Trap, Uni-Trap), which were loaded with insect poison. This type, however, was used in smaller numbers. At each locality, 5 to 9 traps were placed baited with different pheromones. The traps were attached to branches and on the peaks of young bushes and trees (*Sorbus*, *Quercus*, *Carpinus*) at heights of 1 m to 6 m above the ground. Specimens trapped in

delta traps were later soaked in clean gasoline to detach them and prepared. Species identification was based on external morphology and knowledge of the composition of the pheromones used in these pheromone traps. Mitochondrial DNA analysis (iBOL project) was later performed as confirmation, at the Canadian Centre for DNA Barcoding (CCDB, Guelph). The PCR primers used were LepF1 and LepR1; the distance model was Kimura 2 Parameter. For details see the "Barcode of Life Database" (BOLD) web page (http://www.barcodinglife.com/views/login.php). Detailed data can be accessed by login under the project "Global Sesiidae – Clearwing Moths of the World". Representative specimens have been deposited in the private collection of the author. The nomenclature used is according to Laštůvka & Laštůvka (2001).

Identification of trapped males

During faunistic research in Slovenia in the period 2005-2013 (author's data), a large number of males belonging to 38 different clearwing species was caught using pheromone traps baited with various pheromone compositions. Many males showing external morphological characteristics of *S. cephiformis* - *S. loranthi* - *S. spuleri* were found among these. Identifying these trapped males initially represented a problem and they were therefore initially determined as *S. spuleri*, on the presumption that the observed morphological differences represented variability among local populations of this species. Subsequent redetermination, using molecular analysis (I-BOLD), showed that they in fact belong not only to *S. spuleri* but also to *S. cephiformis* and *S. loranthi*. After closer inspection, the re-determined males indeed showed the external characteristic of *S. cephiformis*, *S. loranthi* and *S. spuleri* as referred to in the literature (Bartsch et al., 1997; Špatenka et al., 1999; Lepidopterologen-Arbeitsgruppe, 2000; Laštůvka & Laštůvka, 2001; Bąkowski, 2013a), as well to reared material in the collection of the author. The now reliable identification, supported by DNA analysis, provides important information about which species is attracted to which pheromone composition. This, in combination with the morphological differences and sometimes the ecological characteristics, allows reliable identification of trapped males in the field, even with more damaged specimens. Using the genital morphology, in particular in the closely related sister species *S. cephiformis* / *S. loranthi* and *S. tipuliformis* / *S. spuleri*, it is difficult to find significant differences between these species (Bartsch et al., 1997; Bąkowski, 2013a, 2013b). For example, *S. spuleri* males are in many cases barely distinguishable from *S. cephiformis* and *S. tipuliformis*, although they differ well in pheromone response.

Additional external morphological characters useful in identification: *S. loranthi* has two-thirds of the fore coxa yellow laterally: in *S. cephiformis* this is entirely or at least up to four-fifths yellow laterally. *S. spuleri* has the fore coxa black and yellow on the dorsal margin only, while in *S. tipuliformis* it is yellow dorsally. *S. spuleri* is also distinguishable from other species by the slightly rounded apex of the forewing: the apical area is dark without or, at most, indistinct spots. Populations of *S. tipuliformis* in the submediterranean part of Slovenia have a squarer ETA, the

Table 1: The table includes only those characteristics that, in the opinion of the author, can be helpful in identifying trapped male specimens and only the effective pheromone compositions used in the study.

Morphological properties	External transparent area (ETA)	Discal spot on hindwing	Wingspan	Pheromone
S. cephiformis	Varies in size and in shape. Nearly square, sometimes narrower. Divided into 5 cells.	Broad, cuneiform. More visible than with *S. loranthi* / *S. spuleri*. Almost always extending to the stem of veins M3-Cu1.	Varies in size. 19-22 mm n= 14	ins, fla, myo
S. loranthi	Generally larger than with other species. At least twice as broad as apical area.	Short and narrow, barely visible. Never reaching stem of veins M3-Cu1.	Varies in size. 18-21 mm, n= 10	ins
S. spuleri	Nearly square or narrower. Sometimes similar to *S. cephiformis*. Narrowest of S. *loranthi*	Narrow, barely visible. Wider than with *S. loranthi*. Not extending to the stem of veins M3-Cu1.	Greatly varies in size. Usually smaller than by *S. cephifor*mis / *S. loranthi* 16-21 mm, n= 21	"old api"
S. tipuliformis	Narrow. In submediterranean populations broad, similar to *S. spuleri*	Fairly broad and short. Approximately between *S. loranthi* / *S. spuleri*.	Greatly varies in size. 12-20 mm n= 106	tip, rarely sof
S. conopiformis	Trapezoid, higher than broad. Divided into 5 cells	Narrow, cuneiform. Extending to stem M3-Cu1.	Varies in size. 12-24 mm n= 52	tab, rarely myo, ves

apical area is almost black and they are very similar to *S. spuleri*. In contrast, *S. tipuliformis* from the greater part of the country have a narrow to very narrow ETA, with frequently emphasised yellowish brown stripes between the veins in the apical area (author's data). Males of *S. conopiformis* show very good response to the pheromone tab initially developed for *Paranthrene tabaniformis* (Rottemburg, 1775) (Pühringer & Ryrholm, 2000; author's data) and are well differentiated from other species of the *S. tipuliformis* group by the conspicuously copper-coloured stripes between the veins in the apical area of the forewing and by the typical distinctive yellow transverse spot on the metathorax (Špatenka et al., 1999; Laštůvka & Laštůvka, 2001).

An additional observation is that *S. tipuliformis* males show a very good response to the pheromone tip initially developed for this species (Pühringer & Ryrholm, 2000; author's data) and were trapped by pheromone traps baited exclusively with pheromone tip, rarely with pheromone intended for *Synanthedon soffneri* Špatenka, 1983.

Table 2: Pheromone response.

+++, very good response
++, good response
+, weak response
-, no response

pheromones	ins	fla	myo	tab	tip	sof	"old api"	ves
S. cephiformis	++	+ (++?)	+	-	-	-	-	-
S. loranthi	++	-	-	-	-	-	-	-
S. spuleri	-	-	-	-		-	++	-
S. tipuliformis	-	-	-	-	+++	+	-	-
S. conopiformis	-	-	+	+++	-	-	+	+

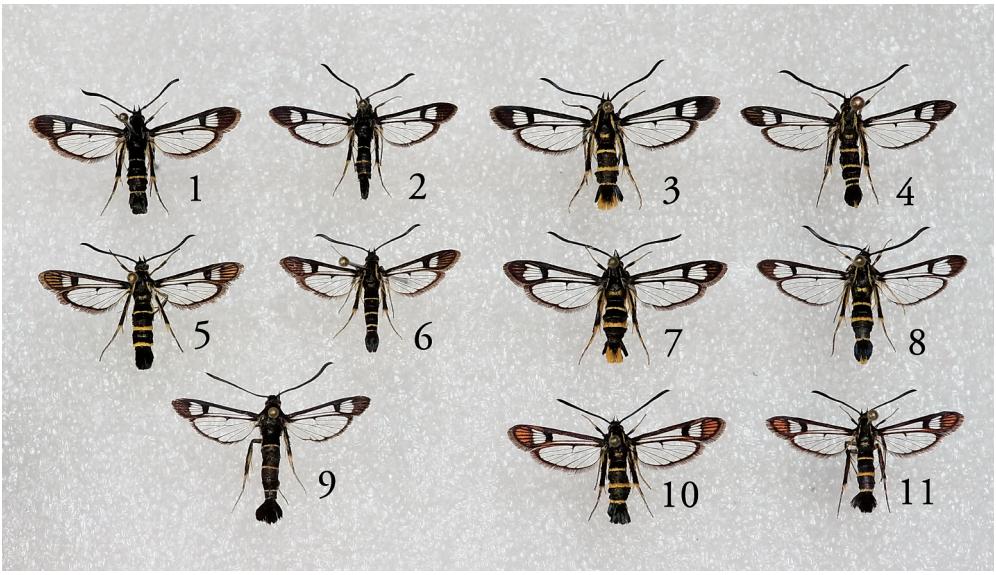

Photo 1-11: *S. spuleri*, 1: ♀, 2: ♂; *S. cephiformis*, 3: ♀, 4: ♂; *S. tipuliformis*, 5: ♀, 6: ♂, 9: ♂; *S. loranthi*, 7: ♀, 8: ♂; *S. conopiformis*, 10: ♀, 11: ♂.

Table 3: Abbreviations of pheromones used.

pher.	pheromone	"old api"	old pheromone apiformis (empi in Lepid.-Arbeitsgruppe, 2000)
pher. trap	pheromone trap	sof	pheromone soffneri
ins	pheromone insolita	tip	pheromone tipuliformis
fla	pheromone flaviventris	tab	pheromone tabaniformis
myo	pheromone myopaeformis	ves	pheromone vespiformis

Results

Synanthedon cephiformis has a European distribution and occurs in central Europe, parts of western Europe and in the Balkan Peninsula (Laštůvka in Laštůvka, 2001). In Slovenia, this clearwing species is well spread, particularly in forests in the hills of the Dinaric Alps and subalpine part (author's data). The host plant of the larvae is silver fir (*Abies alba* Mill., Pinaceae) while in the Southern Balkans it is *A. cephalonica* Loudon and *A. borisii-regis* Mattf. Numerous larvae regularly live together during the one year (less often two-year) development below the bark of succulent tissues of swellings on trunks and branches caused by the fungus *Melampsorella caryophyllacearum* (DC) Schröt on *Abies alba* at all elevations. Rarely *Viscum* (Loranthaceae), growing on *Abies* or in injured parts of trees, is used as host. Infested trees usually serve several generations of the moth and some of them can eventually contain hundreds of old exit holes. The presence of larvae can be observed externally by characteristic orange grain chips on the surface of the bark. The larvae pupate in a compact cocoon. The flight period of the adults is June to August (Špatenka et al., 1999; Laštůvka in Laštůvka, 2001).

The most attractive pheromone for *S. cephiformis* contains a mixture of three compounds: E2,Z13-18:Ac, Z3, Z13-18:Ac and Z3,Z13-18:OH in a ratio 100:100:5. It attracted 14 males in pheromone traps. This interesting pheromone (under the abbreviation ins) is attractive in Slovenia for males of *Synanthedon mesiaeformis* (Herrich-Schäffer, 1846), *Paranthrene insolita* Le Cerf, 1914, *Synanthedon myopaeformis* (Borkhausen, 1789) (Predovnik, 2012), *S. andrenaeformis* (Laspeyres, 1801), *S. loranthi* (Králíček, 1966), *Bembecia albanensis* (Rebel, 1918), *Chamaesphecia schmidtiiformis* (Freyer, 1836) and *C. empiformis* (Esper, [1783]) (author's data).

An alternative attractant for males of *S. cephiformis* was originally designed for *Synanthedon flaviventris* (Staudinger, 1883) (Lepidopterologen-Arbeitsgruppe, 2000) and is made of binary compounds E2,Z13-18:Ac and Z3,Z13-18:Ac in a ratio 100:100. Traps baited with this pheromone (under the abbreviation fla) lured 5 males of *S. cephiformis*. Pheromone fla is also attractive for *P. insolita* and, to a lesser extent for *Pyropteron affinis* (Staudinger, 1856) (author's data).

Lastly, a PRI commercial attractant (under the abbreviation myo) designed for *S. myopaeformis* (Borkhausen, 1789) also lured 5 males, which considering the large number of pheromone traps baited with pheromone, was a poor result. Nevertheless, this is a very effective pheromone for many other clearwing species, in particular of the genus *Bembecia* Hübner, 1919.

Material:

Ljubljansko barje, Jezero, Virje, 300 m, 16.6.-1.7.2005, pher. trap VARs+, pher. fla (in two traps), 3♂ (CCDB-14648 D03, 1♂); Logatec, Zaplana, Strmica, 600 m, 16.6.-1.7.2005, pher. trap VARs+, pher. ins (in two traps), 3♂; Grosuplje, Hočevje, Brujek, 466 m, 14.6.-1.7.2006, pher. trap VARs+, pher. myo, 1♂; Boč, Čača vas, Šekličev vrh, 480 m, 19.-24.6.2007, pher. trap RAG, pher. myo, 2♂ (CCDB-14648 E08, 1♂); Nova Gorica, Ajševica, Lijaško polje, 70 m, 26.6.-2.7.2007, pher. trap

RAG, pher. fla, 1♂; Kranj, Naklo, Dobrava, 438 m, 3.-13.6.2009, pher. trap RAG, pher. ins, 1♂ (CCDB-14648 E03); Dražgoše-Podblica, 808 m, 11.-27.7.2013, pher. trap RAG, pher. ins, 5♂ and pher. trap RAG, pher. myo, 2♂. Mojstrana, Belca, Jurčkov vrh (soteska potoka Belca), 1014 m, pher. trap UNI, pher. myo, 11.-27.7.2013, 1♂. Croatia, Gorski Kotar: Čabar, Gorači, Kajšli, 845 m, 7.-19.7.2009, pher. trap RAG, pher. ins, 4♂; Gerovo, Gerovski Kraj, Mladje, 875 m, 7.-19.7.2009, pher. trap VARs+, pher. fla, 1♂ (CCDB-14648 F06).

Synanthedon loranthi has a European but insufficiently known distribution area. This species occurs in western, central and southern Europe (Laštůvka & Laštůvka, 2001; Bąkowski, 2013a) but is found in Slovenia only in the eastern part of the country (author's data). The larvae live one year - rarely two years - in trunks and branches of *Viscum* spp. and *Loranthus europaeus* Jacq., on various tree and bush species from the top to 1.5 meters from the ground. The larvae pupate in a compact cocoon behind the membrane of the emergency hole. Adults occur from May to August (Špatenka et al., 1999; Laštůvka & Laštůvka, 2001).

Pheromone traps for this clearwing species should be placed as high as possible on the branches or the tops of young trees, since males do not otherwise fly into them (Bartsch et al., 1997). Only a few non-standard pheromone compositions are known to be attractive for this species. In Slovenia, 14 males were lured into traps baited with pheromone ins (E2,Z13-18:Ac/ Z3,Z13-18:Ac/ Z3,Z13-18:OH in a ratio 100/100/5).

Material:

Boč, Čača vas, Šekličev vrh, 480 m, 19.-24.6.2007, pher. trap RAG, pher. ins, 1♂; Ljutomer, Pristava, 172 m, 16.-24.6.2008, pher. trap RAG, pher. ins, 1♂ (CCDB-14648 E01); Orešje na Bizeljskem, Kozja peč, Silovec, 401 m, 20.6.-2.7.2012, pher. trap RAG, pher. ins, 12♂ (in one trap).

Synanthedon spuleri has a Western Palaearctic distribution. It occurs throughout western, central, southern and eastern Europe (Bąkowski, 2013a). This clearwing species is widespread in Slovenia but, nevertheless, not many data are available (author's data). The nearly polyphagous larvae live one year, occasionally two years, under the bark of trunks and branches, especially in damaged places, of many wood species. In Slovenia, it is found on *Fagus sylvatica* L., *Corylus avellana* L., *Carpinus betulus* L., *Acer* sp. (author's data) and *Juniperus* (Predovnik, 2003). Several other casual food plants are mentioned in the literature: *Quercus* spp, *Salix* spp., *Populus* spp., *Ulmus* spp., *Abies* spp., *Salix* spp., *Betula pendula* Roth., *Picea abies* (L.) and *Abies alba* Mill. (Špatenka et al., 1999; Lepidopterologen-Arbeitsgruppe, 2000; Laštůvka & Laštůvka, 2001; Bąkowski, 2013a). During their development, the larvae push granular pellets towards the surface of the bark and they finally pupate in a cocoon constructed from sawdust and silk. Adults fly from May to September (Špatenka et al., 1999; Laštůvka & Laštůvka, 2001) and in warmer years even starting from the end of April (Bartsch et al., 1997).

Thirty-one males of *S. spuleri* were captured only in pheromone traps baited with "old api" pheromone (PRI) with a single compound Z3,Z13-18:OH. This confirmed previous data in the literature about "old api" as a known attractant for this species (Pühringer 1997, www 4.12.2012; Pühringer & Ryrholm, 2000; Lepidopterologen-Arbeitsgruppe, 2000). The recommended pheromone for *S. spuleri*, with a composition E3,Z13-18Ac and E2,Z1-18Ac in a ratio 10:1 (Lepidopterologen-Arbeitsgruppe, 2000 [after Priesner, Dobler & Voerman, 1986]), has proved to be ineffective for this species in Slovenia but appeared strongly attractive for *Synanthedon andrenaeformis* (Laspeyres, 1801). Pühringer (www 4.12.2012) mentioned pheromones ves (PRI), designed for *Synanthedon vespiformis* (Linnaeus, 1761) and tab (PRI) as a poor attractant for *S. spuleri*, which we were unable to confirm.

Material:

Godovič, Črni vrh, Predgriže, 645 m, 26.6.-2.7.2007, pher. trap VARs+, pher. "old api" (in one trap), 9♂ (CCDB-14648 E06, 1♂); Kočevsko hribovje, Štalcerji, Štalcerski hrib, 591 m, 28.5.-11.6.2008, pher. trap RAG, pher. "old api", 1♂ (CCDB-14648 E10); Kočevski Rog, Stari Log, Šenberg, 372 m, 28.5.-11.6.2008, pher. trap RAG, pher. "old api", 1♂; Štanjel, Braniška dolina, Ječmenov hrib, 141 m, 15.-25.6.2009, pher. trap RAG, pher. "old api", 1♂ (CCDB-14648 F01); Spodnja Trenta,V Klancu, 559 m, 12.-26.7.2009, pher. trap RAG, pher. "old api", 2♂; Godovič, Vrh Zale, 505 m, 5.-16.8.2009, pher. trap RAG, pher. "old api", 1 ♂; Slovenske Gorice, Hermanci, Strmec, 256 m, 22.6.-1.7.2010, pher. trap RAG, pher. "old api", 1♂; Col, Podkraj, Križna gora, 894 m, 12.-25.6.2011, pher. trap RAG, pher "old api" (in one trap), 9♂; Kamnik, Županje Njive, Zg. Stahovica, Iverje, 471 m, 8.-17.7.2011, pher. trap RAG, pher. "old api", 5♂; Log pod Mangartom, Predel (Predelska trdnjava), 1148 m, 11.-27.7.2013, pher. trap RAG, pher. "old api", 1♂.

Discussion

The author primarily used pheromone traps baited with pheromone ins in the field to monitor *P. insolita* mainly on sites with *Quercus* spp., in the period from the end of May until the middle of July. Perhaps using these traps in areas with predominantly *Abies alba* present, they would attract large numbers of *S. cephiformis*. In two cases, males of *S. cephiformis* were found in traps baited with pheromone fla, placed in marshes far away from the larval habitat, which confirms reports of the high mobility of males of the genus *Synanthedon* (Špatenka et al., 1999). In one case, at the same locality, 5 males of *S. cephiformis* were caught in a pheromone trap baited with pheromone ins and only two males in a pheromone trap baited with pheromone myo.

The above mentioned trapping results do not give any information about the attraction period or the daily pheromone activity of males of *S. cephiformis* nor about their behaviour. In view of the daily sexual activity of males of other related species of the *S. tipuliformis* group, it can be assumed that males of *S. cephiformis* are also probably active in early or even late afternoon.

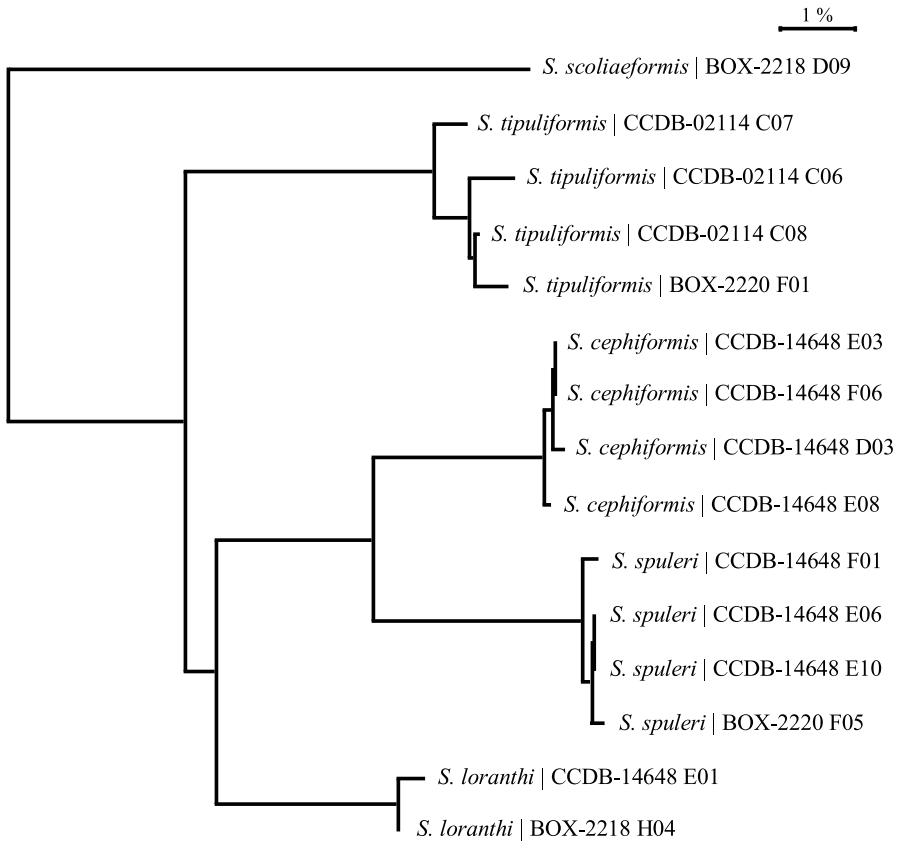

Fig. 12: Neighbour joining tree of DNA barcodes of *Synanthedon* species, showing specimen registry numbers (all specimens from Slovenia, one from Croatia). *S. scoliaeformis* (Borkhausen, 1789) represents an outgroup. The difference (in %) between 2 specimens is given by the total length of the horizontal lines from the first specimen to the second one. In general, a difference of more than 3 % is considered to be an indication of possibly different species. The fact that different species group together and are well separated from each other indicates that DNA confirms the species status and the determination based on other criteria.

Males of *S. cephiformis*, *S. loranthi* and *S. spuleri* have never been found in large quantities in pheromone traps, while males of *S. tipuliformis* and *S. conopiformis* are usually found in large number and, in appropriate places, occasionally trapped in masse. The highest numbers of trapped males per trap are 5 males for *S. cephiformis*, 12 males for *S. loranthi* and 9 males for *S. spuleri*. These 12 males of *S. loranthi* together in single trap baited with pheromone ins suggest that this mixture is perhaps more effective for this species but the available data are insufficient to draw final conclusions.

It is necessary in the future to examine the behaviour, daily rhythm of attraction and field responses of males of *S. cephiformis* to free hanging pheromone lures and to try to find optimal pheromone blends for *S. cephiformis*, *S. loranthi* and *S. spuleri*.

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