SIMULIUM COLOMBASCHENSE AND *S. VOILENSE* (DIPTERA, SIMULIIDAE) IN SLOVAKIA AND AUSTRIA

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Abstract: *Simulium colombaschense* (Scopoli, 1780) had previously been reported in Slovakia in the Danube river between Bratislava and Gabčíkovo, and in Austria in the Danube and Inn rivers. *Simulium voilense* Serban, 1960 had only been found in Slovakia in the Váh, Belá and Danube rivers, and there was no record of its existence in Austria. Between 2007 and 2010, we recorded *S. colombaschense* in the Danube in Slovakia at five sites downstream of the Gabčíkovo dam at Medveďov, Čičov, Klížska Nemá, Moča and Mužla and at one site upstream of the Gabčíkovo dam in Bratislava. In Austrian section of the Danube river we recorded S. colombaschense at one site at Stopfenreuth. *S. voilense*, however we found only at two sites in the Belá river, and we could not confirm its occurrence in the Danube, mainly due to the lack of reliable distinguishing characteristics between these two species. The Belá population of *S. voilense* had only 10 pupal gill filaments and differed significantly from the Danube populations of S. colombaschense with 54.8% of 10-filamentous gills in Austria and 73.2% in Slovakia. The remaining pupae of *S. colombaschense* in Austria and Slovakia had 11 – 14 filaments. The proportion of 10-filamentous pupae in Austrian Danube was significantly higher than those published in the Danube in Serbia (Iron Gate). The difference between pupae from the Danube river from Austria and Slovakia was not statistically significant. The cocoon shape was so variable that *S. colombaschense* and *S. voilense* could not be distinguished by this characteristic.

Key words: *Simulium colombaschense*, *Simulium voilense*, Central Europe, pupal gill branching, Simuliidae, Diptera.

INTRODUCTION

The black fly *Simulium colombaschense* (Scopoli, 1780) was first recognized in the Djerdap area around the Iron Gate Gorge (Serbia and Romania) and it was noted to be a dangerous pest causing big losses in cattle and in other domestic animals (Babić et al. 1935, Živković 1955). In the past, *S. colombaschense* occurred in huge masses in the Djerdap area, and it was considered to be bound only to the Danube and some of its tributaries in this area (Živković 1955, Rubtsov 1956). However, it was later also recorded in other sections of the Danube river in Slovakia (Halgoš & Jedlička 1987) and in Austria (Humpesch et al. 1987, Humpesch & Anderwald 1988), as well as in the Austrian section of the Inn river (Supperer & Kutzer 1964, Kowald 1971). It was also found in some other European rivers, with its distribution stretching from northern Italy and eastern Bavaria (Kowald 1971, Seitz 1992, Rivosecchi 1978) to the Ukraine (Rubtsov 1956), and a single larva was also identified in the Harz Mountains in central Germany (Werner & Adler 2004). When the Iron Gate I hydroelectric power

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Table 1. Material used for the comparison of the number of pupal gill filaments.

River	Site	Date	Number of pupae
Danube (Slovakia)	Medveďov	3.5.2009	5
	Moča	3.5.2009	12
		10.5.2009	4
	Mužla	10.5.2009	20
Danube (Austria)	Stopfenreuth	17.4.2011	42
Belá	Dovalovo	18.6.2007	1
	Liptovský Hrádok	18.6.2009	5
		22.6.2011	5

station was built in 1969, the occurrence of *S. colombaschense* in Djerdap decreased dramatically (Živković 1975), so that this species is no longer a threat to that area.

Simulium voilense Serban, 1960 was described from the Romanian river Doftana (Serban 1960), and also later reported from Serbia, Italy and Slovakia (Živković 1975, Rivosecchi & Lipparoni 1965, Jedlička & Halgoš 1982). Although *S. voilense* was considered for a long time to be bound to smaller streams, in contrast to *S. colombaschense* which preferentially bred only in large and fast flowing rivers (Rubzow 1959 – 1964, Rivosecchi 1978), *S. voilense* was later recorded at the same sites as *S. colombaschense* in the Danube in Serbia and Slovakia (Živković 1975, Illéšová et al. 1994), and in the Adige (Rivosecchi 1978).

S. voilense is morphologicaly very similar to *S. colombaschense*. According to several authors, such as Rubzow (1959 – 1964) and Rivosecchi (1978), the main morphological characteristic in which those two species should differ is the number of filaments in the pupal gills. *S. voilense* should have 10 filaments which are never secondarily branched while *S. colombaschense* can have 10 – 16 filaments, and the increase in filament number is due to secondary branching (Živković 1955). The shape of the cocoon was another character which was sometimes used to discriminate between the species, where the cocoon of *S. colombaschense* should have a longer collar (Jedlička et al. 2004). However, differentiation between *S. voilense* and *S. colombaschense* is very complicated since both species can have 10 pupal gill filaments, and cocoon shape varies (Rubzow 1959 – 1964). According to Rivosecchi et al. (2007), the filament number is the only reliable morphological characteristic in which these two species differ. Therefore, revision of both species is strongly needed (Jedlička & Seitz 2008).

The aim of this work is to summarize existing data on the distribution of *S. colombaschense* and *S. voilense* in Slovakia and Austria, and to supply new information from our black fly research in 2007 – 2010. This research includes also data on the number of gill filaments and the cocoon shape, since these characteristics have always been considered important in the species identification.

MATERIAL AND METHODS

Material was collected from the river Danube in Slovakia and Austria and the river Belá in Slovakia, during 2007 – 2010. Here, larvae and pupae were collected individually from plants, stones or other submerged objects in the flowing waters. Most of this material was stored in 96% ethanol, with some of the larvae preserved in a 3:1 mixture of ethanol and acethic acid. Larvae and pupae were identified by stereo-microscope and identification keys (Jedlička et al. 2004, Rubzow 1959 – 1964). The number of gill filaments was counted in all pupae, and the higher number was recorded where there was an unequal number of filaments on the left and right sides. The filament number was compared between five populations in the Danube in Slovakia (Danube SK), Danube in Austria (Danube AT) and Belá. The Danube SK population consisted of pupae collected from the Slovak-Hungarian section of the Danube near Medveďov, Moča and Mužla, while the Danube AT population contained individuals from the Austrian section of the Danube near Stopfenreuth and the Belá population consisted of pupae collected near Liptovský Hrádok and Dovalovo (Table 1). The χ^2 test of contingency tables was used to compare the number of individuals with at least 11 secondarily branched filaments and individuals with 10 filaments and no secondary branching. Subsequently, the numbers of individuals which had 10 filaments and those with at least 11 were compared to the proportion of 10 filamentous individuals published for *S. colombaschense* from the Iron Gate area referred to as the Danube RS population (Živković 1955). This comparison was performed using the χ^2 goodness-of-fit test, and both tests were evaluated at the 0.05 level of significance.

RESULTS

Distribution of *S. colombaschense* and *S. voilense* in Slovakia and Austria

Prior to Gabčíkovo dam construction on the Danube, *S. colombaschense* was recorded in Slovakia only in the Danube river in Bratislava and Bratislava – Čunovo (Halgoš & Jedlička 1987). After this damming, its presence was reported in the old Danube channel in Dobrohošť (Illéšová 1995), Bodíky (Illéšová et al. 1994, Illéšová 1995) and Gabčíkovo (Illéšová 1995, Jedlička et al. 1997). *S. colombaschense* was also registered in Austria in the river Inn (Supperer & Kutzer 1964, Kowald 1971), and in the Danube near Krems (Humpesch et al. 1987, Humpesch & Anderwald 1988), presence downstream of Vienna mentioned Car (1988). Another record of *S. colombaschense* in Austria was published from Schaida-Sattel in Carinthia (Zwick 1976). *S. voilense* has been recorded in Slovakia in the river Váh near Čemice (Jedlička & Halgoš 1982), which is now a part of the Liptovská Mara water reservoir, and in the river Belá near Dovalovo (Jedlička & Halgoš 1982, Jedlička 1984). Later, *S. voilense* was recorded in the old Danube channel with *S. colombaschense*, near Dobrohošť, Bodíky (Illéšová 1995) and Gabčíkovo (Illéšová 1995, Jedlička et al. 1997). There is no record of *S. voilense* presence in Austria.

Between 2007 and 2010, *S. colombaschense* was recorded at six sites in the Slovak section of the Danube (one site upstream and five sites downstream of the Gabčíkovo dam) and at one site in the Austrian section of the Danube. *S. voilense* was found at two sites in the river Belá. Since it was impossible to discriminate between *S. colombaschense* and *S. voilense* by pupal filament number or cocoon shape in the Danube individuals, we registered them all as *S. colombaschense*. However, all individuals in the river Belá had only 10 filaments and generally quite short cocoon collars, so in accordance with previous published data, these were identified as *S. voilense*. Recent records of both species are as follows:

Simulium colombaschense (Scopoli, 1780)**:**

Danube river – Stopfenreuth, river kilometer (rkm) 1887 (48.144° N, 16.901° E), 138 m a. s. l., Marchfeld Plain, 17. 4. 2011: 4 larvae, 42 pupae

Danube river – Bratislava, Karloveské rameno distributary, rkm 1873 (48.146° N, 17.052° E), 133 m a. s. l., Podunajská rovina Plain, 1. 5. 2009: 2 pupae

Danube river – Medveďov, rkm 1805 (47.789° N, 17.664° E), 110 m a. s. l., Podunajská rovina Plain, 3. 5. 2009: 5 pupae

Danube river – Čičov, rkm 1796 (47.750° N, 17.763° E), 109 m a. s. l., Podunajská rovina Plain, 10. 4. 2010: 8 larvae

Danube river – Klížska Nemá, rkm 1791 (47.743° N, 17.839° E), 108 m a. s. l., Podunajská rovina Plain, 11. 4. 2010: 4 larvae

Danube river – Moča, rkm 1744 (47.758° N, 18.424° E), 102 m a. s. l., Hronská pahorkatina Hills, 3. 5. 2009: 12 pupae, 10. 5. 2009: 4 pupae

Danube river – Mužla, rkm 1734 (47.768° N, 18.539° E), 102 m a. s. l., Hronská pahorkatina Hills, 10. 5. 2010: 20 pupae.

Simulium voilense Serban, 1960:

Belá river – Liptovský Hrádok (49.041° N, 19.716°E), 640 m a. s. l., Liptovská kotlina Basin, 16. 6. 2009: 5 pupae, 22. 6. 2011: 4 larvae, 5 pupae

Belá river – Dovalovo (49.049° N, 19.752° E), 680 m a. s. l., Liptovská kotlina Basin, 18. 6. 2007: 1 pupa.

Variability in the number of pupal gill filaments

Two types of pupal gills were identified in the studied material: (i) gills with 10 filaments, branched in 5 pairs only, and (ii) gills with 11 – 14 filaments,

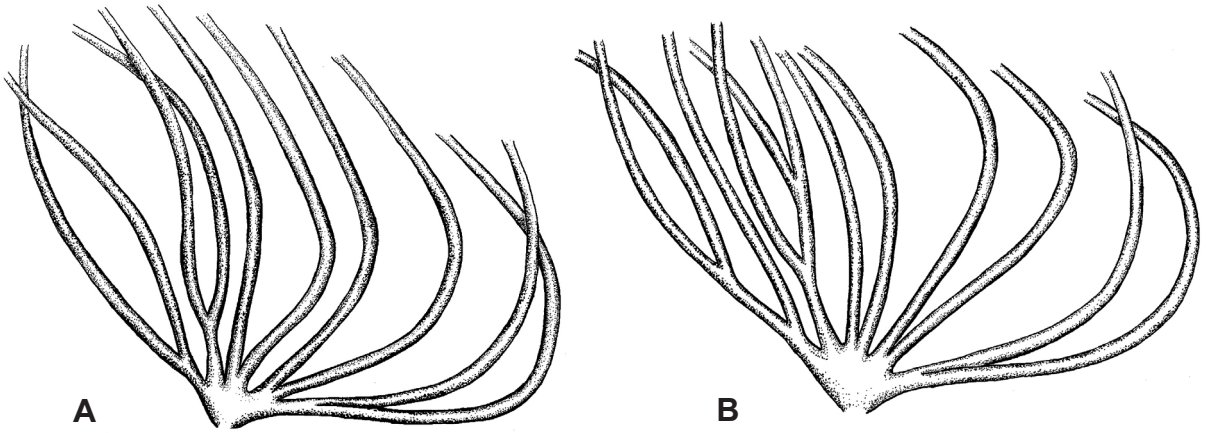

Figure 1. Branching of the pupal gill filaments of *Simulium colombaschense*: A – gill filaments with no secondary branching, B – secondarily branched gill filaments.

Table 2. Number and proportion of pupae of *Simulium colombaschense* and *S. voilense* with particular numbers of pupal gill filaments.

Populations: Belá – Belá river near Liptovský Hrádok and Dovalovo; Danube SK – Danube river near Medveďov, Moča and Mužla (Slovakia); Danube AT – Danube river near Stopfenreuth (Austria); Danube RS – Danube river in the Iron Gate, proportions published by Živković (1955).

Population	Pupae with particular number of gill filaments						
	10	11	12	13	14	15	16
Belá	11 (100%)	0	0	0	0	0	0
Danube SK	30 (73.2%)	7 (17.1%)	3 (7.3%)	1 (2.4%)	0	0	0
Danube AT	23 (54.8%)	3 (7.1%)	13 (30.9%)	2 (4.8%)	1 (2.4%)	0	0
Danube RS	20.6%	5.5%	65.7%	4.1%	2.7%	1.2%	0.2%

with some of the basic 10 filaments secondarily branched (Figure 1).

We compared the number of pupal gill filaments among individuals from two populations of *S. colombaschense* from the river Danube (Danube AT, Danube SK) and one population of *S. voilense* (Belá). Pupae with 10 gill filaments, whose gill filaments were not secondarily branched, formed the majority of the populations of Danube SK (73.2%) and Danube AT (64.8%). However, in population Danube RS these individuals comprised only 20.6% of all the pupae, with the great majority having more than 10 gill filaments. The details with particular numbers of gill filaments are listed in Table 2. All Belá river individuals had 10 pupal gill filaments without secondary branching (Table 2). Although analysis of contingency tables showed that filament numbers are depended on the site ($\chi^2 = 9.08$, $p = 0.011$), no significant difference was found between the Danube AT and Danube SK populations ($\chi^2 = 3.05$, $p = 0.081$). Of all populations in this study, the Danube AT population registered the highest proportion of pupae with 11 or more filaments, however, it was significantly lower then the proportion published for the Danube RS population ($\chi^2 = 29.97$, $p < 0.001$).

Variability in cocoon shape

Cocoons had a highly variable shape in all studied populations, exhibiting a different number and shape of openings, two individuals are displayed on Figure 2. The height of the collar, formed by the anterior lower edge of the cocoon was also very variable. This corresponded with the variability in the front part of the cocoon noted above. Although the Belá population collars were rather small, we were unable to distinguish two clearly distinct types of cocoons in the pooled material, or to find a discriminating characteristic in cocoon shape. Therefore, neither cocoon character enabled certain species distinction.

DISCUSSION

Simulium colombaschense was first recorded in Slovakia in 1982 in Bratislava (Halgoš & Jedlička 1987). The first record of *S. voilense* in Slovakia was from the river Váh near Čemice (Jedlička & Halgoš 1982), but due to building of the Liptovská Mara dam this site does not exist anymore. The second locality, where *S. voilense* has been found was the river Belá near Dovalovo (Jedlička & Halgoš 1982). For a long time it was thought that *S. voilense* bred only in smaller sub-mountain streams, however *S. voilense* was later recorded also in the Danube in Slovakia at the same sites as *S. colombaschense* (Illéšová 1995). Discrimination between *S. colombaschense* and *S. voilense* is very difficult, and when they both occur at the same site it is almost impossible to distinguish between them because both species can have the same number of pupal gill filaments (10) and the cocoon shape varies with only minor differences. Therefore the recordings of *S. voilense* in the Danube may be doubtful. On the other hand, we consider all individuals from the river Belá to be *S. voilense*. This is based on the facts that (i) the occurrence of *S. colombaschense* in smaller streams has never yet been published and (ii) because several authors have asserted that *S. voilense* presence is typical in smaller streams (Rubzow

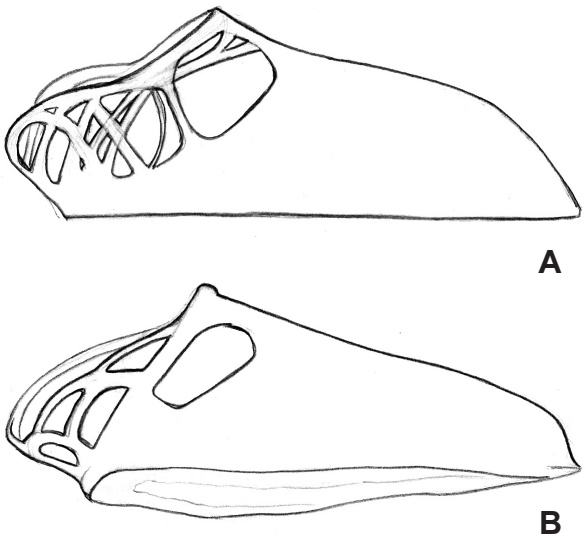

Figure 2. The shape of cocoon. A – *S. colombaschense*, Danube (Moča); B – *S. voilense*, Belá (Liptovský Hrádok).

1959 – 1964, Rivosecchi 1978) (iii) all individuals from Belá had 10 pupal filaments and (iv) all the old records of that species here were published as *S. voilense*.

S. colombaschense was recorded in Austria at first in the river Inn (Supperer & Kutzer 1964, Kowald 1971). Later records of this species come from the Danube near Krems (Humpesch et al. 1987, Humpesch & Anderwald 1988), and it has been mentioned to occur also downstream of Vienna (Car 1988, see also Seitz 1992). This present study confirms its occurrence in the larger reaches of the Danube, especially downstream from the Gabčíkovo dam at Medveďov, Čičov, Klížska Nemá, Moča and Mužla, where it was not previously known. These records of its presence there proved that *S. colombaschense* is not exclusively limited to the upper very fast flowing mountainous reaches of the Danube. The Medveďov site is at the lower part of the former Danube inland delta which was mostly destroyed by the Gabčíkovo dam, while the Moča and Mužla sites are situated where the river has a lowland character and is 2 – 3 times wider than it is at Medveďov and Stopfenreuth. The recent record of *S. colombaschense* in Bratislava is from the Karloveské rameno distributary, the last free flowing distributary of the Danube in the Slovak section of the river. The distributary has a fluctuating flow, and is often drying out.

Secondary branching of the pupal gills and subsequent variability in the number of gill filaments is recognized in species with a relatively high number of gills. For example, this has been documented in *Prosimulium rufipes* (Meigen, 1830) (Stloukalová 2005) and also in *Simulium degrangei* Dorier & Grenier, 1960 (Hackbarth 2004). It was noted in our study that branching of pupal gill filaments in *S. colombaschense* varies at different sites. Secondarily branched gill filaments in *S. colombaschense* pupae increased the number of gill filaments above 10, and these formed the majority only in the Iron Gate Gorge. Here, the Danube resembled a mountain river with an extraordinarily fast stream before it was dammed. Compared to this site, pupae with secondary branched gills were less abundant in the Slovak and Austrian sections of the Danube river. These individuals formed almost half of the population in the Austrian Danube, where there was higher stream velocity. Individuals with secondarily branched pupal gills formed only 26.8% of all pupae in the Slovak section of the Danube, where the river has a more lowland character; most notably in the Moča and Mužla sites. However, the difference between these populations was not statistically significant. No individuals with secondarily branched pupal gills corresponding to descriptions of *S. voilense* by Rubzow (1959 – 1964) and Rivosecchi (1978) were recorded in the Belá river, these individuals differed significantly from the ones found in the Danube. On the other hand, when we take into account the variability of the number of filaments, plus the relatively low number of 11 researched individuals, we can not completely exclude that individuals with secondarily branched filaments also occur in Belá river.

Variability in the branching of pupal gill filaments and the knowledge that *S. colombaschense* can also have pupal gill filaments without secondary branches indicate that this characteristic is quite inappropriate for discrimination between *S. colombaschense* and *S. voilense*. Because of the lack of other reliable distinguishing features, we could not confirm the record of *S. voilense* in the Danube and therefore we support the necessity for revision of these species, as proposed by Jedlička & Seitz (2008).

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