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## Phylogenetic notes on the rare Mediterranean digger wasp *Psenulus fulvicornis* (Schenck, 1857) (Hymenoptera: Crabronidae) new to Switzerland

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**Abstract:** Identifying alien species is important to detect invaders early and to survey shifts in species ranges in the context of global change. Here we present the first recorded occurrences of the Mediterranean digger wasp *Psenulus fulvicornis* (Schenck, 1857) (Crabronidae) in Switzerland. To aid species identification and separation from the morphologically similar congener *P. schencki* (Tournier, 1889), which is known to occur in Switzerland, we show discriminating morphological characters and deliver the first DNA barcode sequences and a molecular phylogenetic tree of the mitochondrial *cox1* locus from specimens sampled at the European scale. While the species can be readily separated by morphological characters, maximum likelihood and Bayesian inferred phylogenetic trees revealed the existence of polyphyly. Thus, we could not identify a barcoding gap at the European scale, which may hamper taxon identification. Nevertheless, *cox1* sequences were diagnostic for all Central European specimens. Finally, an exhaustive revision of *P. schencki* accessions in the Swiss historical museum and private collections did not reveal overlooked specimens of *P. fulvicornis*. This confirms the status of *P. fulvicornis* as a new species to Switzerland, where it is currently only known from the Cantons of Zurich and Geneva; inhabiting warm lowland habitats such as urban gardens.

**Keywords:** Alien species - Cytochrome oxidase 1 - first record - Hymenoptera - identification - introduction - invasive - urban garden.

### INTRODUCTION

*Tous les entomologistes qui ont tenté de déterminer des hyménoptères appartenant au groupe des Psenini ont pu se rendre compte des difficultés que présente ce travail.*

*Jacques de Beaumont (1937-1939)*

Diversity and distribution of organisms across taxonomic groups are changing at fast rates worldwide (e.g. Pereira *et al.*, 2010). The correct identification of new arrivals is important: not only to conduct risk assessments of

potential pest organisms at an early stage of invasion, but also to elucidate range shifts of non-invasive species in the context of global change (e.g. climate change or urbanization; Blackburn *et al.*, 2011; Simberloff *et al.*, 2013; Hawkins *et al.*, 2015).

Cities are port of entries for alien species due to extensive trade, traffic and transport, which can facilitate (long distance) dispersal (Rebele, 1994; von der Lippe & Kowarik, 2007). Moreover, climatic barriers limiting the establishment and spread of such species may be overcome due to the urban heat island effect (Kowarik,

2011; Aronson *et al.*, 2016). For instance, surveys of urban arthropod communities in northern temperate cities frequently reveal new alien species that originate from warmer climates (e.g. Germann *et al.*, 2015; Frey *et al.*, 2016; Zanetta *et al.*, 2016). One prerequisite for assessing naturalization status and/or environmental impact of such species is their rapid detection and identification (Comtet *et al.*, 2015).

DNA barcoding of the mitochondrial *cox1* locus can be a simple and cost-effective method to detect and identify alien organisms: especially arthropods. For example, it may enable their identification during life stages in which determination based on morphology is difficult (Comtet *et al.*, 2015). However, this approach needs a reference database composed of barcode sequences of correctly determined voucher specimens against which unknown specimens can be compared (e.g. the Barcode of Life Data System BOLD, Ratnasingham & Hebert, 2007). Moreover, several methodological (e.g. sampling scale and intensity) and evolutionary factors (e.g. introgression) can limit the diagnostic ability of the *cox1* marker (Funk & Omland, 2003; Meyer & Paulay, 2005; DeSalle *et al.*, 2005; Petit & Excoffier, 2009; Nicholls *et al.*, 2012; Patten *et al.*, 2015; Barley *et al.*, 2016).

Here we report the Mediterranean digger wasp *Psenulus fulvicornis* (Schenck, 1857) (Crabronidae) as a new species to Switzerland by combining genetic, morphological and historical biogeographic evidence. The species was recently found for the first time during an extensive arthropod survey in urban gardens in the city of Zurich as well as in the Canton of Geneva (Boillat, 2012), but, so far, lacked a description and entry in national faunistic databases. We provide microphotographs of the discriminating morphological characters between *P. fulvicornis* and its morphologically similar congener *P. schencki* (Tournier, 1889), which already occurs in Switzerland, and investigate, for the first time, sequences of the mitochondrial *cox1* locus of both species to infer taxon identity and phylogenetic relationships among them and other Central European *Psenulus*. Finally, to confirm its status as a new species to Switzerland, we checked Swiss *P. schencki* accessions in all major Swiss museums and private collections for potentially overlooked specimens of *P. fulvicornis*.

## MATERIAL AND METHODS

### Study species

The cosmopolitan genus *Psenulus* Kohl, 1897 comprises more than 160 species, with the Oriental biogeographic region being particularly species rich (Bohart & Menke, 1976; Pulawski, 2018). About 26 species occur in the Palearctic region; with eight taxa found in Central Europe and Switzerland (Bohart & Menke, 1976; Blösch, 2000; Herrmann, 2005; Artmann-Graf, 2006; Pulawski, 2018; Info Fauna, 2018).

Ecological information is available for a small number of taxa, of which all nest in hollow or pithy stems (e.g. *Sambucus* spp.) or beetle borings, and prey on adult or nymph arthropod herbivores such as aphids, leafhoppers, delphacids and psyllids (Bohart & Menke, 1976; Blösch, 2000). Some species, e.g. *P. fuscipennis* (Dahlbom, 1843), accept artificial cavities and can serve as indicators to assess the effect of land-use (change) on hymenopteran-based food-webs (e.g. Tschamtker *et al.*, 1998; Fabian *et al.*, 2013); a research approach for which DNA barcoding can be useful (Turčinavičienė *et al.*, 2016).

*Psenulus fulvicornis* has been reported from Algeria, Andorra, Bulgaria, Croatia, France, Germany, Greece, Hungary, Italy (Aosta), Russia, Spain, Switzerland, Syria and Turkey (Gayubo *et al.*, 2002; Schmid-Egger, 2002; Nieves-Aldrey *et al.*, 2003; Boillat, 2012; Cruz-Sanchez *et al.*, 2005; Gayubo *et al.*, 2006; Zsolt, 2008; González *et al.*, 2009; Standfuss & Standfuss, 2012; Reder & Niehuis, 2014; Yildirim *et al.*, 2015; Mokrousov & Popov, 2016) (Fig. 1). Older observations are given from Austria (Tirol), Italy (Trentino – Alto Adige) and Poland (Pomeriana) (Brischke, 1864; von Aichinger, 1870; Kohl, 1880, 1888). Taken together, these occurrences indicate a geographic range spanning from the Mediterranean to the Anatolian region, with additional occurrences in central and southern alpine valleys, and continental and steppe zones of Europe. Schmid-Egger (2002) observed a slight morphological divergence in several taxonomically important characters (e.g. the texture of the propodeal surface) between females from Algeria, Turkey and Syria on the one hand, and females from Central Europe on the other hand; a pattern which may indicate phylogeographic structuring.

In Central Europe *P. fulvicornis* is found in warm lowland habitats such as abandoned vineyards in the Upper Rhine Plain in Germany, which is a region characterized by a relatively mild climate (Blösch, 2000). In the Mediterranean area, specimens have been captured in olive and oak (*Quercus ilex* L.) groves, and vineyards. In Spain and in the North Caucasus they also occur on (shrubby) coastal sand dunes (see Results).

In the past, the taxonomic status of *P. fulvicornis* as a separate species has been doubted due to its morphological similarity with other taxa, the low number of observations and the lack of males, which have been discovered only recently (Schenck, 1861; De Beaumont, 1937-1939; Schmidt, 1971; Schmid-Egger, 2002). *Psenulus fulvicornis* type material has been studied by the latter two authors, who highlighted the close morphological relationship with *P. schencki* (Tournier, 1889) and delivered a detailed species diagnosis. Due to the close morphological relationship between the two taxa, and since *P. fulvicornis* was described first, we here refer to them as the *Psenulus fulvicornis*-Group. Currently, the status of *P. fulvicornis* as a separate species is accepted, and the taxon is included in the standard



Fig. 1. Sampling sites of *Psenulus fulvicornis* (Schenck, 1857) (large filled circles) and *Psenulus schencki* (Tournier, 1889) (squares) used for the DNA barcode sequencing of the mitochondrial *cox1* locus. Small filled circles show georeferenced distribution records of *P. fulvicornis* with confirmed determinations by Schmid-Egger (2002) together with newer records by Gayubo *et al.* (2012), Mokrousov & Popov (2016), Reder & Niehuis (2014), Standfuss & Standfuss (2012) and Zsolt (2008). The triangle represents the undetermined specimen (PSC-7) from Pisa, Italy.

literature (Bohart & Menke, 1976; Blösch, 2000; Jacobs, 2007; de Jong *et al.*, 2014; Pulawski, 2018).

*Psenulus fulvicornis* is considered rare in Germany and red-listed under the category “Vulnerable” due to a predicted (moderate) long-term population decline (Schmid-Egger, 2010a). In contrast, the number of records has slightly increased in recent years (Schmid-Egger, 2010a, b, 2015; Frommer, 2012; Reder & Niehuis, 2014) but it is unclear whether this is due to temporary and local population size increases, for example because of exceptionally favourable weather conditions, an increased sampling effort, or whether this represents an ongoing population size increase and/or range expansion, e.g. favoured by climate warming (Schmid-Egger, 2010a).

### Sampling

Flying arthropods were sampled in 85 urban gardens in the city of Zurich between May 18 and August 19 2015 with three bowl traps fixed on a triangular wooden pole. Each bowl was sprayed with either UV-bright blue, white or yellow paint (Sparvar Leuchtfarbe, Spray-Color GmbH, Merzenich, Germany) (Westphal *et al.*, 2008) and three quarters filled with 0.2% Rocima solution (Acima, Buchs, Switzerland). Aculeate hymenopterans (Formicidae excluded) were identified by one of the authors (M. Řiha), and vouchers of all taxa were deposited in the reference collection

of the Swiss Federal Research Institute for Forest Snow and Landscape (WSL). One female of each of *P. fulvicornis* and *P. schencki* was caught and identified during this survey. In addition, to complete the dataset of specimens found in Switzerland, we were kindly allowed to sample one hind leg of the *P. fulvicornis* female captured in 2005 in the Canton of Geneva at the Swiss-French border (Boillat, 2012). Finally, to increase the geographic scale of the genetic analysis, specimens of both *P. fulvicornis* and *P. schencki* from areas of sympatry across almost the entire known distribution range of the *P. fulvicornis* were added from the collections of two of the authors (S. F. Gayubo and M. Mokrousov). Note that one such sample (PSC-7) from Pisa, Italy, which was originally determined as *P. schencki*, was genetically so divergent from all other samples of the *P. fulvicornis*-Group (2.3% sequence divergence over 614 base pairs) that we considered it an unknown species (see Results). Genetic analyses were further completed with publicly available *cox1* sequences of *Psenulus* taxa in GenBank and BOLD (Ratnasingham & Hebert, 2007) (supplementary Table S1 in the Appendix).

Photographs of specimens and their discriminating characters were made with a Leica Digital Microscope DVM6 by using image-stacking. Georeferenced sampling sites and distribution records were mapped with ArcGIS (version 10.2; ESRI).



### DNA extraction and *cox1* sequencing

The DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used for the extraction following the manufacturer's instructions. The partial *cox1* locus (approx. 650 nu) was sampled using the primer pair LepF1 and LepR1 (Hebert *et al.*, 2003) following the published thermal regime. The JumpStart REDTaq ReadyMix Reaction Mix (Sigma, St. Louis, Missouri, U.S.A.) was used in the PCR, and its products were purified enzymatically using Illustra ExoProStar (GE, Chicago, Illinois, U.S.A.) following the manufacturer's instructions. PCR products were sequenced in both forward and reverse directions on an ABI 3130 sequencer (Applied Biosystems, Foster City, California, U.S.A.) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and PCR primers, following the manufacturer's indications. The cycle sequencing products were purified using the BigDye XTerminator Purification Kit (Applied Biosystems). The corresponding forward and reverse electropherograms were assembled with CLC Main Workbench 7 (Qiagen) and visually corrected.

### Genetic diversity

The *cox1* polymorphism of the *P. fulvicornis*-Group was analysed using the software DnaSP v.5.10.01 (Librado & Rozas, 2009) under the option for haploid mitochondrial DNA. No position contained gaps or missing data. Three codon positions were included with a total of 187 codon positions in the final dataset. We calculated the haplotype diversity (Hd; the probability that two randomly chosen haplotypes are different within the sample), the nucleotide diversity per site (Pi; the probability that two randomly taken nucleotides from the same position are different, and the average nucleotide differences (K). As we found that the inferred haplotypes belonged to divergent clades (see Results), genetic diversity was analysed in the entire sample (i.e. clades I-VI) and within each of the statistically well supported clades I-II (*P. fulvicornis*) and III (*P. schencki*).

### Phylogenetic tree

The model of nucleotide substitutions was computed with the software jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2006, 2008) and the best-fit model was selected based on the Akaike information criterion (Akaike, 1973). A maximum likelihood (ML) analysis used the RAxML-HPC BlackBox (Stamatakis, 2006) and a Bayesian analysis was performed with MrBayes 3.2.6 on the CIPRES Science Gateway (Miller *et al.*, 2010). The statistical support of branches in the ML analysis was calculated based on 1,000 bootstrap replicates (Felsenstein, 1985). Bayesian analysis was run with 5 million generations and sampled every

100th generation, following a discarded burn-in of 12,500 generations. Convergence and the consequent proportion of burn-in were assessed using Tracer v1.5 (available from <http://beast.bio.ed.ac.uk/>). Output tree files, with each containing the best tree found and labelled with the statistical support for each branch, were graphically represented with TreeGraph 2 (Stöver & Müller, 2010).

### Review of Swiss museum and private collections of *P. schencki* accessions

The (undetected) presence of *P. fulvicornis* in Switzerland was checked in all major Swiss museums as well as in the most important private collections. To achieve this, we controlled the correct identification of *P. schencki* specimens since older identification keys do not discriminate the taxa (e.g. de Beaumont, 1937-1939). We followed Schmidt (1971) and Schmid-Egger (2002) for taxon identification. The subsequent collections were visited or contacted: Bündner Naturmuseum, ETH Zürich Entomological Collection (ETHZ), Muséum d'histoire naturelle de Genève (MHNG), Muséum d'histoire naturelle de Neuchâtel, Musée de la nature de Sion, Musée de zoologie, Lausanne (MZL), Museo cantonale di storia naturale (LUG), Naturhistorisches Museum Basel (NMB), Naturhistorisches Museum Bern (NMBE), Naturmuseum Luzern and Naturmuseum St. Gallen. Only collections harbouring *P. schencki* were visited except for the Musée de la nature de Sion, where the Crabronidae of Maurice Paul (1835-1898) were reviewed. Additionally, the following private collectors were contacted: Neumeyer, Rainer; Artmann-Graf, Georg; Müller, Andreas; Herrmann, Mike; Salzmann, Irene. The material examined is given in the Appendix.

## RESULTS

### Successfully barcoded material:

#### *Psenulus fulvicornis* (Schenck, 1857)

1 female; city of Zurich (CH: ZH); garden lot in the "Juchhof" allotment garden area; 47.3997°N, 8.4794°E; 395 m a.s.l.; 16.06.2015; D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll.; DNA-ID PHP16-0418; GenBank accession Nr. KY039438 (Figs 2, 3). – 1 female; Liencres dunes national parc (ESP: Cantabria); 01. – 30.06.2004; S. F. Gayubo leg. & det.; DNA-ID PFU-7; GenBank accession Nr. MG872065. – 1 female; Gametxo (ESP: Vizcaya); oak grove; 43.4054°N, 2.6722°W; ca. 160 m a.s.l.; 1 - 15.06.2004; S. F. Gayubo leg. & det.; DNA-ID PFU-8; GenBank accession Nr. MG872066. – 1 male; Miranda del Castañar, Las Madroñeras (ESP: Salamanca); vineyard; 05.2005; S. F. Gayubo leg. & det.; DNA-ID PFU-1; GenBank accession Nr. MG872062. – 1 female; Miranda del Castañar, Las

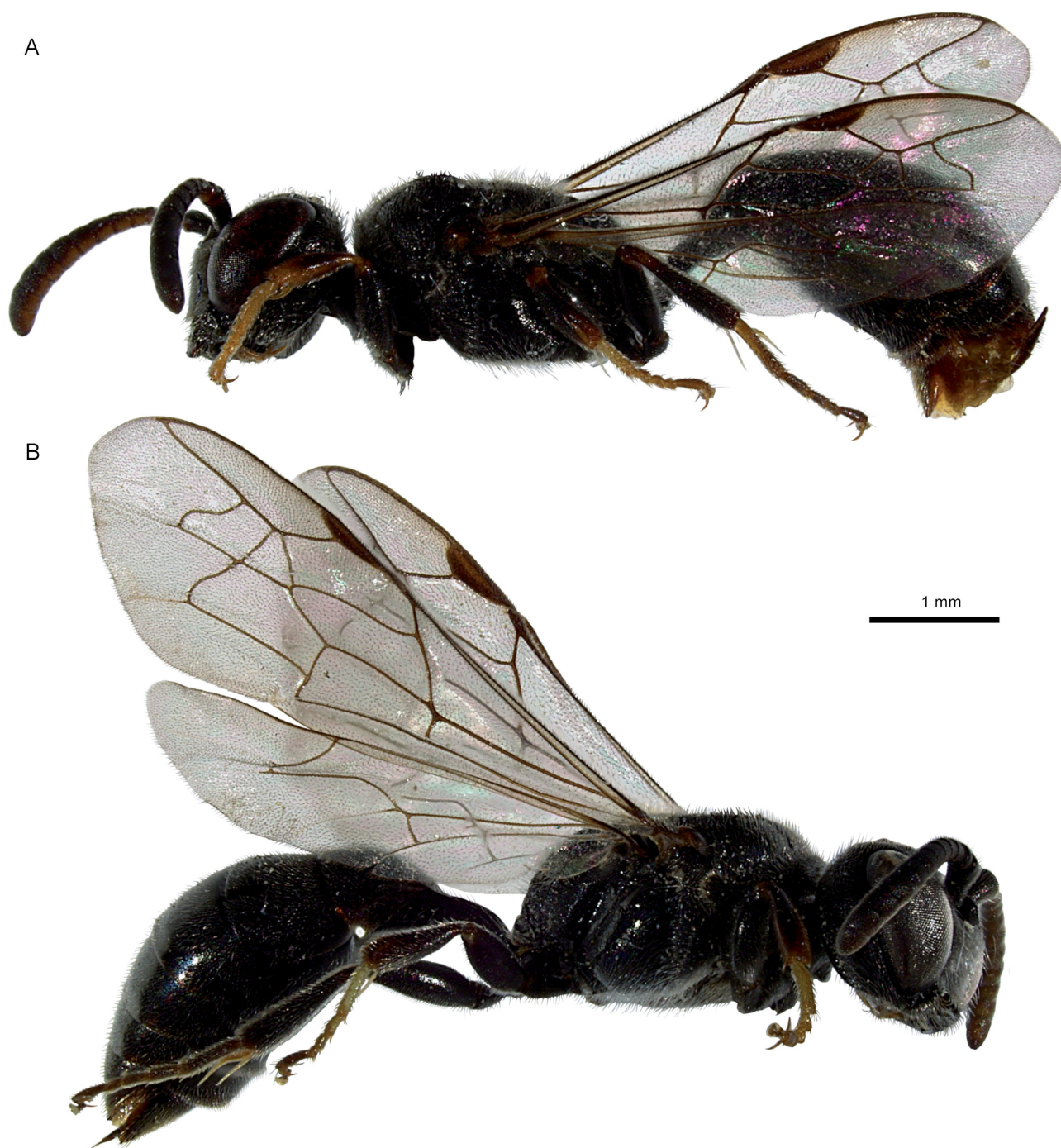


Fig. 2. (A) Female *Psenulus fulvicornis* (Schenck, 1857) from the City of Zurich, Switzerland (D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll; GenBank accession Nr. KY039438). (B) Female *Psenulus schencki* (Tournier, 1889) from the City of Zurich (25.05.2015; D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll; GenBank accession Nr. KY039439). Note the (barely visible) oblong spot at the lower end of the mid-tibia, which is a unique and shared character for both taxa among Central European *Psenulus* species (Jacobs, 2007).

Madroñeras (ESP: Salamanca); vineyard; 06.2005; S. F. Gayubo leg. & det.; DNA-ID PFU-2; GenBank accession Nr. MG872063. – 1 female; Miranda del Castañar, Las Matos (ESP: Salamanca); olive grove; 8.2005; S. F. Gayubo leg. & det.; DNA-ID PFU-3;

GenBank accession Nr. MG872064. – 1 female; Anapa, Djemete (RU: Krasnodar); shrubby coastal sand dunes; 44.961107°N, 37.281649°E; 17.06.2014; M. Mokrousov leg. & det.; DNA-ID PFU-9; GenBank accession Nr. MG872072.

***Psenulus schencki* (Tournier, 1889)**

1 female; city of Zurich (CH: ZH); private garden; 47.3692°N, 8.5061°E, ca. 428 m a.s.l.; 25.05.2015; D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll.; DNA-ID PHP16-0493; GenBank accession Nr. KY039439 (Figs 2, 3). – 1 female; Anapa, Djemete (RU: Krasnodar); shrubby coastal sand dunes; 44.961107°N, 37.281649°E; ca. 15.06.2014; M. Mokrousov leg. & det.; DNA-ID PSC-10; GenBank accession Nr. MG872073. 1 female; Barkhan Sarykum (RU: Dagestan); on the leaves of *Ailanthus altissima*; 43.002435°N, 47.237245°E; ca. 30.05.2017; M. Mokrousov leg. & det.; DNA-ID PSC-11; GenBank accession Nr. MG872074.

***Psenulus* sp.**

1 female; La Sterpaia, Parco San Rossore (IT: Pisa); 31.05.2005- 09.06.2005; L. Strumina leg.; S. F. Gayubo det.; DNA-ID; PSC-7; GenBank accession Nr. MG872069.

**Discriminating morphological characters**

The main discriminating characters between female *P. fulvicornis* and *P. schencki* are depicted in Fig. 3. In brief, the lateral surface of the propodeum of *P. fulvicornis* has short crosswise carinas that give the texture the characteristic coarse appearance (Fig. 3A). This trait is unique among females of Central European *Psenulus*. In *P. schencki* crosswise carinas are lacking, and the texture of the propodeum is smoother (Fig. 3C). Moreover, the pygidial area is usually longer and broader in *P. fulvicornis* (Fig. 3B) than in *P. schencki* (Fig. 3D). Likewise, the colour of the foretibia tends to be lighter in *P. fulvicornis* than in *P. schencki* (Fig. 2). More characters and a description of males are given by Schmid-Egger (2002) and Jacobs (2007).

***cox1* sequence variation**

The *cox1* sequences of *P. fulvicornis* and *P. schencki* from Zurich were sampled successfully and sequences were deposited in GenBank (supplementary Table S1 in the Appendix). However, since the *P. fulvicornis* specimen from Geneva was collected several years ago, the extracted DNA was of poor quality and *cox1* amplification failed in that sample, despite several amplification attempts with varying PCR parameters. All obtained sequences met barcode quality criteria with more than 500 bp sampled and no uncertain base calls (N's).

We found 22 polymorphic sites within a 561 bp alignment of the *P. fulvicornis*-Group, which excluded gap positions on the flanking regions (Table 1). All variable sites were synonymous single-nucleotide polymorphisms (SNPs), except two nonsynonymous replacements found in the specimens from Krasnodar (Russia) and Salamanca (Spain) (Fig. 4). The overall haplotype diversity was relatively high (Table 1). The three samples from the Caucasus (clades IV, V, and VI) strongly contributed to the overall genetic diversity as each sample represented a unique haplotype and contained a large proportion of polymorphic sites (Table 1; Fig. 4).

***cox1* phylogenetic tree**

In total, eleven *cox1* sequences were sampled (supplementary Table S1 in the Appendix). The dataset for the analysis of the genus *Psenulus* comprised 637 nucleotide sites and 42 sequences, including five sequences of two outgroup species, *Diodontus minutus* (Fabricius, 1793) and *Pemphredon lethifer* (Shuckard, 1837). Of the 637 sites, 204 were polymorphic. Both ML and Bayesian analyses resulted in highly similar topologies for *Psenulus* species (ML tree: Fig. 5; Bayesian tree: supplementary Fig. S1 in the Appendix), and the genus obtained maximal support under both analyses. Bootstrap values and posterior probabilities were high for most taxa, but while

Table 1. *Cox1* polymorphism in the *Psenulus fulvicornis*-Group and within each of the statistically well supported Central and South-Western European clades I-II (*P. fulvicornis*) and III (*P. schencki*) based on a 561 bp alignment.

	<i>P. fulvicornis</i> -Group Clades I-VI	<i>P. fulvicornis</i> Clades I-II	<i>P. schencki</i> Clade III
Number of sequences	17	6	8
Polymorphic sites	22	7	2
Parsimony informative sites	12	4	2
Number of haplotypes (H)	11	5	3
Haplotype diversity (Hd)	0.934	0.933	0.714
Variance of haplotype diversity	0.00181	0.01481	0.01507
Nucleotide diversity per site (Pi)	0.01020	0.00606	0.00178
Average of nucleotide differences (K)	5.721	3.400	1.000



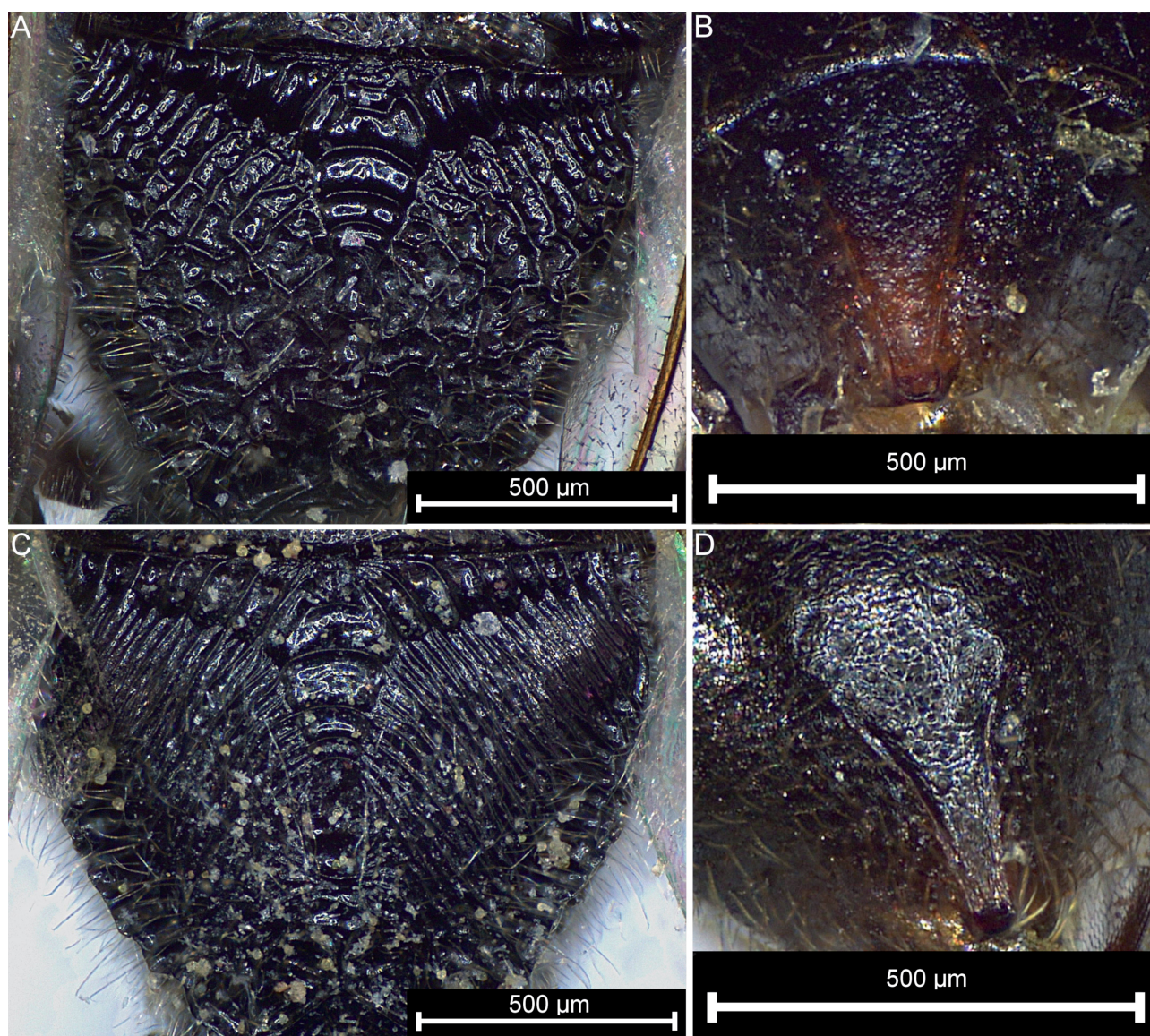


Fig. 3. Main discriminant characters between females of *Psenulus fulvicornis* (Schenck, 1857) and *P. schencki* (Tournier, 1889). (A) Propodeal surface and (B) pygidial area of *P. fulvicornis* from the City of Zurich, Switzerland (D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll; GenBank accession Nr. KY039438). (C) Propodeal surface and (D) pygidial area of *P. schencki* from the City of Zurich (25.05.2015; D. Frey & A. Zanetta leg.; M. Řiha det.; R. Neumeyer conf.; WSL coll; GenBank accession Nr. KY039439). In *P. fulvicornis*, the lateral surface of the propodeum (A) has short crosswise carinas which gives the texture coarse appearance. This character is unique among females of Central European *Psenulus*. In *P. schencki* such crosswise carinas are lacking (C), and the texture is finer. The pygidial area is longer and broader in *P. fulvicornis* (B) than in *P. schencki* (D), but this character can be difficult to observe in certain specimens.

Bayesian analysis highly supported a closer relationship between *P. pallipes*, *P. trisulcus*, *P. meridionalis* and *P. fuscipennis*, RAxML calculated only a low bootstrap value of 63% for this branching. Additionally, the position of the accession JN934379 within the *P. pallipes* clade was poorly supported in both analyses. The unidentified specimen PSC-7 from Pisa formed a highly supported sister branch to all other *P. fulvicornis* and *P. schencki* samples in both analyses (RAxML: 94%; MrBayes: 0.99), confirming the unknown status

of PSC-7 whose species identification failed with 2.3% dissimilarity from other *Psenulus* species in a BOLD System search (<http://www.boldsystems.org>; July 10th, 2018). All samples of the *P. fulvicornis*-Group formed a highly supported monophyletic clade (RAxML: 81%; MrBayes: 0.99). The samples of the *P. fulvicornis*-Group were subdivided in six clades, all of which were congruent with taxonomic boundaries based on morphology (Fig. 5): On the one hand, *P. fulvicornis* was split in two well supported clades in Central and South-



Position of segregation sites	12	45	108	111	117	144	168	178*	192	213	243	270	285	321	330	456	472*	474	486	496	516	543	
PFU-1	A	G	G	T	T	G	A	A	A	T	A	A	T	A	A	C	T	A	A	C	T	A	G
PFU-3	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-
PFU-2	-	-	-	-	-	A	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	
PFU-7	-	-	-	-	A	-	-	-	C	-	G	-	-	-	A	-	T	-	-	-	-	-	
PFU-8	-	-	-	-	A	-	-	-	C	-	G	-	-	-	A	-	T	-	-	-	-	-	
PHP16-0418	-	-	-	-	A	-	-	-	C	-	G	-	-	C	-	G	-	-	A	-	T	-	
PSC-11	-	-	C	A	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T	-	G	T	
OPPFM1833	-	-	-	A	-	G	-	G	-	-	-	-	-	-	A	-	-	C	-	-	-	-	
OPPFM3380	-	-	-	A	-	G	-	G	-	-	-	-	-	-	A	-	-	C	-	-	-	-	
OPPEE5705	-	-	-	A	-	G	-	G	-	-	-	-	-	-	A	-	T	C	-	-	-	-	
KR791646	-	-	-	A	-	G	-	G	-	-	-	-	-	-	A	-	T	C	-	-	-	-	
PHP16-0493	-	-	-	A	-	G	-	G	C	-	-	-	-	-	A	-	-	C	-	-	-	-	
GMGMA1097	-	-	-	A	-	G	-	G	C	-	-	-	-	-	A	-	-	C	-	-	-	-	
KR792963	-	-	-	A	-	G	-	G	C	-	-	-	-	-	A	-	-	C	-	-	-	-	
GMGMA1095	-	-	-	A	-	G	-	G	C	-	-	-	-	-	A	-	-	C	-	-	-	-	
PFU-9	-	-	A	-	A	-	G	-	C	G	-	A	-	-	G	A	G	T	-	-	A	-	
PSC-10	T	A	-	A	-	G	-	G	-	-	-	T	-	A	-	T	-	-	A	-	-	-	

Fig. 4. *Cox1* polymorphic sites within a 561 bp alignment of the *P. fulvicornis*-Group. Vertical numbers indicate the position of the segregation site in the alignment. Of 561 sites 539 were invariable, 10 singleton variable, and 12 parsimony informative sites. At position 543 of the alignment were three mutations, and replacements at the positions 178 and 472 were nonsynonymous (asterisks). Specimens are arranged according to their genetic distance. The Caucasian specimen PSC-11 was divergent from *P. fulvicornis* and *P. schencki* but it was similarly distant to both. Specimens are labelled with the specimen ID, the GenBank accession number or the Barcode Identification Number of the BOLD System (see supplementary Table S1). Rectangles include specimens belonging to the clades I–VI in the phylogenetic analysis (Fig. 5).

Western Europe, with clade I including the samples from Salamanca (RAxML: 88%; MrBayes: 0.99), and clade II including the samples from Zurich and Northern Spain (RAxML: 86%; MrBayes: 0.92). On the other hand, the seven *P. schencki* accessions from Canada, Germany and Zurich formed the well-supported monophyletic clade III (RAxML: 86%; MrBayes: 1.0). Finally, the three Caucasian specimens (one attributed morphologically to *P. fulvicornis* and the others to *P. schencki*) represented isolated branches (clades IV, V, and VI) whose relationships with one another and with Central European specimens remained unresolved.

#### Review of museum and private collections

No *P. fulvicornis* were found among *P. schencki* accessions in Swiss museums and private collections. A total of 221 museum and eight specimens from private collections could be reviewed, of which 98% had an indication of the sampling site on the label (see Appendix). All major Swiss museum and private collections harbouring *P. schencki* could be visited apart from the Naturmuseum Luzern, which has three specimens that were not accessible for visitors in February 2018. The Bündner Naturmuseum, Muséum

d'histoire naturelle Neuchâtel, Musée de la nature de Sion, Museo cantonale di storia naturale and Naturmuseum St. Gallen did not harbour *P. schencki* accessions.

## DISCUSSION

### *Psenulus fulvicornis* new to Switzerland

Surveys of urban arthropod communities in northern temperate cities frequently reveal alien species that originate from warmer climates (e.g. Germann *et al.*, 2015; Frey *et al.*, 2016; Zanetta *et al.*, 2016). The urban heat island effect, which provides warmer temperatures in the city when compared to the rural surroundings, and, presumably, the availability of nesting sites (e.g. *Sambucus* and *Rubus* wood, and artificial nests) and feeding resources (e.g. aphids) in large allotment garden areas, could explain the finding of *P. fulvicornis* in an urban garden lot (e.g. Hall *et al.*, 2017). Similarly, in Central Europe, *P. schencki* occurs in settlement areas and gardens (Blösch, 2000). Finally, discovering new cavity-nesting hymenopterans in anthropogenic habitats such as gardens may not be surprising since nests can be easily transported by humans over distances that are far beyond the dispersal capacity of adults. However, the naturalization status of *P. fulvicornis* in Switzerland remains unclear. This inconspicuous species is relatively rarely observed throughout its distribution range and it could have simply been overlooked. Yet our review of *P. schencki* accessions in Swiss museum and private collections indicates that *P. fulvicornis* could represent a recent colonizer, with to date, only two known observations: the two females that were caught in warm lowland habitats of the Cantons of Zurich and Geneva.

### Taxon identification using *cox1* sequence information

While at least females of *P. fulvicornis* and *P. schencki* can be readily identified morphologically (Fig. 3), we found that neither SNPs (Fig. 4) nor the phylogeny (Fig. 5) could consistently separate the two taxa at the large geographic scale investigated. Nevertheless, specimens sampled in Central Europe belonged either to the well supported clades II (*P. fulvicornis*) or III (*P. schencki*) and may also be separated by several diagnostic SNPs (Figs 4, 5). The existence of a such spatial pattern in *cox1* sequence divergence within and among the taxa needs, however, further corroboration.

### Phylogenetic relationships within the *Psenulus fulvicornis*-Group

We found that the *P. fulvicornis*-Group formed a highly supported monophyletic branch within the Central European *Psenulus* (note, however, that our phylogeny lacked *P. chevrieri* (Tournier, 1889) and the rare *P. berlandi* de Beaumont, 1937). This result

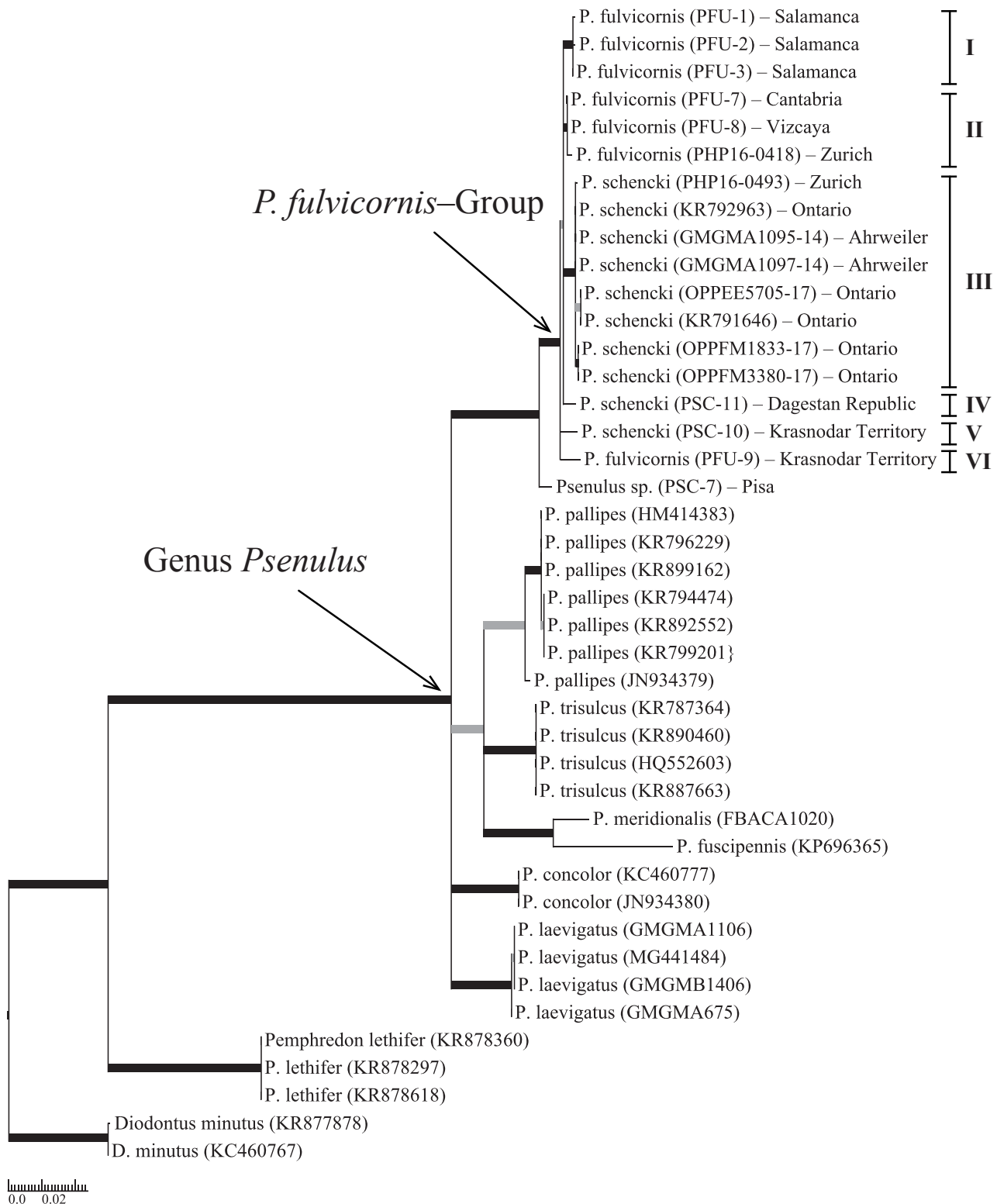


Fig. 5. Maximum likelihood tree showing the phylogenetic relationships within *Psenulus* taxa based on *cox1* and the GTR+G substitution model. All bifurcations with bootstrap values below 60% were collapsed. The bold black lines indicate bootstrap of at least 70% and the thick grey bifurcations 60-70%. Both *Diodontus minutus* (Fabricius, 1793) and *Pemphredon lethifer* (Shuckard, 1837) sequences root this tree. Specimens are labelled with the sample ID, their GenBank accession number or the Barcode Identification Number of the BOLD System, and the collection site if known (see supplementary Table S1). Six lineages I–VI were found within the *P. fulvicornis*-Group.



confirms the morphology-based hypothesis of their close relationship (Schmidt, 1971; Schmid-Egger, 2002) and is in contrast to former authors, which grouped *P. fulvicornis* with *P. fuscipennis* (Schenck, 1861; de Beaumont, 1939). However, we found polyphyly within the *P. fulvicornis*-Group, which was split in at least three well supported clades in South-Western and Central Europe. All clades were coherent with the taxonomic classification of the samples, which excludes imperfect taxonomy as a cause for polyphyly. The unresolved phylogenetic relationship between the Caucasian specimens and the South-Western and Central European clades may be interpreted as a sampling gap (i.e. incomplete sampling) in the eastern part of the distribution range of *P. fulvicornis* (Fig. 1) (Cho *et al.*, 2011; Wiens & Tiu, 2012).

Previous studies have shown that many taxa considered as valid species are polyphyletic at the *cox1* locus, especially when sampled comprehensively across (large) distribution ranges, and intra- and interspecific variability (Funk & Omland, 2003; Meyer & Pauley, 2005). Specifically, it was also found in hymenopterans (e.g. Jansen *et al.*, 2009; Nicholls *et al.*, 2012; Eimanifar *et al.*, 2018). The rather small morphological and genetic differences (Figs 2, 5) suggest that *P. fulvicornis* and *P. schencki* could be interpreted as incipient species, which are not yet reciprocally monophyletic at the *cox1* locus (i.e. incomplete lineage sorting; Funk & Omland, 2003). Similar findings have also been made in other putatively closely related Central European hymenopterans (e.g. Schmidt *et al.*, 2015). Other possible reasons for polyphyly include hybridisation and mitochondrial DNA introgression (Funk & Omland, 2003): the geographic range overlap and potentially similar habitat requirements between *P. fulvicornis* and *P. schencki* could set the stage for occasional hybridisation, and Gauss (1974) indeed observed a putative *P. fulvicornis* female *in copula* with a *P. schencki* male in South Western Germany. Yet we did not find a genetic sign of interspecific gene flow here, and, moreover, other (nuclear) loci may be more effective to detect hybrids (Petit & Excoffier, 2009; Nicholls *et al.*, 2012; Patten *et al.*, 2015; Beresford *et al.*, 2017).

## CONCLUSION

We found extensive and overlapping sequence variability at the *cox1* locus between *P. fulvicornis* and *P. schencki* at the European scale. Hence, we could not identify a barcoding gap, which may hamper taxon identification. Nevertheless, in Central Europe, we found small but well supported genetic and morphological differentiation among co-occurring specimens, which corroborates their separate taxonomic treatment, consents identification and confirms *P. fulvicornis* as a new element of the Swiss hymenopteran fauna.

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## APPENDIX

### Museum and private collection material examined

Ambiguous information or barely readable text on a label is given in square brackets.

#### *Psenulus fulvicornis* Schenck, 1857

1 female; Avusy, Moulin de la Grave (CH: GE); 12.06.2005; leg. & det. H. Boillat; R. Neumeyer conf.; MHNG coll.

#### *Psenulus schencki* Tournier, 1889

1 female; Brusio (CH: GE); 31.07.1935 - 05.08.1935; A. Nadig leg.; M. Hermann det.; ETHZ coll. - 1 male; Pfynwald (CH: VS); 24.05.3[5]; J. de Beaumont det. 1936; ETHZ (Schulthess) coll. - 1 female; CH: VS; J. de Beaumont det. 1936; ETHZ (Schulthess) coll. - 1 male; Ardez (CH: GR); 22.06.1921; A. Breitenstein det. 2002; ETHZ coll. - 1 female; Zernez (CH: GR); 08.08.1935; A. Nadig leg.; S. Bieri det. 1996; ETHZ coll. - 1 female & 1 male; Winterthur, Töss, Rosenau (CH: ZH); 05.06.1971; A. Krebs leg. & det.; ETHZ coll. - 6 males; Winterthur, Töss, Chrugeler (CH: ZH); 09-10.02.1972; A. Krebs leg. & det.; ETHZ coll. - 3 males; Unterstammheim (CH: ZH); 02-06.04.1972; A. Krebs leg. & det.; ETHZ coll. - 1 male & 1 female; Flaach (CH: ZH); 20-25.03.1973; A. Krebs leg. & det.; ETHZ coll. - 1 male; Winterthur (CH: ZH); 19.06.1973; A. Krebs leg. & det.; ETHZ coll. - 1 female; Winterthur (CH: ZH); 26.06.1973; A. Krebs leg. & det.; ETHZ coll. - 1 female; Winterthur (CH: ZH); 04.07.1973; A. Krebs leg. & det.; ETHZ coll. - 1 female; Winterthur (CH: ZH); 11.02.1974; A. Krebs leg. & det.; ETHZ coll. - 2 females; Dielsdorf (CH: ZH); A. Krebs leg. & det.; ETHZ coll. - 1 female & 3 males; Winterthur (CH: ZH); 10-15.03.1974; A. Krebs leg. & det.; ETHZ coll. - 1 female; Flaach (CH: ZH); 27.03.1974; A. Krebs leg. & det.; ETHZ coll. - 3 females & 3 males; Nürensdorf (CH: ZH); 15-17.04.1975; A. Krebs leg. & det.; ETHZ coll. - 1 male; Eglisau (CH: ZH); 16.04.1975; A. Krebs leg. & det.; ETHZ coll. - 2 females; Kleinandelfingen

(CH: ZH); 20.04.1975; A. Krebs leg. & det.; ETHZ coll. - 6 females; CH: GR; 2-27.06.1975; A. Krebs leg. & det.; ETHZ coll. - 8 females; Uerschhausen (CH: TG); 25-26.02.1976; A. Krebs leg. & det.; ETHZ coll. - 4 females & 2 males; CH: GR; 11-14.06.1976; A. Krebs leg. & det.; ETHZ coll. - 1 female; Randen (CH: SH); 28.07.1992; 800 m a.s.l.; H. Bernasconi leg. & det.; ETHZ coll. - 2 males; Rickenbach (CH: SO); 23.06.1994; F. Amiet leg. & det.; ETHZ coll. - 1 female; Rickenbach (CH: SO); 07.07.1994; F. Amiet leg. & det.; ETHZ coll. - 1 female; city of Zurich, Zürichberg (CH: ZH); 30.05.1995; S. Ungricht leg. & det.; ETHZ coll. - 1 male; city of Zurich, Zürichberg (CH: ZH); 28.06.1995; S. Ungricht leg. & det.; ETHZ coll. - 1 male; San Vittore (CH: GR); 320 m a.s.l.; 08.04.1997; A. Salvioni leg. & det.; ETHZ coll. - 1 male; San Vittore (CH: GR); 320 m a.s.l.; 13.07.1997; A. Salvioni leg. & det.; ETHZ coll. - 1 female; Neunform, Bahnhof (CH: TG); 47.59460°N, 8.75661°E; 25.05.2012; M. Herrmann leg. & det.; ETHZ coll. - 1 female; city of Berne, Kirchenfeld (CH: BE); 12.07.1925; T. Steck leg., J. de Beaumont det. 1944; MZL coll.; GBIFCH00502859. - 1 female; city of Berne, Kirchenfeld (CH: BE); 13.08.1925; T. Steck leg., J. de Beaumont det. 1944; MZL coll.; GBIFCH00502864. - 1 female; city of Berne, Kirchenfeld (CH: BE); 13.08.1925; T. Steck leg., J. de Beaumont det. 1944; MZL coll.; GBIFCH00502856. - 1 female; city of Berne, Kirchenfeld (CH: BE); 21.08.1925; T. Steck leg., J. de Beaumont det. 1944; MZL coll.; GBIFCH00502860. - 1 female; city of Berne, Kirchenfeld (CH: BE); 31.07.1927; T. Steck leg., J. de Beaumont det. 1944; MZL coll.; GBIFCH00502858. - 1 female; Coligny (CH: GE); 30.06.1931; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502872. - 1 female; Peney (CH: GE); 05.07.1931; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502871. - 1 female; Coligny (CH: GE); 20.09.1931; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502873. - 1 female; Peney (CH: GE); 20.05.1932; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502870. - 1 male; Coligny (CH: GE); 26.06.1932; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502886. - 1 male; Coligny (CH: GE); 26.06.1932; J. de Beaumont leg. &



det.; MZL coll.; GBIFCH00502887. – 1 male; Cologny (CH: GE); 11.06.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502875. – 1 male; Cologny (CH: GE); 11.06.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502888. – 1 male; Cologny (CH: GE); 16.06.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502881. – 1 male; Cologny (CH: GE); 30.06.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502880. – 1 male; Cologny (CH: GE); 30.06.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502883. – 1 male; Cologny (CH: GE); 01.07.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502882. – 1 male; Cologny (CH: GE); 01.07.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502885. – 1 male; Cologny (CH: GE); 01.07.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502889. – 1 female; Versoix (CH: GE); 05.08.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502867. – 1 female; Allondon (CH: GE); 19.08.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502869. – 1 female; Cologny (CH: GE); 01.10.1933; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502874. – 1 female; Thun (CH: BE); 04.08.1937; Naef leg.; J. de Beaumont det.; MZL coll.; GBIFCH00502865. – 1 male; Grimontz (CH: VS); 27.07.1944-12.08.1944; J. de Beaumont leg.; J. de Beaumont det. 1957; MZL coll.; GBIFCH00502879. – 1 female; Grimontz (CH: VS); 24.06.1949-08.07.1949; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502861. – 1 female; city of Lausanne, Vidy (CH: VD); 23.07.1955; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502866. – 1 male; Losone (CH: TI); 30.06.1959-02.07.1959; J. de Beaumont leg. & det.; GBIFCH00502878. – 1 male; Gordola (CH: TI); 01.07.1959; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502877. – 1 male; Pampigny (CH: VD); 22.06.1960; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502876. – 1 male; Ecublens (CH: VD); 18.07.1962; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502884. – 1 female; La Sarraz (CH: VD); 04.08.1963; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502862. – 1 female; La Sauge (CH: VD); 16.08.1963; J. de Beaumont leg. & det.; MZL coll.; GBIFCH00502863. – 1 female; P. [Peney-le-Jorat (CH: VD)]; 02.06.1878; leg. & det. H. Tournier; MHNG coll. (Typus!). – 1 female; P. [Peney-le-Jorat (CH: VD)]; 21.06.1878; leg. & det. H. Tournier; MHNG coll. (Typus!). – 1 female; P. [Peney-le-Jorat (CH: VD)]; 05.05.1884; leg. & det. H. Tournier; MHNG coll. – 1 female; P. [Peney-le-Jorat (CH: VD)]; 05.06.1889; leg. & det. H. Tournier; MHNG coll. – 1 female; Pen. [Peney-le-Jorat (CH: VD)]; 23.05.1890; leg. & det. H. Tournier; MHNG coll. – 1 female; Nyon (CH: VD); leg. & det. H. Tournier; MHNG coll. – 1 female; Allondon (CH: GE); 16.08.1995; H. Boillat leg. & det.; MHNG coll. – 1 female; Sézegnin (CH: GE); 06.07.1997; H. Boillat leg. & det.; MHNG coll. – 1 female; Biasca (CH: TI); B. Merz leg.; A. Salvioni det.; 360 m a.s.l.; 08.07.1997; MHNG coll. – 1 female; Biel-Benken (CH: BL); 360 m a.s.l.; 30-31.05.1998; B. Merz leg.; H. Boillat det. 2006; MHNG coll. – 1 male; Leuk, Brentjong (CH: VS); 920 m a.s.l.; 15.05.2000; B. Merz leg.; H. Boillat det.; MHNG coll. – 1 male; Jussy, Les Prés de Villette (CH: GE); 475 m a.s.l.; B. Merz & Bächli leg.; H. Boillat det.; MHNG coll. – 1 female; Avusy, Moulin de la Grave (CH: GE); 22.07.2005; H. Boillat leg.; H. Boillat det. 2006; MHNG coll. – 1 female; Avusy, Moulin de la Grave (CH: GE); 10.08.2005; H. Boillat leg.; H. Boillat det. 2006; MHNG coll. – 1 male; CH: BE; 13.07.1891; T. Steck leg. & det.; NMB coll. – 1 female; Bätterkinden (CH: BE); 13.08.1892; T. Steck leg. & det.; NMB coll. – 1 female; CH: BE; 21.08.1896; T. Steck leg. & det.; NMB coll. – 1

female; Unterbäch (CH: VS); 12.06.1915; T. Steck leg. & det.; NMB coll. 1 male; Unterbäch (CH: VS); 19.06.1915; T. Steck leg. & det.; NMB coll. – 1 female; city of Berne, Kirchenfeld (CH: BE); 16.08.1915; T. Steck leg. & det.; NMB coll. – 6 females; city of Berne, Kirchenfeld (CH: BE); 23.06.1918-26.09.1918; T. Steck leg. & det.; NMB coll. – 1 female; Berne, Kirchenfeld (CH: BE); [07?].06.1920; T. Steck leg. & det.; NMB coll. – 2 females; Berne, Kirchenfeld (CH: BE); 12.08.1920-16.10.1920; T. Steck leg. & det.; NMB coll. – 6 males; Lyss (CH: BE); 26.05.1921; T. Steck leg. & det.; NMB coll. – 17 females and 5 males; Kirchenfeld (CH: BE); 11.06.1921-15.09.1921; T. Steck leg. & det.; NMB coll. – 1 male; Ayent (CH: VS); 25.06.19[21?]; T. Steck leg. & det.; NMB coll. – 2 females; Kirchenfeld (CH: BE); 12.06.1922-14.06.1922; T. Steck leg. & det.; NMB coll. – 1 female; Euseigne (CH: VS); 06.08.1922; T. Steck leg. & det.; NMB coll. – 4 females and 1 male; Kirchenfeld (CH: BE); 10.06.1924-09.09.1924; T. Steck leg. & det.; NMB coll. – 31 females and 1 male; Kirchenfeld (CH: BE); 10.06.1925-21.09.1925; T. Steck leg. & det.; NMB coll. – 4 females and 1 male; 11.07.1926-24.09.1926; T. Steck leg. & det.; NMB coll. – 2 females; Kirchenfeld (CH: BE); 30.05.1927-28.06.1927; T. Steck leg. & det.; NMB coll. – 1 female; Gümligen (CH: BE); 21.06.1927; T. Steck leg. & det.; NMB coll. – 1 male; Lötschental (CH: VS); 16.07.1934; T. Steck leg. & det.; NMB coll. – 1 female; Kirchenfeld (CH: BE); [22].08.19[?]; T. Steck leg. & det.; NMB coll. – 1 female; Kirchenfeld (CH: BE); 14.06.19[?]; T. Steck leg. & det.; NMB coll. – 1 female; Martigny (CH: VS); 15.06.1889; J. de Beaumont det. 193[0]; NMBE coll. – 1 female and 1 male; Mörel (CH: VS); 28.05.[19]50; Bucher leg. & det.; NMBE coll. – 1 female; Arch (CH: BE); 24.07.1987; F. Amiet leg.; F. Amiet det. 1989; NMBE coll. – 1 female; Attisholz (CH: SO); 28-29.03.1977; F. Amiet leg.; F. Amiet det. 1989; NMBE coll. – 1 female; Rickenbach (CH: SO); 04.08.1994; F. Amiet leg. & det.; NMBE coll. – 1 female; Lostorf, Mahren (CH: SO); 28.09.1995; F. Amiet leg.; F. Amiet det. 1997; NMBE coll.

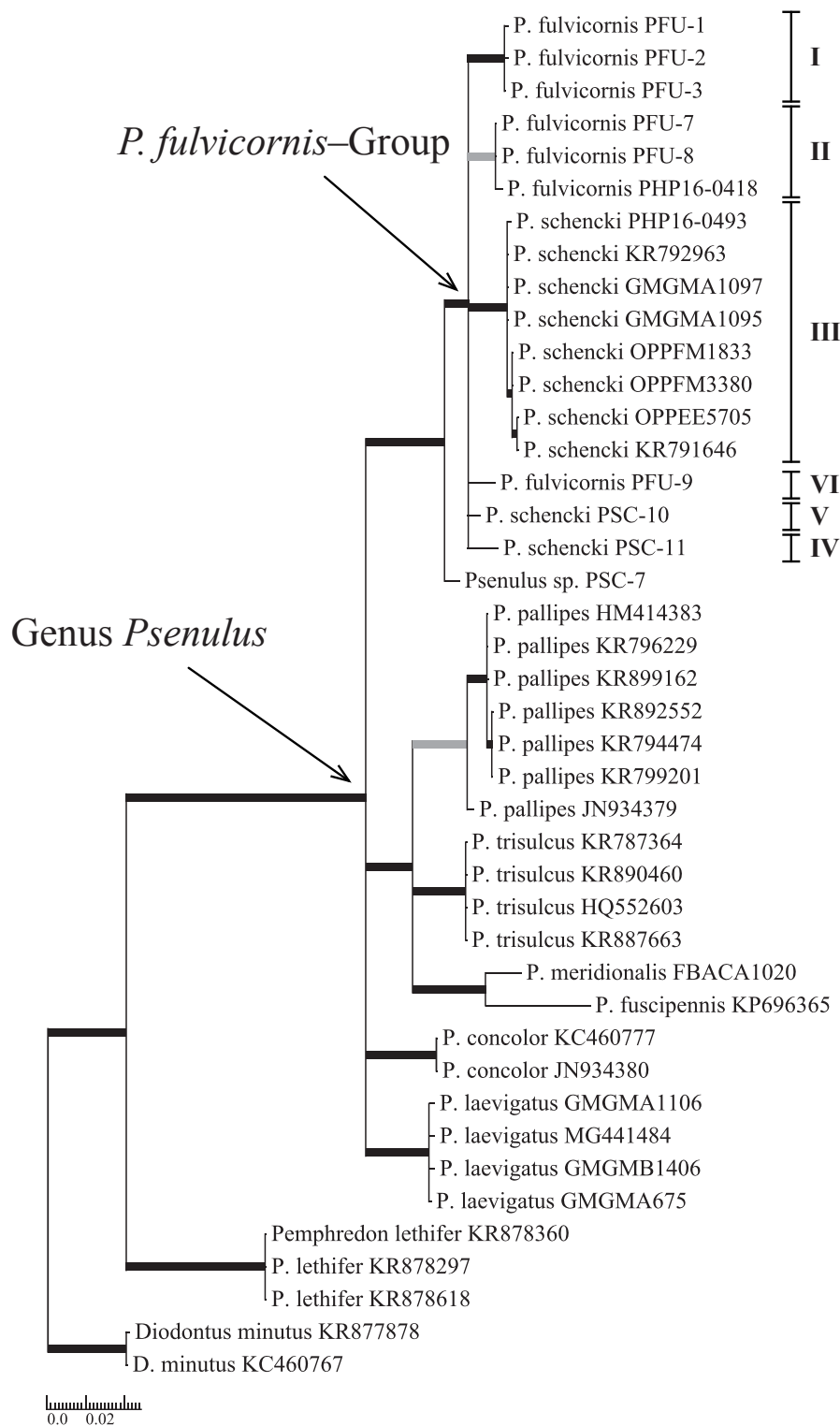
#### Private collection material examined

1 female; Jona, Mittlere Tägernau (CH: SG); horse pasture; 47.24109°N, 8.86645°E; 475 m a.s.l.; 25.08.1999; R. Neumeyer leg. & det.; C. Schmid-Egger conf. 2012; R. Neumeyer coll. – 1 female; Trimbach, Miesernbach (CH: SO); moist herbaceous fringe (Convolvulion); 47.36540°N, 7.87152°E; 530 m a.s.l.; 04.08.2002; H. Baur & G. Artmann-Graf leg. & det.; G. Artmann-Graf coll. 1 female; Härkingen, Untere Allmend (CH: SO); mesophilous ruderal vegetation in a gravel pit (Dauco-Melilotion); 47.29499°N, 7.82467°E; 430 m a.s.l.; 11.07.2010; G. Artmann-Graf leg., det. & coll. – 1 female; Holderbank, Chrüzacher (CH: SO); herbaceous fringe at the forest margin (Trifolion medii); 47.32849°N, 7.73273°E; 660 m a.s.l.; 26.06.2014; G. Artmann-Graf leg., det. & coll. – 1 female; Vordemwald (CH: AG); in private garden on honeydew; 47.27992°N, 7.90045°E; 446 m a.s.l.; 20.06.1982; I. Salzmann leg., det. & coll. – 1 female; Vordemwald (CH: AG); in the house at the window; 47.27992°N, 7.90045°E; 446 m a.s.l.; 08.06.1983; I. Salzmann leg., det. & coll. – 1 female; Vordemwald (CH: AG); in the house at the window; 47.27992°N, 7.90045°E; 446 m a.s.l.; 08.07.1984; I. Salzmann leg., det. & coll. – 1 male; Vordemwald (CH: AG); in the house at the window; 47.27992°N, 7.90045°E; 446 m a.s.l.; 13.06.1999; I. Salzmann leg., det. & coll.

## Supplementary Table S1

Collection information for sequences produced in our lab. Sequences acquired from GenBank (GB) or the Barcode of Life Data System (BOLD) are listed below.

Taxon	Voucher (DNA-ID) / Reference	GB accession number	BOLD sequence-ID
Specimens analyzed in this study			
<i>P. fulvicornis</i> (Schenck, 1857)	Salamanca, Spain, S.F. Gayubo (PFU-1)	MG872062	–
	Salamanca, Spain, S.F. Gayubo (PFU-2)	MG872063	–
	Salamanca, Spain, S.F. Gayubo (PFU-3)	MG872064	–
	Cantabria, Spain, S.F. Gayubo (PFU-7)	MG872065	–
	Vizcaya, Spain, S.F. Gayubo (PFU-8)	MG872066	–
	City of Zurich, Switzerland, D. Frey (PHP16-0418)	KY039438	–
	Krasnodar Territory, Russia, M. Mokrousov (PFU-9)	MG872072	–
<i>P. schencki</i> (Tournier, 1889)	City of Zurich, Switzerland, D. Frey (PHP16-0493)	KY039439	–
	Krasnodar Territory, Russia, M. Mokrousov (PSC-10)	MG872073	–
	Dagestan Republic, Russia, M. Mokrousov (PSC-11)	MG872074	–
<i>Psenulus</i> sp.	Pisa, Italy, L. Strumina (PSC-7)	MG872069	–
<i>Psenulus</i> species sequences extracted either from GenBank or BOLD			
<i>P. concolor</i> (Dahlbom, 1843)	Turčinavičienė <i>et al.</i> (2016)	JN934380	GBMIN8968-12
	Turčinavičienė <i>et al.</i> (2016)	KC460777	GBMIN11495-13
<i>P. fuscipennis</i> (Dahlbom, 1843)	Becker & Keller (2015)	KP696365	GBAH11915-15
<i>P. laevigatus</i> (Schenck, 1857)	Biodiversity Institute of Ontario (2018)	MG441484	RRSSC1796-15
	Biodiversity Institute of Ontario (2018)	–	GMGMA1106_14
	Biodiversity Institute of Ontario (2018)	–	GMGMA675_14
	Biodiversity Institute of Ontario (2018)	–	GMGMB1406_14
<i>P. pallipes</i> (Panzer, 1798)	Biodiversity Institute of Ontario (2010)	HM414383	BBHEC732-10
	Hebert <i>et al.</i> (2016)	KR799201	HPPPD314-13
	Hebert <i>et al.</i> (2016)	KR892552	SMTPD1227-13
	Hebert <i>et al.</i> (2016)	KR899162	SMTPI5851-14
	Hebert <i>et al.</i> (2016)	KR796229	HEJUL005-12
	Hebert <i>et al.</i> (2016)	KR794474	JSHYM764-11
	Turčinavičienė <i>et al.</i> (2016)	JN934379	GBMIN29695-13
	Hebert <i>et al.</i> (2016)	KR791646	MBIOD104-13
<i>P. schencki</i> (Tournier, 1889)	Hebert <i>et al.</i> (2016)	KR792963	MBIOE1294-13
	Biodiversity Institute of Ontario (2018)	–	GMGMA1095-14
	Biodiversity Institute of Ontario (2018)	–	GMGMA1097-14
	Biodiversity Institute of Ontario (2018)	–	OPPEE5705-17
	Biodiversity Institute of Ontario (2018)	–	OPPFM1833-17
	Biodiversity Institute of Ontario (2018)	–	OPPFM3380-179
	Hebert <i>et al.</i> (2016)	KR890460	PHMTX721-11
<i>P. trisulcus</i> (Fox, 1898)	Hebert <i>et al.</i> (2016)	KR887663	TTMHY660-11
	Hebert <i>et al.</i> (2016)	KR787364	JSHYM986-11
	Biodiversity Institute of Ontario (2010)	HQ552603	BBHYF250-10
	Hebert <i>et al.</i> (2016)	KR877878	NGNAX5018-14
<i>Diodontus minutus</i> (Fabricius, 1793)	Turčinavičienė <i>et al.</i> (2016)	KC460767	GBMIN11500-13
<i>Pemphredon lethifer</i> (Shuckard, 1837)	Hebert <i>et al.</i> (2016)	KR878360	NGAAD389-14
	Hebert <i>et al.</i> (2016)	KR878297	NGAAF255-14
	Hebert <i>et al.</i> (2016)	KR878618	NGNAN043-14



Supplementary Figure S1

Phylogenetic tree representing species of the wasp genus *Psenulus* (Kohl, 1897). Bayesian analysis was based on *cox1* sequences (201 polymorphic nucleotides out of 637 sites), including 37 *Psenulus* and five outgroup specimens [*Diodontus minutus* (Fabricius, 1793) and *Pemphredon lethifer* (Shuckard, 1837)]. The analysis was performed with the substitution model GTR+G, estimated with jModelTest 0.1.1, and run in two chains with 5 million generations each. After discarding the burn-in, 37,501 trees were sampled of a total of 100,002 trees from both chain files. Bifurcations with posterior probabilities of <0.9 were collapsed, except for branches with ambiguous Maximum Likelihood and Bayesian results (thick grey bifurcations). All thick black branches represent posterior probabilities of at least 0.99. Specimens are labelled with the specimen ID, the GenBank accession number or the Barcode Index Number BIN of the BOLD System.