Dtsch. Entomol. Z. 68 (2) 2021, 249–259 | DOI 10.3897/dez.68.70814

PENSOFT

für Natur MUSEUM FÜR NATURKUNDE BERLIN

Where did the Central European populations of *Ornatoraphidia flavilabris* (Costa) come from? (Neuropterida, Raphidioptera, Raphidiidae)

Horst Aspöck[1], Ulrike Aspöck[2,3], Julia Walochnik[1], Edwin Kniha[1]

1 *Medical University Vienna (MUW), Institute of Specific Prophylaxis and Tropical Medicine, Kinderspitalgasse 15, A-1090 Vienna, Austria*

2 *Natural History Museum, 2nd Zoological Department, Burgring 7, A-1010 Vienna, Austria*

3 *University of Vienna, Department of Evolutionary Biology, Althanstraße 14, A-1090 Vienna, Austria*

http://zoobank.org/20935557-2FF2-49F6-BA61-266323E22F77

Corresponding author: Edwin Kniha (edwin.kniha@meduniwien.ac.at)

Academic editor: Susanne Randolf ♦ **Received** 29 June 2021 ♦ **Accepted** 5 August 2021 ♦ **Published** 30 August 2021

Abstract

Ornatoraphidia flavilabris (Costa, 1851) is one of 15 snakefly species occurring in southern parts of Central Europe. It is a polycentric Mediterranean faunal element with refugia in the Apennine Peninsula and the Balkan Peninsula. Two phylogeographic questions are dealt with in this paper:

(1) Is it possible to differentiate, morphologically or genetically, the Balkanic populations from the Italian?

(2) Did the species reach Central Europe from the Balkan or Apennine Peninsula?

These questions were investigated using morphological and molecular biological methods. No morphological characters were uncovered which could serve to differentiate specimens from either distribution center. However, differences were detected in *cox1*, *cox3* and *28S* genes which allow for a reliable differentiation. Central European populations were largely identical with populations from Italy, but distinctly different from specimens from Greece. This could lead one to assume that the species migrated from Italy to Central Europe, although colonization from the southeast would appear easier due to more favorable orographic conditions. This discrepancy may be explained by the apparent absence of *O. flavilabris* from the large central part of the Balkan Peninsula, so that a gap exists between the southern and northern areas inhabited by *O. flavilabris*. Moreover, the species does not occur in eastern parts of Europe. Thus it would be more probable to assume that the occurrence of the species in the northwest Balkan Peninsula can be traced to migrations from the Apennine Peninsula to areas north and northeast of the Adriatic Sea, where *O. flavilabris* may have colonized the southeast of Central Europe. A migration of Adriatomediterranean faunal elements from the northwest Balkan Peninsula to Central Europe might be of more significance than previously assumed.

Key Words

Apennine Peninsula, Balkan Peninsula, Mediterranean refugial centers, phylogeography, snakeflies

Introduction

Raphidioptera (snakeflies) is the smallest order of holometabolous insects. So far, around 250 valid described species are known world-wide: ca. 210 species of Raphidiidae and >40 species of Inocelliidae. The distribution of snakeflies is confined to the temperate zone of the Holarctic, with hotspots of high biodiversity in the Mediterranean region, Central Asia, Southeast Asia and southern North America. Europe harbors 82 species, and 57 of these are confined to the Mediterranean region (H. Aspöck et al. 1991, 2019, U. Aspöck and H. Aspöck 2009, H. Aspöck and U. Aspöck 2013).

Compared to the high number of snakefly species in the Mediterranean parts of Europe, Central Europe harbors only a moderate Raphidioptera fauna. It comprises

altogether 15 species: 12 Raphidiidae and 3 Inocelliidae. Six of these species have postglacially expanded their distribution originating from extramediterranean refugial centers and 9 species from Mediterranean refugia. The ranges of most snakeflies in the Mediterranean have not undergone significant expansion but are confined to one of the three peninsulas (Balkan, Apennine, Iberian), and in many cases to small parts only (H. Aspöck et al. 2001).

Few species have shown considerable expansivity and moved northwards after the last glacial period and colonized Central Europe. Among these are two species which occur on two peninsulas: *Ornatoraphidia flavilabris* (Costa, 1851) and *Venustoraphidia nigricollis* (Albarda, 1891). Of these, only *O. flavilabris* (Figs 1, 2) has been recorded from various parts of the Balkan Peninsula, most parts of the Apennine Peninsula (i.e., the southernmost parts to the north) and, moreover, the southern parts of Central Europe (Fig. 3). Since almost all Mediterranean snakeflies are restricted to mostly limited areas on either or both peninsulas, two questions arise. First, do the populations of *O. flavilabris* belong to a single species? And secondly, where were the Central European populations derived from, the Balkan or Apennine Peninsula? To clarify these questions, we repeated classical morphological examinations and comparisons — as performed years ago, and in particular, complemented this with a phylogeographic study.

Material and methods

Morphological examinations

Altogether about 75 specimens from 10 localities of the Balkan Peninsula, from 10 localities of the Apennine Peninsula, and from 5 localities in Central Europe were selected for investigation and comparison of characters related to the head, thorax, wings, legs and genitalia of males and females.

Molecular analysis

Six *Ornatoraphidia flavilabris* specimens originating from three countries, namely Austria, Italy and Greece (Table 1) were investigated by molecular methods. Tissue was removed from legs of adult specimens preserved in ethanol (96%).

Table 1. Specimens of *Ornatoraphidia flavilabris* used in the molecular analysis.

voucher	geographic origin (altitude m a.s.l.)	coordinates
Ofla1	Greece, Simos (800 m)	38°31.78'N, 21°49.91'E
Ofla2	Greece, Parnon Mountain (958 m)	37°06.56'N, 22°43.77'E
Ofla3	Italy, Calabria, Aspromonte (1740 m)	38°09.39'N, 15°55.98'E
Ofla4	Italy, Calabria, Sila Grande (1298 m)	39°23.61'N, 16°36.43'E
Ofla5	Austria, Lower Austria, Eichkogel (358 m)	48°03.75'N, 16°17.55'E
Ofla6	Austria, Lower Austria, Gaming (430 m)	47°56.57'N, 15°06.59'E

Mapping of *O. flavilabris* distribution

Coordinates or locations of previously published *O. flavilabris* records (Navas 1929, H. Aspöck and U. Aspöck 1971, Joost 1973, Kofler 1977, Hölzel et al. 1980, Devetak 1984, Gepp 1986, H. Aspöck et al. 1991, Sziráki et al. 1992, Popov 1993, 1997, 2000, Letardi 1994, 2003, 2018, 2021, Letardi and Pantaleoni 1996, Pantaleoni and Letardi 1998, Letardi and Migliaccio 2002, Letardi and Biscaccianti 2007, Badano 2008, Aistleitner and Gruppe 2009, Klokočovnik et al. 2010, Letardi et al. 2010, Badano and Letardi 2010, Tillier 2013, 2015, Sziráki 2014, Devetak et al. 2015, Devetak and Rausch 2016, Hiermann et al. 2018, Letardi and Scalercio 2018) as well as personal unpublished records (Suppl. material 1) were georeferenced into a distribution map using Quantum GIS 3.4.11 (QGIS Development Team 2019) (Fig. 3).

DNA extraction, PCR and sequencing

DNA was isolated with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), strictly following the manufacturer's instructions. DNA was stored in a final volume of 100 µl in elution buffer at -20 °C.

PCR amplification of fragments of three different gene fragments, namely cytochrome c oxidase subunit 1 (*cox1*), cytochrome c oxidase subunit 3 (*cox3*) and 28S rRNA gene (*28S*), was performed with an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany) containing 10 × Reaction Buffer B, 2.5 mM $MgCl_2$, 1.6 mM dNTPs, 1 µM primers, 1.25 units DNA polymerase and 1–5 µl DNA. Sterile H_2O was added to a final volume of 50 µl. For negative controls microbial DNA free water (Qiagen, Hilden, Germany) was added instead of template DNA.

For *cox1* a 658 bp fragment was amplified using the primers LCO1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') published by Folmer et al. (1994) for *cox3* also a 658 bp fragment was amplified using the primers Raph-cox3fw (5'-TAG TCC ATG ACC HTT AAC AGG-3') and cox3-rev (5'-ACA TCA ACA AAA TGT CAA TAT CA-3') published by Haring and U. Aspöck (2004). The final *28S* sequence was obtained by the amplification of overlapping fragments obtained by the primer pairs Raph28S-1+ (5'-CAG GGG TAA ACC TGA GAA A-3')/Raph28S-4- (5'-AGC GCC AGT TCT GCT TAC C-3') and Raph28S3+ (5'-AGC TTT GGG TAC TTT CAG GA-3')/Raph28S6- (5'-GGA ATA GGA ACC GGA TTC CC-3') published by Haring et al. (2011). Amplification of all three genes was carried out using the following conditions: 95 °C for 15 min, followed by 35 cycles of 95 °C for 1 min (denaturation), 52 °C for 1:30 min (annealing) and 72 °C for 2 min (elongation), followed by a final extension of 72 °C for 10 min.

Figure 1. *Ornatoraphidia flavilabris*, male. Austria, Eichkogel, 48°03.75'N, 16°17.55'E, 358 m, 10 May 2021, H. & U. Aspöck leg., now in coll. NHMW. (Photo: H. Bruckner, NHMW). Length of forewing: 8.4 mm.

Figure 2. *Ornatoraphidia flavilabris*, female. Greece, Phokis, S Pendayi, 38°34.95'N, 22°03.45'E, 960 m, 31 May 2008, H. & U. Aspöck leg., now in coll. NHMW. (Photo: P. Sehnal, NHMW). Length of forewing: 10.5 mm.

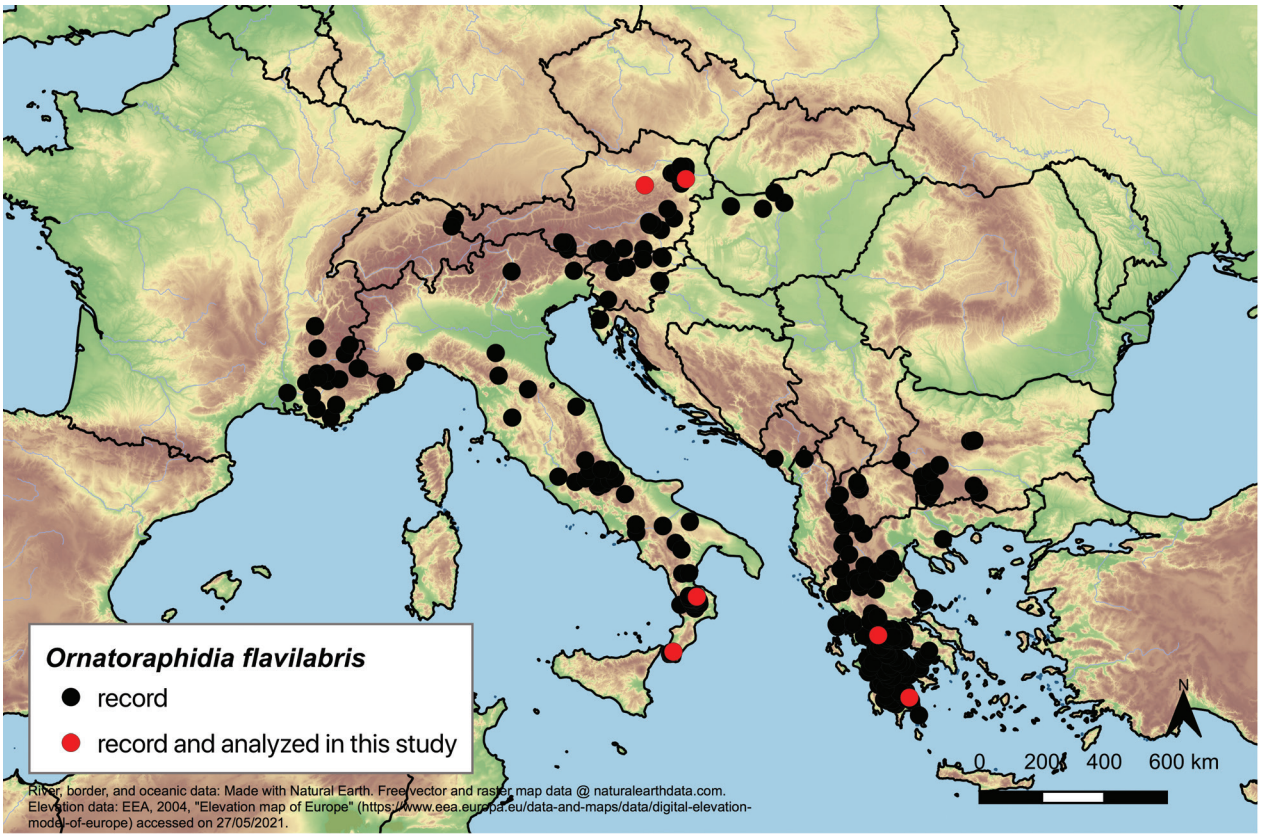

Figure 3. Distribution map of *Ornatoraphidia flavilabris*.

The PCR products were subjected to electrophoresis in 2% agarose gels stained with GelRed Nucleic Acid Gel Stain (Biotium, Inc., CA, USA). For further sequencing, bands were analyzed with a Gel DocTM XR+ Imager (Bio-Rad Laboratories, Inc., CA, USA), cut out from the gel and purified with the IllustraTM GFXTM PCR DNA and Gel Purification Kit (GE Healthcare, Buckinghamshire, UK).

Sanger sequencing was performed with a Thermo Fisher Scientific SeqStudio (Thermo Fisher Scientific, MA, USA). Sequences were obtained from both DNA strands and consensus sequences were generated in GenDoc 2.7.0. Obtained sequences were stored in GenBank (MZ313518.1–MZ313535.1) and compared to available sequences using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in GenBank.

DNA sequence analysis

Obtained sequences were aligned with ClustalX 2.1 (Larkin et al. 2007) and manually edited with GeneDoc 2.7.0 (Nicholas 1997) for further analysis. The three included genes (*cox1*, *cox3* and *28S*) were analyzed independently. Alignments of *cox1* and *cox3* both showed a length of 658 bp without any insertions or deletions. Translation to amino acid sequences for both genes (*cox1* and *cox3*) showed intact reading frames for all included sequences. 28S sequences, which are variable in length, were prealigned with ClustalX. Sequence gaps and ambiguously aligned sections were checked and edited manually with GeneDoc. This manual alignment approach was compared to a computer approach by Haring et al. (2011) and was considered appropriate for this study. After removal of these sections the final alignment resulted in a length of 1602 bp. Final sequence alignments of all three genes are given in supplementary files (Suppl. material 2–4).

Pairwise distances

Based on the best fit evolutionary model selection, pairwise Tamura-3-parameter + G (gamma distributed) distances were calculated individually for all three genes in MEGAX (Kumar et al. 2018). Therefore, available sequences of Raphidioptera species were downloaded from GenBank. Available sequences of species belonging to the "*Phaeostigma* clade" proposed by Haring et al. (2011), to which also the genus *Ornatoraphidia* belongs, were chosen as well as sequences of species belonging to the "*Puncha* clade" which were used as outgroups for tree calculation (Table 2).

Phylogenetic analysis

To infer the phylogenetic position of the analyzed *O. flavilabris* samples, maximum likelihood (ML) analysis using the Tamura 3-parameter + G + I model with four discrete gamma values was applied. Nodal support was evaluated by 1000 bootstrap replicates.

Table 2. Raphidioptera species included in the phylogenetic analysis.

Taxon	GenBank accession		
	cox1	*cox3*	*28S*
***Dichrostigma* Navás, 1909**			
Dichrostigma flavipes (Stein, 1863)	KJ592551.1	HM543286.1	HM543378.1
***Ornatoraphidia* H. Aspöck & U. Aspöck, 1968**			
Ornatoraphidia flavilabris (Costa, 1855)			
Austria (Ofla5)	MZ313522.1	MZ313528.1	MZ313534.1
Austria (Ofla6)	MZ313523.1	MZ313529.1	MZ313535.1
Greece (Ofla1)	MZ313518.1	MZ313524.1	MZ313530.1
Greece (Ofla2)	MZ313519.1	MZ313525.1	MZ313531.1
Italy (Ofla3)	MZ313520.1	MZ313526.1	MZ313532.1
Italy (Ofla4)	MZ313521.1	MZ313527.1	MZ313533.1
Italy	–	HM543306.1	HM543373.1
Italy	–	HM543307.1	–
Italy	–	HM543308.1	–
***Parvoraphidia* H. Aspöck & U. Aspöck, 1968**			
Parvoraphidia microstigma (Stein, 1863)	–	HM543319.1	HM543366.1
***Phaeostigma* Navás, 1909**			
Phaeostigma notata (Fabricius, 1781)	KJ592465.1	–	–
***Puncha* Navás, 1915**			
Puncha ratzeburgi (Brauer, 1876)	–	HM543321.1	HM543358.1
***Subilla* Navás, 1916**			
Subilla confinis (Stephens, 1836)	–	HM543325.1	HM543375.1
***Turcoraphidia* H. Aspöck & U. Aspöck, 1968**			
Turcoraphidia amara H. Aspöck & U. Aspöck, 1964	–	HM543328.1	HM543372.1
***Xanthostigma* Navás, 1909**			
Xanthostigma xanthostigma (Schummel, 1832)	KJ592580.1	HM543337.1	HM543360.1

Results

Morphological examinations

Specimens of *O. flavilabris* from Greece had already been carefully studied and compared with specimens from Italy and Central Europe by us in the late 1960s, and again in the following decades. These examinations consistently led to the conclusion that constant differences do not exist, and thus all populations were assigned to one species (H. Aspöck et al. 1991, 2001). In the course of the present study we again thoroughly examined many specimens from many localities and could only confirm what we had already concluded half a century ago.

Thus, on a morphological basis it is impossible to answer the question whether the Central European populations (at least those of Lower Austria) can be traced back to migrations from the Apennine Peninsula or from the Balkan Peninsula.

Molecular analysis of *O. flavilabris* specimens

Sequences of all six included *O. flavilabris* specimens were successfully amplified by PCR and sequenced. The sequence length was 658 bp for all *cox1* (GenBank accession: MZ313518.1–MZ313523.1) and *cox3* (GenBank accession: MZ313524.1– MZ313529.1) sequences. The *28S* sequences of specimens originating from Greece had lengths of 1741 bp (Ofla1, GenBank: MZ313530.1) and 1749 bp (Ofla2, GenBank: MZ313531.1), those from Austria had lengths of 1763 bp (Ofla5, GenBank: MZ313534.1) and 1765 bp (Ofla6, GenBank: MZ313535.1) and those from Italy had a length of 1763 bp (Ofla3, GenBank: MZ313532.1; Ofla4, GenBank: MZ313533.1).

After alignment, *cox1* sequences showed 96 (96/658; 14.6%) variable positions, of which 81 were parsimony informative. Amino acid sequences of all six *O. flavilabris* specimens showed no differences. The *Cox3* sequences showed 91 (91/658; 13.8%) variable positions, of which 71 were parsimony informative, which resulted in nine differences at amino acid sequence level. The *28S* sequences showed 25 (25/1602; 1.6%) variable positions, of which 18 were parsimony informative.

Pairwise distance analysis

In total, nine *cox1* sequences of Raphidioptera with a length of 658 bp were analyzed (Table 2). The overall mean pairwise Tamura-3-parameter distance was 16.0%. Pairwise distances between the six *O. flavilabris* sequences analyzed ranged from 0.0% to 15.2% with a mean distance of 8.4%. While genetic distances between the *O. flavilabris* specimens (Ofla5, Ofla6) from Austria and the specimens from Italy were 2.8% (Ofla3) and 3.0% (Ofla4) respectively, genetic distances to the specimens from Greece were considerably higher, being 12.9% (Ofla1) and 13.8% (Ofla2), respectively (Table 3). Pairwise distances of *O. flavilabris* to other included Raphidioptera species ranged from 15.6% to 19.4% (Suppl. material 5).

Altogether, 15 *cox3* sequences of Raphidioptera with a length of 658 bp were included in the analysis (Table 2). The overall Tamura-3-parameter distance was 12.8%. Pairwise distances between nine analyzed *O. flavilabris*

Table 3. Pairwise Tamura-3-parameter distances (%) of *Ornatoraphidia flavilabris* based on all three included genes (*cox1*/*cox3*/*28S*).

	1	2	3	4	5	6	7	8	9
1 ***O. flavilabris* (Eichkogel, Austria)***	–								
2 ***O. flavilabris* (Gaming, Austria)***	0.0/0.0/0.0	–							
3 ***O. flavilabris* (Simos, Greece)***	12.9/11.5/1.1	12.9/11.5/1.1	–						
4 ***O. flavilabris* (Parnon Mt., Greece)***	13.8/12.8/1.5	13.8/12.8/1.5	2.5/3.0/0.5	–					
5 ***O. flavilabris* (Calabria, Italy)***	3.0/2.5/0.3	3.0/2.5/0.3	14.3/12.0/1.2	15.2/13.3/1.3	–				
6 ***O. flavilabris* (Calabria, Italy)***	2.8/2.5/0.3	2.8/2.5/0.3	13.9/12.4/1.1	14.8/13.3/1.2	0.5/0.6/0.1	–			
7 ***O. flavilabris* (Calabria, Italy)⁺**	-/2.5/0.4	-/2.5/0.4	-/12.0/1.3	-/13.3/1.3	-/0.3/0.2	-/0.3/0.1	–		
8 ***O. flavilabris* (Emilia-Romagna, Italy)⁺**	-/2.8/-	-/2.8/-	-/11.0/-	-/12.1/-	-/1.9/-	-/1.9/-	-/1.9/-	–	
9 ***O. flavilabris* (Emilia-Romagna, Italy)⁺**	-/3.2/-	-/2.1/-	-/11.4/-	-/12.5/-	-/2.2/-	-/2.2/-	-/2.2/-	-/0.3/-	–

*specimens molecularly analysed in this study, ⁺specimens from GenBank.

sequences ranged from 0.0% to 13.3% with a mean distance of 6.0%. Pairwise distances between the *O. flavilabris* specimens from Austria and Italy ranged from 2.5% to 3.2%. Again, much higher pairwise distances were observed between the specimens from Austria with either of those from Greece (11.5% Ofla1 and 12.8% Ofla2) (Table 3). Pairwise distances of *O. flavilabris* to other included Raphidioptera species ranged from 13.0% to 21.4% (Suppl. material 6).

Overall, 13 *28S* sequences of Raphidioptera with a length of 1602 bp were included in the analysis (Table 3). The overall mean pairwise intraspecific Tamura-3-parameter distance was 2.9%. Pairwise distances between seven analyzed *O. flavilabris* sequences ranged from 0.0% to 1.5% with a mean distance of 0.7%. While pairwise distances between the specimens from Austria and Italy ranged from 0.3% to 0.4%, distances between the specimens from Austria and Greece were 1.1% (Ofla1) and 1.5% (Ofla2) (Table 3). Pairwise distances of *O. flavilabris* to other included Raphidioptera species were comparably higher and ranged from 2.6% to 4.7% (Suppl. material 7).

Phylogenetic analysis

All sequences included in pairwise sequence calculations were used for the maximum likelihood analysis. *Xanthostigma xanthostigma* and *Puncha ratzeburgi* belonging to the *Puncha* clade were used as outgroups with one exception. For *cox1*, only *X. xanthostigma* was used as an outgroup, since no other sequence of the *Puncha* clade was available from GenBank.

The *cox1* tree resulted in two major clades. Clade 1 included all *O. flavilabris* sequences, clade 2 comprised *Phaeostigma notata* and *Dichrostigma flavipes* (Fig. 4A). Clade 1 was further divided into an *O. flavilabris* lineage from Austria and a lineage from Italy forming a monophyletic group that was well supported by high bootstrap values. Together they represent the sister group of a *O. flavilabris* lineage from Greece (Fig. 4A).

The *cox3* tree showed three *O. flavilabris* lineages (Austria, Italy and Greece) with high bootstrap support. Again, the lineages from Austria and Italy formed a sister group, and together they were the sister group of the lineage from Greece. In addition, the lineage from Italy was subdivided into two sublineages originating from the Calabria and one from the Emilia-Romagna regions of Italy (Fig. 4B). *Parvoraphidia microstigma* arose as the sister species of *O. flavilabris* and *P. microstigma* + *O. flavilabris* formed the sister group of *Turcoraphidia amara*, all together representing a monophyletic group. However, the phylogenetic positions of *P. microstigma* and *T. amara* were only supported by comparably low bootstrap values (Fig. 4B). Also in a recent paper on the phylogeny of Neuropterida based on the transcriptomic dataset (Vasilikopoulos et al. 2020) *Ornatoraphidia* emerged as sister of *Parvoraphidia* and both being sister of *Turcoraphidia*.

The 28S tree comprised two major clades (Fig. 4C). Clade 1 included all *O. flavilabris* sequences and was subdivided into three *O. flavilabris* lineages (from Austria, Italy and Greece). Clade 2 comprised all other included members of the *Phaeostigma* group. As already observed in the *cox1* and *cox3* tree, the *O. flavilabris* lineage from Austria formed the sister group of the lineage from Italy, and together they formed the sister group of the lineage from Greece (Fig. 4C). In the *28S* tree, *T. amara* and *P. microstigma* belonged to clade 2, unlike tree *cox3*, however, again the bootstrap support was low (Fig. 4C).

Discussion

Phylogeographic studies have not previously been carried out in Raphidioptera — but for one exception: the Mediterranean *Raphidia* (*R.*) *mediterranea* H. Aspöck, U. Aspöck & Rausch, 1977. Surprisingly in 2013, this species was found in the yard of an old farmhouse in Upper Austria at an altitude of 800 m thriving at an extraordinarily high population density (Rausch et al. 2016, H. Aspöck et al. 2017). The larvae developed in the straw of the thatched roof of the farmhouse (Gruppe et al. 2017). This Central European record is distinctly isolated from the distribution of *R.* (*R.*) *mediterranea* in Italy, the Balkan Peninsula and southeast Europe. We concluded that the species must have colonized the farmhouse in Upper Austria by anthropogenic dispersal. However, where did they come from, the Balkan Peninsula or Italy? A morphological comparison of both sexes from various populations of the Balkan Peninsula and Apennine Peninsula to specimens from Upper Austria revealed no charac-

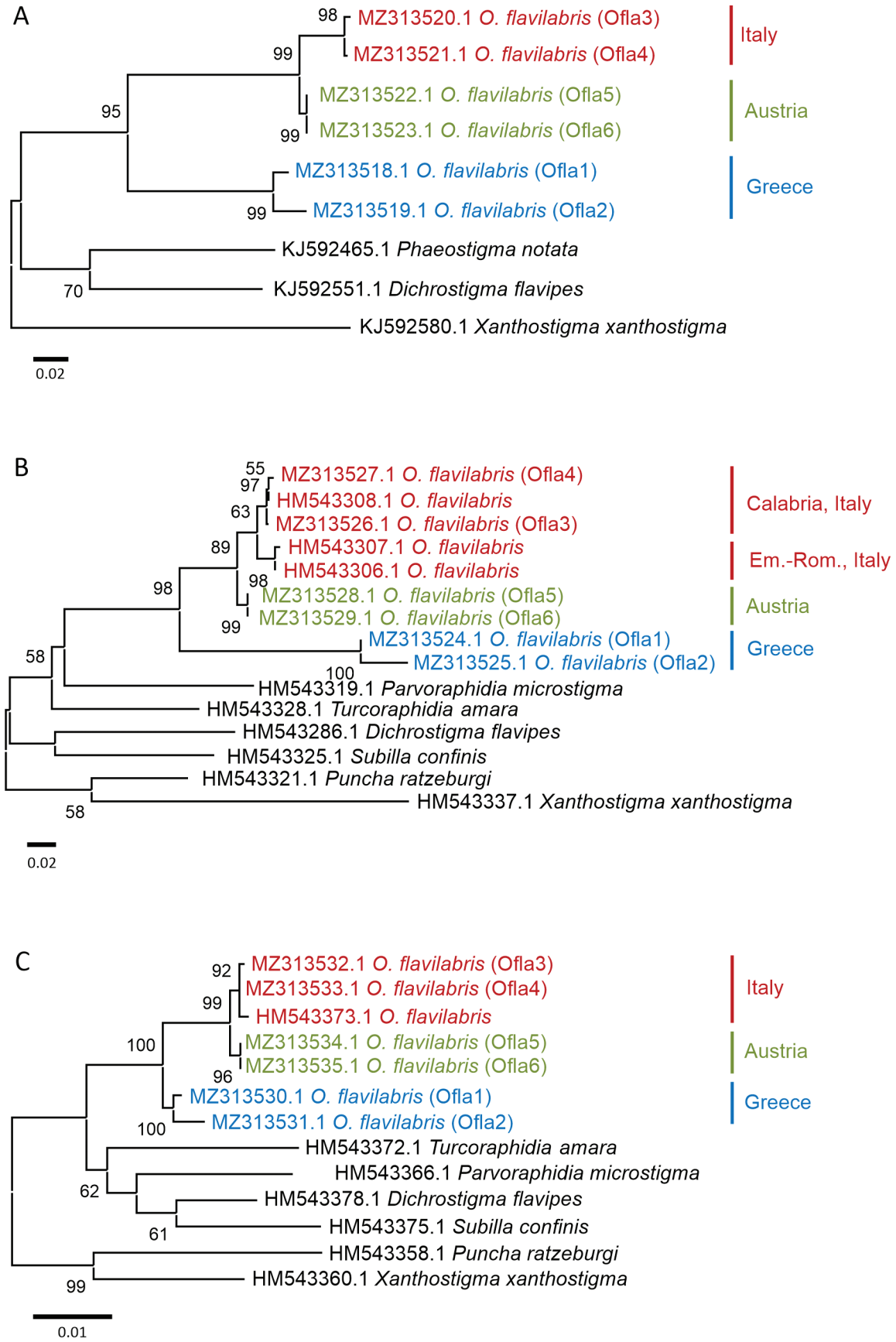

Figure 4. Maximum likelihood trees based on *cox1* (**A.**), *cox3* (**B.**), and *28S* (**C.**) sequences of Raphidioptera species. *Puncha ratzeburgi* (except *cox1* tree) and *Xanthostigma xanthostigma* were used as outgroups. Vertical colored bars represent *Ornatoraphidia flavilabris* sequences originating from Austria (green), Italy (red), and Greece (blue). Em.-Rom.=Emilia-Romagna, Italy. Bootstrap values >50% are shown.

teristic differences. Thus a molecular study was carried out which showed homogeneity among all populations. These results led to the conclusion that the dispersal of *R.* (*R.*) *mediterranea* occurred from a primary refugial center (perhaps somewhere in the south of Greece) in recent times by means of human activity.

The phylogeography of *Ornatoraphidia flavilabris* is quite different. Since there are numerous records of this species in Central Europe, the likelihood of anthropogenic dispersal is out of the question. However, a post-glacial natural expansion from Mediterranean refugial centers could have led to the present distribution. If so, the question arises whether Central Europe was colonized via the Balkan or the Apennine Peninsula. *O. flavilabris* has been biogeographically characterized as a polycentric Balkanopontomediterranean-Adriatomediterranean faunal element (H. Aspöck et al. 1991), which means that populations of the species were at least present during the last glacial period (about 100,000 to 10,000 years BP) in the Balkan as well as the Apennine Peninsula. The colonization pathway was clarified by phylogenomics involving an analysis of three genes (*cox1*, *cox3* and *28S*). The study clearly showed that:

1. Greek populations (at least from two far distant localities) form a monophyletic group.
2. Italian populations (at least from two far distant localities) are closely related.
3. The two Greek populations can clearly be differentiated from the Italian populations.
4. The two specimens of *O. flavilabris* from Central Europe (Austria) are identical to each other (with one exception of the 28S rRNA gene sequences) and closely related to the populations from Italy.

The conclusion drawn is that postglacial migration to Central Europe had its origin in the Apennine Peninsula. This is astounding since migration from the Balkan Peninsula would seem to be easier with regard to orography (cf. Schmitt 2020). Nevertheless, *O. flavilabris* could still have colonized Central Europe from the northwest Balkan Peninsula, if we assume that present-day populations, e.g. in Slovenia, are to be traced back to (possibly postglacial, if not even earlier) migrations from Italy. This assumption is strongly corroborated by the fact that there is a large gap comprising central parts of the Balkan Peninsula between the range of *O. flavilabris* in the south (Greece, Albania, Macedonia, Bulgaria) and that in the north (Slovenia).

Aside from phylogeography, a final taxonomic aspect must be discussed. The molecular results show considerable differences between the populations from Greece and those from Italy and Central Europe. Thus, the question arises whether both population groups represent one species. In the recent past an increasing number of cryptic species has been detected in various groups of arthropods on the basis of molecular differences. However, in these cases, morphological differences could be found that, although slight and often inconspicuous, were constant, and thus would justify the description of a new taxon (species) or re-installment of a species previously regarded as a synonym (Ronkay and Huemer 2018). Regarding *Ornatoraphidia flavilabris* the conditions are quite different. We have not found any constant morphological characters which would allow a safe differentiation. Interestingly, the high observed genetic pairwise distances of Italian and Austrian *O. flavilabris* mitochondrial gene sequences (*cox1* and *cox3*) to both Greek specimens were only marginally lower than those to other included Raphidioptera species, thus, pointing towards the molecular identification of a new (cryptic) species. However, mitochondrial genes might not optimally present taxonomic or phylogenetic relationships among Raphidioptera due to sequence saturation as observed by Haring et al. (2011). While intraspecific *28S* genetic distances generally supported the results obtained for *cox1* and *cox3*, all three genes showing low distances between Austrian and Italian specimens and about 3-fold higher distances to Greek specimens, this gene revealed a better separation between *O. flavilabris* and the other included Raphidioptera species. Thus, the 28S ribosomal RNA gene possibly reflects the taxonomic status of *O. flavilabris* more accurately than the two mitochondrial marker genes, thereby justifying our decision of differentiating *O. flavilabris* into two lineages rather than two (sub)species.

A description of new taxa solely on the basis of genomic differences is in our opinion – at least in Neuropterida – unjustified. Therefore, we continue to regard all populations of *O. flavilabris* as a single – morphologically monotypic – species, which means that there is no reason to differentiate subspecies.

Nevertheless, further studies on the genomic and morphological characters of *O. flavilabris* including other populations will be useful to corroborate the conclusions presented here. Moreover, the search for cryptic species (taxa) in Raphidioptera should be enhanced and may yield surprising results.

Conclusion

The occurrence of *Ornatoraphidia flavilabris* in Central Europe can be traced to a postglacial migration of the species from the Apennine Peninsula via the northwest Balkan Peninsula. The distribution of *O. flavilabris* in the south of the Balkan Peninsula represents an isolated refugial center and is of no relevance for the migration of the species to extramediterranean parts of Europe.

Acknowledgements

Hubert Rausch (Scheibbs, Austria) has kindly provided the specimen of *O. flavilabris* from Gaming (Lower Austria) and has made available several records of *O. flavilabris* from the Balkan Peninsula. Mag. Harald Bruckner and Peter Sehnal (Natural History Museum Vienna)

provided the photographs of adult *O. flavilabris*. Dr. Dr. John Plant (Madison, Connecticut, USA) critically read the manuscript and polished the English. Grateful thanks to all these colleagues! Sincere thanks also to Dr. Davide Badano (Sapienza University of Rome, Italy), Prof. Dr. Dušan Devetak (University of Maribor, Slovenia), and Prof. Dr. Alexi Popov (National Museum of Natural History, Sofia, Bulgaria) for thoroughly reviewing and improving the manuscript and to Mag. Dr. Susanne Randolf (Natural History Museum Vienna), Subject Editor, for carefully supervising the review process. We gratefully acknowledge the Museum für Naturkunde Berlin for waiving the authors' fees.

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Supplementary material 1

Personal unpublished *O. flavilabris* records used for the distribution map

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha

Data type: locations

Link: https://doi.org/10.3897/dez.68.70814.suppl1

Supplementary material 2

Multiple sequence alignment based on cytochrome c oxidase subunit 1 (*cox*1) sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha

Data type: Sequence alignment

Link: https://doi.org/10.3897/dez.68.70814.suppl2

Supplementary material 3

Multiple sequence alignment based on cytochrome c oxidase subunit 3 (*cox*3) sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha
Data type: Sequence alignment

Link: https://doi.org/10.3897/dez.68.70814.suppl3

Supplementary material 4

Multiple sequence alignment based on 28S rRNA gene (*28S*) sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha
Data type: Sequence alignment

Link: https://doi.org/10.3897/dez.68.70814.suppl4

Supplementary material 5

Pairwise Tamura-3-parameter distances (%) based on cytochrome c oxidase subunit 1 (*cox*1) gene sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha
Data type: Pairwise genetic distances

Link: https://doi.org/10.3897/dez.68.70814.suppl5

Supplementary material 6

Pairwise Tamura-3-parameter distances (%) based on cytochrome c oxidase subunit 3 (*cox*3) gene sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha
Data type: Pairwise genetic distances

Link: https://doi.org/10.3897/dez.68.70814.suppl6

Supplementary material 7

Pairwise Tamura-3-parameter distances (%) based on 28S rRNA gene (*28S*) gene sequences

Authors: Horst Aspöck, Ulrike Aspöck, Julia Walochnik, Edwin Kniha
Data type: Pairwise genetic distances

Link: https://doi.org/10.3897/dez.68.70814.suppl7