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# Anthropogenic dispersal of a snakefly (Insecta, Neuropterida) – a singular phenomenon or a model case in Raphidioptera?

Horst Aspöck<sup>1</sup>, Ulrike Aspöck<sup>2,3</sup>, Axel Gruppe<sup>4</sup>, Marcia Sittenthaler<sup>5,6</sup>, Elisabeth Haring<sup>3,5</sup>

- 2 Natural History Museum Vienna, Department of Entomology, Burgring 7, 1010 Vienna, Austria
- 3 Department of Integrative Zoology, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
- 4 Department of Animal Sciences, Chair of Zoology-Entomology, Technical University of Munich (TUM), Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany
- 5 Natural History Museum Vienna, Central Research Laboratories, Burgring 7, 1010 Vienna, Austria
- 6 University of Natural Resources and Life Sciences, Vienna, Institute of Wildlife Biology and Game Management, Gregor-Mendel-Strasse 33, 1180 Vienna, Austria

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Corresponding author: Ulrike Aspöck (ulrike.aspoeck@nhm-wien.ac.at)

## Abstract

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## Key Words

Raphidia mediterranea man-made dispersal neozoon Raphidia phylogeny The Mediterranean snakefly *Raphidia mediterranea* H. Aspöck, U. Aspöck & Rausch, 1977 – known from many parts of the Balkan Peninsula, several Aegean islands, southern parts of Italy, northwest of Anatolia and a few localities in Eastern Europe, yet not recorded in Central Europe – was surprisingly found with an astoundingly high population density on bushes in the yard of an old farmhouse at a comparatively high altitude (800 m) in Upper Austria, north of the Danube River, in 2013. This spectacular phenomenon was again observed in the following years (2014, 2015, 2016, 2017), and in 2016 the suspicion that the larvae develop in the straw of the thatched roof of the farmhouse could be confirmed by findings of larvae, pupae, and exuviae.It is most likely, that the occurrence of this *Raphidia* species in Austria is to be traced back to a human-caused introduction at some point in time. It remains, however, unknown when and specifically how this event might have occurred.

Morphologically no substantial differences were found between specimens from Greece, Italy and Upper Austria. In addition, the genetic uniformity (using 3 genes: cox1, cox3, and 28S) of the populations was verified. This supports an earlier hypothesis that the occurrence of the species, as well as that of *R. mediterranea* in Italy, Anatolia and perhaps elsewhere, may be related to importation of goods involving wood or soil.

A molecular genetic analysis of several *Raphidia* species confirmed the present morphology-based concept of their systematic position.

The means of dispersal of Raphidioptera are largely unknown. We do not know of any other similar cases of anthropogenic dispersal of a snakefly, but it cannot be excluded that human activities may have played a greater role in the dispersal of Raphidioptera than previously assumed. Phylogenomic studies would therefore be promising to solve some of these questions.

## Introduction

By the 1960s, it was revealed that the Balkan Peninsula was a distribution (and evolution) centre of the order Raphidioptera with an incredibly high number of species (H. Aspöck and U. Aspöck 1965). In the course of the following years, extensive field studies were carried out in various parts of the Balkan Peninsula (H. Aspöck 1987, H. Aspöck et al. 1989, H. Rausch and R. Rausch 2004). These investigations led to the discovery of nu-

<sup>1</sup> Institute of Specific Prophylaxis and Tropical Medicine, Medical Parasitology, Medical University of Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria

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merous new species and among them a snakefly species, which - despite slight differences - was morphologically so similar to Raphidia ophiopsis Linnaeus, 1758, that we hesitated to separate it from R. ophiopsis. However, after discovery of unusually large populations of this taxon in various parts of Greece and in biotopes (e.g. in coastal areas with maquis vegetation) ecologically entirely different from those of R. ophiopsis, which is associated with coniferous trees, we decided to describe it as a subspecies of Raphidia ophiopsis: R. ophiopsis mediterranea (H. Aspöck et al. 1977). In the meantime, the taxon was surprisingly found in Italy (Apulia) and later in northwest Anatolia. In our monograph (H. Aspöck et al. 1991) we argued that the disjunct distribution could hardly be explained by natural dispersal and we therefore considered that human activities might have been a significant cause of the amplification of the distribution of R. o. mediterranea. One of our arguments was that R. o. mediterranea occurs on the eastern coast of the Apennine Peninsula around Brindisi, a region known for its intensive ship traffic with Greece, which dates back to antiquity. Subsequently, entomologists from Italy found R. o. mediterranea in western parts of the Apennine Peninsula, and from this they concluded that R. o. mediterranea had not been introduced from Greece to Italy by human activities, but that its occurrence in Italy was due to natural dispersal (Letardi 2002, Letardi and Pantaleoni 1996, Pantaleoni 2005). Meanwhile, the taxon was unexpectedly found in Romania (Kis 1984) and Hungary (Sziráki 1993a, b, 2010). Both latter authors studied the taxon carefully and arrived at the conclusion that R. mediterranea is a good species and not a subspecies of R. ophiopsis. Aside from the known and corroborated morphological differences, an important argument for the status of a separate species was the sympatry of both taxa in Romania and Hungary. The arguments of Kis (1984) and Sziráki (1993a) were convincing and accepted by us (H. Aspöck and U. Aspöck 2007, 2013, 2014). Finally, in 2013 R. mediterranea was found in the yard and on the outer walls of an old farmhouse, now representing an open-air museum, at a considerably high altitude (800 m) in Upper Austria (Rausch et al. 2016). It was an absolute surprise to find this Mediterranean snakefly in a comparatively cold region of Austria (Figs 1, 2). Moreover, R. mediterranea occurs there in an extremely high population density. It was suspected that the larvae develop within the straw covering the roof (Rausch et al. 2016), and this could recently be confirmed (Gruppe et al. 2017) (Figs 3, 4). Thus, the question arose: How has R. mediterranea achieved the establishment of a stable population in a locality in Central Europe, which offers unfavourable climatic conditions compared to many other parts of Austria (Fig. 5)? To better evaluate the phylogeographic scenarios of this species, i.e. natural expansion of the distribution range vs. human mediated dispersal, we performed molecular genetic analyses of specimens from Austria, Greece and Italy. The specimens analysed genetically were compared morphologically with specimens from many localities covering the currently known distribution. Moreover, specimens of R. ophiopsis from Upper Austria and other parts of Central Europe were included to corroborate the morphological differences between the two taxa.



Figure 1. Raphidia mediterranea, male, from Pelmberg (Upper Austria). Photo H. Bruckner.



Figure 2. Raphidia mediterranea, female, from Pelmberg (Upper Austria). Photo H. Bruckner.



Figure 3. Raphidia mediterranea, full-grown larva, from Pelmberg (Upper Austria). Photo H. Bruckner.



Figure 4. Raphidia mediterranea, pupa, from Pelmberg (Upper Austria). Photo H. Bruckner.



Figure 5. Known distribution of Raphidia mediterranea H.A. & U.A. & Rausch. Source of the map see under Material and methods.

## Material and methods

#### **Morphological studies**

Numerous adults of both sexes of *Raphidia mediterranea* from many localities in Greece, Italy, Anatolia, as well as specimens of *R. ophiopsis* from Upper Austria and other parts of Central Europe were compared with imagines from Pelmberg (Upper Austria) based on the well-known morphological characters of male and female genitalia (H. Aspöck et al. 1991). Genital segments were cleared in KOH and processed in the usual manner described elsewhere.

The distribution map was provided with ArcGis/ ArcMap ver. 10.3.1.4959 based on the distribution records provided in the Suppl. material 2. Source of the map: National Geographic-Weltkarte - Content may not reflect National Geographic's current map policy. Sources: National Geographic, Esri, DeLorme, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp.

#### Molecular genetic analysis

For DNA analysis samples of four individuals of *Raphidia mediterranea* were selected, which had been collected in Pelmberg (Upper Austria), Gargano (Italy) and Zachlorou (Peloponnesus, Greece). Moreover, five representatives of the genus were included: *Raphidia ophiopsis* Linnaeus, 1758, *Raphidia alcoholica* H. Aspöck & U. Aspöck, 1969, *Raphidia ulrikae* H. Aspöck, 1964, *Raphidia ariadne* H. Aspöck & U. Aspöck, 1964, and *Raphidia ligurica* Albarda, 1891. A list of specimens analysed with exact localities is given in Table 1. Tissue samples were taken from one leg of alcohol-preserved specimens with sterile forceps. Vouchers are stored at the Entomological Department of the Museum of Natural History Vienna (NHMW). Remaining DNA is stored in the DNA and Tissue Collection of the Central Research Laboratories at the NHMW.

#### Marker sequences and laboratory procedures

Two mitochondrial marker sequences were amplified using primers listed in Table 2: (1) A partial sequence of the *cytochrome c oxidase subunit 3* gene (cox3) which has been also used in a previous study on Neuropterida, as well as Raphidioptera (Haring and Aspöck 2004; Haring et al. 2011) and (2) the complete sequence of the *cytochrome c oxidase subunit 1* gene (cox1) plus partial sequences of the adjacent tRNA genes. In addition, a partial sequence of the 28S rRNA gene (28S) was used as a nuclear marker sequence. The fragment lengths of cox1sequences ranged from 1604 -1610 bp (due to indels in the flanking tRNA genes). The amplicon length of the cox3 sequence was 712 bp. The amplicon length of the 28S sequence was 1155–1161 bp.

DNA extraction was performed using the DNeasy-Blood and Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The final volume of elution buffer was 40 µl. DNA solutions were stored in aliquots to avoid too frequent thawing. Control extractions with pure extraction buffer (without tissue) were prepared. PCR was carried out in an Eppendorf Thermocycler in a volume of 25 µl, containing Taq Polymerase (1.25 units/reaction; QIAGEN, Hilden, Germany) 1 µM of each primer, and 0.2 mM of each dNTP, 1.5 mM MgCl,, 5 µl Q-Solution, 2.5 µl 10x PCR buffer and 1 µl of template DNA. The PCR protocols were as follows: cox3: initial denaturation 94° C (3 min); 35 cycles: 94° C (60 s) / 50° C (30 s) / 72° C (60 sec); final extension at 72° C (10 min). cox1: initial denaturation 94° C (3 min); 35 cycles: 94° C (60 s) /  $50^{\circ}$  C (30 s) /  $72^{\circ}$  C (60 sec); final extension at  $72^{\circ}$  C (10 min). 28S: initial denaturation 94° C (3 min); 35 cycles: 94° C (60 s) / 55° C (30 s) / 72° C (60 sec); final extension at 72° C (10 min). Negative PCR controls were carried out to screen for contaminated reagents: (1) control extractions without tissue and subsequent PCR reactions (i.e. instead of template DNA); (2) PCR reactions with distilled water instead of template. PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) prior to sequencing. Sequencing (both directions) was performed at Microsynth (Vienna, Austria) using the PCR primes as well as various internal primers (Table 2). Sequences obtained in the present study are deposited in GenBank under the accession numbers listed in Table 1.

Species	Compling locality	Labaada		GenBank	
Species	Sampling locality	Labcoue	Cox1	Cox3	285
Raphidia mediterranea	Austria, Upper Austria, Pelmberg near Hellmonsödt	Ramed-1	MF975675	MF975666	MF975657
Raphidia mediterranea	Austria, Upper Austria, Pelmberg near Hellmonsödt	Ramed-2	MF975676	MF97567	MF975658
Raphidia mediterranea	Greece, Peloponnesus, Kato Zachlorou	Ramed-3	MF975677	MF975668	MF975659
Raphidia mediterranea	Italy, Puglia, Gargano, Punta Lunga	Ramed-4	MF975678	MF975669	MF975660
Raphidia ariadne	Greece, Crete, W Omalos	Raari-1	MF975679	MF975672	MF975661
Raphidia alcoholica	Greece, Phokis, S Pendayi	Raalc-1	MF975680	MF975671	MF975662
Raphidia ophiopsis	Germany, Bayerischer Wald, Ruckwiesberg	Raoph-1	MF975681	MF975670	MF975663
Raphidia ulrikae	Austria, Styria, Gulsen near Kraubath	Raulr-1	MF975682	MF975673	MF975664
Raphidia ligurica	Italy, Sila Grande, near Viváio	Ralig-1	MF975683	MF975674	MF975665

Table 1. Specimens analysed genetically in the present study and GenBank accession numbers for Cox1, Cox3 and 28S sequences.

Gene	Primer	Sequence (5´-3´)	Reference
Cox3 external primers	L.		
	Arth-cox3-fwd	5'-TAGTTGATTATAGACCATGACC-3'	а
	Arth- cox3-rev	5'-ACATCAACAAAATGTCAATATCA-3'	а
Cox3 internal primers			
	Raph- cox3-1+	5'-ACAAATTCCTTTATTAAATAC-3'	present study
	Raph- cox3-2-	5'-CAWGTAATTGTTAATCCTGA-3'	present study
Cox1 external primers			
	Tyr-myr-1+	5'-CCCATAAATAAATTTACAGTTTA-3'	present study
	Leu-Myr-1-	5'-GCACTATTCTGCCATATTAG-3'	present study
Cox1 internal primers			
	Raph. cox1.int1+	5'-TAGCAGGAGCTATCACTATACT-3'	present study
	Raph- cox1-int2-	5'-ATATAAACTTCTGGATGTCC-3'	present study
	Raph- cox1-int5+	5'-CGAATACCTTTATTTGTATGATC-3'	present study
	Raph- cox1-int4-	5'-AGAATAGGGTCTCCTCCTCC-3'	present study
28S internal primers			
	Raph-28S1+	5'-CAGGGGTAAACCTGAGAAA-3'	b
	Raph-28S-4-	5'-AGCGCCAGTTCTGCTTACC-3'	b
28S external primers			
	Raph28S-3+	5'-AGCTTTGGGTACTTTCAGGA-3'	b
	Raph28S-2-	5'-ACATGCTAGACTCCTTGGT-3'	h

#### Table 2. Primers used.

a: Haring and Aspöck (2004); b: Haring et al. (2011)

#### **Phylogenetic analyses**

Raw sequences were manually aligned in BioEdit v.7.1.3 (Hall 1999) and checked for errors. The alignment was straightforward for the three marker sequences and was done in BioEdit v.7.1.3 In cox1 and cox3 sequences there were no insertions or deletions, and in the 28S gene there were only a few indels allowing clear assessment of positional homology. The final alignment of the complete cox1 gene had a length of 1534 positions. The cox3 gene had 667 positions and the 28S gene 1144 positions. As outgroup, Agulla, another genus of Raphidiidae was used. The cox3 and 28S sequences have been published in our previous paper (Haring et al. 2011; HM543275; HM543340; Agul*la adnixa*). The complete *cox1* sequence was derived from GenBank (FJ207460.1; Agulla sp.). By comparing this sequence with published partial cox1 sequences of Agulla adnixa (e.g., KR141904.1), we deduced that the sequence FJ207460.1 is derived from Agulla adnixa (which has an identical sequence). As a result, in the concatenated data set, the outgroup sequence was derived from different individuals of the same species, which however appears to be unproblematic in this case.

Bayesian Inference (BI) was used for calculating phylogenetic trees. For BI the best fitting substitution model was determined for each of the three genes as well as codon positions of the protein coding genes by jModelTest v.2.1.5 (Darriba et al. 2012) with the corrected Akaike information criterion (AICc). The BI analyses were calculated using MrBayes v.3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Phylogenetic trees were also calculated from a combined alignment in which all three marker sequences were concatenated (length of alignment: 3345 positions). BI analyses were run for 7x10<sup>6</sup> generations (2 runs each with 4 chains, one of which was heated), sampling every hundredth tree. The first 25% of trees were discarded as burnin and from the remaining trees a 50% majority rule consensus tree was calculated. In addition, Neighbour Joining (NJ) trees (Saitou and Nei 1987) were calculated. Nodal support of NJ trees was evaluated with nonparametric bootstrapping based on 1000 replicates. These trees are shown to illustrate p distances among taxa in comparison of the three marker sequences.

## Results

In former studies (H. Aspöck et al. 1991 and unpublished), based on male and female genitalia, populations of *Raphidia mediterranea* from various localities in Greece, Anatolia, and Italy could not be differentiated from each other. This was confirmed again on the basis of more material, particularly specimens from Pelmberg (Upper Austria) whose morphological characters coincide perfectly with those figured in H. Aspöck et al. (1991). Specimens of the genus *Raphidia* from other parts of Central Europe (except *Raphidia ulrikae*) proved to be conspecific with *Raphidia ophiopsis*.

The DNA sequence analysis revealed that the four specimens of *R. mediterranea* are identical in cox1 and 28S, while in cox3 a single substitution differentiating Ramed-4 from the other (identical) sequences was found. In general, the variation within 28S was extremely low. Except *R. ligurica*, which shows distances to the other ingroup taxa of 1.08 and 1.35%, respectively, sequences of all other ingroup species differ with p distances below

1% or are even identical. Between *Raphidia* and *Agulla* 28S distances ranged from 6.0 to 6.5%. Concerning the mitochondrial marker sequences, p distances between *R. mediterranea* and *R. ophiopsis* (the closest relative) were 5.08% in *cox1* and 5.62% in *cox3*, while the other species of *Raphidia* differed between 8.63–14.83% (*cox1*) and 7.46–16.94% (*cox3*) from *R. mediterranea*. Distances between species in the various gene sequences are illustrated by the NJ trees in Suppl. material 1.

To assess the systematic position of R. mediterranea not only on the basis of morphological characters, we performed a phylogenetic analysis based on three genes (cox1, cox3, 28S). The two mt sequences resulted in trees in which the sister group of R. mediterranea is R. ophiopsis. In most analyses, R. alcoholica is the sister group of those two lineages, followed by R. ariadne; only in the BI tree of cox1 the relationships were unresolved (Suppl. material 1). With respect to the relationships of the other species there is a difference concerning R. ligurica and R. ulrikae depending on the marker sequence and the method applied. In some trees R. ulrikae splits from the most basal node, in others it is R. ligurica. Yet, in all trees this node is poorly supported. The tree based on 28S sequences (Suppl. material 1) is congruent with the mt based trees, yet, due to the low variation within this gene, the amount of phylogenetic information is limited. In a BI tree based on the combined marker genes (Fig. 6) all nodes are highly supported.

## Discussion

The discovery of an isolated and unusually large population of *Raphidia mediterranea* – a Mediterranean species which has never been found elsewhere in Central Europe - in a farmhouse in a comparatively climatically unfavourable part of Upper Austria had raised the question concerning the origin of this population. It was assumed that morphological and/or genetic differences would be found, if the species had reached Upper Austria long ago by natural means of expansion of the distribution range. Therefore, specimens of the population from Upper Austria were compared with specimens from Greece and Italy. In the present study, the morphology-based results were clearly confirmed by molecular genetic analyses: The four specimens of R. mediterranea (two from Pelmberg (Austria), one from Greece, one from Italy) had almost identical sequences. It is legitimate to conclude that these populations were not separated long ago. The other species of Raphidia are clearly separated (see Fig. 6).

Substantial differences could not be found in morphological characters, particularly in male and female genitalia, or in the sequences of three genes (cox1, cox3, 28S). This implies that all presently known and examined populations of *R. mediterranea* originated from a single glacial refugium. This refugium can reasonably be assumed to be in the south of the Balkan Peninsula as a part of the large balkanopontomediterranean refugium (H. Aspöck et al. 1991). From there the species reached other parts of Europe (and Anatolia) not long ago. Natural dispersal from the south of the Balkan Peninsula to isolated areas of the north of the Balkan Peninsula, to southern parts of Italy, to parts of Eastern Europe and particularly parts of Central Europe is highly unlikely. Consequently, an anthropogenic introduction into various regions is highly probable. *Raphidia mediterranea* is a euryoecious species, whose larvae live mainly in the detritus of roots of bushes, but sometimes also under bark.

In Greece, the species occurs in many regions, in various habitats at altitudes of 10 - 1200 m and often in high population densities. Thus, it might have been occasionally transported to new habitats by ships carrying wood or soil. This could have occurred already in antiquity and throughout the past centuries.

The discovery of the isolated population of R. mediterranea in Upper Austria and the failure to detect any morphological or genetic differences between these vastly distant populations supports our previous hypothesis (Aspöck et al. 1977, 1980, 1991, 2001) of unintentional introduction by human activities. A natural dispersal - per continuitatem or by wind - can convincingly be excluded. How did R. mediterranea come to Upper Austria? We now know definitely that the larvae develop within the straw of the roof (Gruppe et al. 2017). Thus, it is a reasonable assumption that this snakefly was introduced with straw from somewhere on the Balkan Peninsula. The straw presently on the roof is from Austria, but in the past it may have been imported. It is also possible that live adults (theoretically one female would be sufficient) were introduced (e.g. via car, truck or bus) from the Mediterranean region to Pelmberg and subsequently the female laid eggs in the straw. Until now, no studies have been published indicating that larvae develop in straw on roofs. In Central Europe thatched roofs have become rare, but in eastern and southeastern parts of Europe such roofs are still frequent in certain regions. It would be easy and exciting to examine these habitats for snakeflies. It would particularly be interesting to know whether other species of Raphidioptera can also develop in straw of thatched roofs where they would feed on mites, spring-tails, Psocoptera, larvae of beetles and other small arthropods living in the straw.

Concerning the systematic position of *R. mediterranea*, the phylogenetic analysis based on three genes confirmed our view of the systematics of *R. ophiopsis*, *R. mediterranea*, *R. alcoholica*, *R. ariadne*, *R. ulrikae* and *R. ligurica* (H. Aspöck et al. 1991, 2001). It is of particular interest that *R. alcoholica* is the sister species of *R. ophiopsis* + *R. mediterranea*, thus confirming the close relationship of the three taxa once regarded as subspecies of *R. ophiopsis*.

We know little about the formation of distribution patterns of Raphidioptera. Snakeflies are generally characterised by low, in many cases extremely low, expansivity, and many species have hardly enlarged their distribution beyond their glacial refugial areas. In Central Europe, 16



Figure 6. Phylogenetic tree based on BI analysis of six species of *Raphidia* (with *Agulla adnixa* as outgroup). Posterior probability values are indicated at the nodes.

species of Raphidioptera (13 Raphidiidae and 3 Inocelliidae) occur, 10 of these are of Mediterranean origin and presumably have reached Central Europe after the last glacial period, i.e. within the past 10,000 years (H. Aspöck 2008, 2010, H. Aspöck et al. 1991, 2001, H. Aspöck and U. Aspöck 2015). At least in Austria, *R. mediterranea* must be regarded as a human introduced neozoon.

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## References

- Aspöck H (1987) The Raphidioptera of the Middle East: A Review (Insecta: Neuropteroidea). In: Krupp F, Schneider W, Kinzelbach R (Eds) Proceedings of the Symposium on the fauna and Zoogeography of the Middle East. Beihefte zum Tübinger Atlas des Vorderen Orients, Reihe A, 28: 148–159.
- Aspöck H (2008) Postglacial formation and fluctuations of the biodiversity of Central Europe in the light of climate change. Parasitology

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research (Suppl. 1) 103: S7–S10. https://doi.org/10.1007/s00436-008-1057-6

- Aspöck H (2010) Fluctuations of Biodiversity in Europe in Light of Climate Change. In: Friedrich B, Hacker J, Hasnain SE, Mettenleiter Th, Schell J (Eds) Climate Change and Infectious Diseases. Nova Acta Leopoldina NF 111, Nr. 381: 35–44.
- Aspöck H, Aspöck U (1965) Zur Kenntnis der Raphidiiden von Südosteuropa und Kleinasien. (Mit kritischen Bemerkungen zur Klassifikation der Familie). Annalen des Naturhistorischen Museums Wien 68: 309–364.
- Aspöck H, Aspöck U (2007) The Raphidioptera of the Apennines Peninsula: a biogeographical analysis. In Pantaleoni RA, Letardi A, Corazza C (Eds) Proceedings of the Ninth International Symposium on Neuropterology. Ferrara, Italy, 20–23 June 2005. Annali del Museo Civico di Storia Naturale 8: 95–106.
- Aspöck H, Aspöck U (2013) Woher kommen die Namen? Die validen rezenten Taxa der Kamelhalsfliegen der Erde: Systematisches Verzeichnis und Etymologie (Insecta: Endopterygota: Neuropterida: Raphidioptera). Entomologica Austriaca 20: 9–155.
- Aspöck H, Aspöck U (2014) Die Autoren der Taxa der rezenten Raphidioptera (Insecta: Endopterygota). Entomologica Austriaca 21: 9–152.
- Aspöck H, Aspöck U (2015) [2016] Die Neuropterida Europas aus der Sicht von Klimawandel und Globalisierung. DGaaE (Deutsche Gesellschaft für allgemeine und angewandte Entomologie) Nachrichten 29(2): 103–108.
- Aspöck H, Aspöck U, Hölzel H (unter Mitarbeit von Rausch H) (1980) Die Neuropteren Europas. 2 vols. Goecke and Evers, Krefeld, 495 pp, 355 pp.
- Aspöck H, Aspöck U, Rausch H (1977) Polyzentrische Ausbreitung eines "sibirisch-mediterranen" Faunenelements am Beispiel der

polytypischen Kamelhalsfliege Raphidia ophiopsis L. (Neuroptera, Raphidioptera, Raphidiidae). Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 28(4): 89–105.

- Aspöck H, Aspöck U, Rausch H (1989) The Raphidioptera of the Eastern Mediterranean: A Zoogeographical Analysis. Biologia Gallo-Hellenica 15: 67–112.
- Aspöck H, Aspöck U, Rausch H (1991) Die Raphidiopteren der Erde. Eine monographische Darstellung der Systematik, Taxonomie, Biologie, Ökologie und Chorologie der rezenten Raphidiopteren der Erde, mit einer zusammenfassenden Übersicht der fossilen Raphidiopteren (Insecta: Neuropteroidea). Goecke and Evers, Krefeld, 730 pp, 550 pp.
- Aspöck H, Aspöck U, Hölzel H (2001) Kommentierter Katalog der Neuropterida (Insecta: Raphidioptera, Megaloptera, Neuroptera) der Westpaläarktis. Denisia 2: 1–606.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModel-Test 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109
- Gruppe A, Aspöck H, Aspöck U (2017) Das Strohdach des Mittermayrhofs in Pelmberg (Oberösterreich) – Kinderstube und Puppenwiege einer mediterranen Kamelhalsfliege (Insecta: Neuropterida: Raphidioptera: Raphidiidae). Linzer biologische Beiträge 48 (1).
- Güsten R (1998) Segnalazioni faunistiche italiane. 331 Raphidia mediterranea H. Aspöck, U. Aspöck & Rausch, 1977 (Raphidioptera Raphidiidae). Bollettino della Società Entomologica Italiana 130: 80–81.
- Hall TA (1999) BioEdit: a user-friendly biological sequences alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series 41: 95–98.
- Haring E, Aspöck U (2004) Phylogeny of Neuropterida: a first molecular approach. Systematic Entomology 29: 415–430. https://doi. org/10.1111/j.0307-6970.2004.00263.x
- Haring E, Aspöck H, Bartel D, Aspöck U (2011) Molecular phylogeny of the Raphidiidae (Raphidioptera). Systematic Entomology 36: 16–30. https://doi.org/10.1111/j.1365-3113.2010.00542.x
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference for phylogeny. Bioinformatics 17: 754–755. https://doi.org/10.1093/ bioinformatics/17.8.754
- Kis B (1984) L'ordre Raphidioptera (Insecta) en Roumanie. Travaux du Muséum d'Histoire Naturelle "Grigore Antipa" 26: 84–97.
- Letardi A (2002) A web page on Italian Neuropterida. In Sziráki G (ed). Proceedings of the Seventh International Symposium on Neuropterology. Budapest, Hungary 6–9 August 2000. Acta Zoologica Academiae Scientiarum Hungaricae 48 (Supplement 2): 155–158.
- Letardi A, Pantaleoni RA (1996) I Neurotteroidei W-Paleartici della collezione del Museo di Zoologia dell'Università di Roma (Neuropteroidea). Fragmenta Entomologica 28: 277–305.
- Pantaleoni RA (2005) Interpretation of Achille Costa's data on Neuropterida. Bulletin of Insectology 58: 71–92.
- Rausch H, Aspöck H, Aspöck U (2016) Rätselhaftes Massenauftreten einer mediterranen Kamelhalsfliege im Mühlviertel, Oberösterreich (Insecta: Neuropterida: Raphidioptera: Raphidiidae). Linzer biologische Beiträge 48(1): 523–534.
- Rausch H, Rausch R (2004) 1000 Tage auf entomologischen Forschungsreisen. Unsere gemeinsamen Unternehmungen zur Erforschung der Neuropterida von 1969 bis 2000. In Aspöck U (wiss. Red.). Entomologie und Parasitologie. Festschrift zum 65. Geburtstag von Horst Aspöck. Denisia 13: 79–105.

- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425.
- Sziráki G (1993a) Taxonomic status of Raphidia mediterranea H. Aspöck, U. Aspöck et Rausch, 1977 (Raphidioptera: Raphidiidae).
  Folia Entomologica Hungarica [= Rovartani Közlemények] 54: 147–150.
- Sziráki G (1993b) Néhány, Magyarország faunájára új rovarfaj (Psocoptera, Raphidioptera, Neuroptera). [= Some insect species new to the fauna of Hungary (Psocoptera, Raphidioptera, Neuroptera).] Folia Entomologica Hungarica [= Rovartani Közlemények] 54: 187–189.
- Sziráki G (2010) Néhány kisebb fajszámú rovarrend szigetközi vizsgálata. In: Gubányi A, Mészáros F (Eds) A Szigetköz Állattani Értékei. Magyar Természettudományi Múzeum, Budapest, 49–56.
- Sziráki G (2014) Data to the Raphidioptera fauna of the Balkan Peninsula and Crete. Folia Historico-Naturalia Musei Matraensis 38: 87–90.

## Supplementary material 1

#### **BI and NJ trees**

- Authors: Horst Aspöck, Ulrike Aspöck, Axel Gruppe, Marcia Sittenthaler, Elisabeth Haring
- Data type: molecular data
- Explanation note: BI and NJ trees calculated with *cox1*, *cox3* and *28S* sequences, respectively. Posterior probability values (BI trees) and bootstrap values (in %, NJ trees) are indicated at the nodes.
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- Link: https://doi.org/10.3897/dez.64.19859.suppl1

## Supplementary material 2

#### Records of Raphidia mediterranea

- Authors: Horst Aspöck, Ulrike Aspöck, Axel Gruppe, Marcia Sittenthaler, Elisabeth Haring
- Data type: Microsoft Excel Worksheet (.xlsx)
- Explanation note: Records of *Raphidia mediterranea* documented in the distribution map (Fig. 5).
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons. org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/dez.64.19859.suppl2

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