

## Research article

[urn:lsid:zoobank.org:pub:DA4E1A0C-723E-4A6F-A214-955E8BBCB81F](https://zoobank.org/pub:DA4E1A0C-723E-4A6F-A214-955E8BBCB81F)**DNA barcoding reveals an unexpected diversity in Old World Vermileonidae (Insecta: Diptera)**

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D–01109 Dresden, GermanyEmail: [kehlmaier@web.de](mailto:kehlmaier@web.de)[urn:lsid:zoobank.org:author:3BD2A72C-A88E-4B2F-A22C-7CF522DE6F4D](https://zoobank.org/author:3BD2A72C-A88E-4B2F-A22C-7CF522DE6F4D)

**Abstract.** Bayesian Inference and Maximum Likelihood were used to analyse the DNA sequences of the mitochondrial *COI* gene of 69 wormlion specimens (Diptera: Vermileonidae). Most of the studied individuals belong to Old World taxa and belong to four genera, i.e., *Vermileo* (n=53), *Lampromyia* (n=13), *Vermipardus* (n=2), and *Leptynoma* (n=1). Species of the genus *Vermileo* were resolved into two distinct groups, with the analysed Nearctic species placed in a distinct grouping from the Palaearctic and Afrotropic taxa. Old World *Vermileo* species were divided into a western and eastern species group. Eight unnamed genetic lineages within the Palaearctic members of the genus *Vermileo* were identified, separated by a *p* distance of at least 0.038, and are regarded as putative species. *Vermileo cylindraceus* (Costa, 1844) stat. rev. is reinstated as a valid species.

**Key words.** Diptera, Vermileonidae, wormlions, systematics, COI, DNA barcoding.

**INTRODUCTION**

The family Vermileonidae (Insecta: Diptera) originated about 150 Ma ago in the Upper Jurassic, and thus represents an old lineage of brachyceran flies (Wiegmann et al. 2011). Being nowadays absent from Antarctica, Australia and South America, 63 described extant species placed in 11 genera are distributed globally, predominantly in arid habitats of mainly subtropical to temperate climates (Nagatomi et al. 1999; Kehlmaier 2014; Swart et al. 2015; Woodley & Swart 2017; Carles-Tolrá & Cuesta-Segura 2020; Bueno et al. unpubl.). Whereas adults are nectar feeding, their larvae are predators; hence their common name ‘wormlions’. Similar to many Myrmeleontidae larvae known as antlions (Insecta: Neuroptera), wormlions construct small pitfalls in fine grained soil or sand at rain-protected sites, waiting for prey at the deepest point of their trap.

The discovery of the genus *Vermileo* Macquart, 1834, the type genus of the family, is a story of travelling among the old Europe. The first published mention of this genus as “*Formica-Vulpes*” (literally antfox), to differentiate it from the “*Formica-Leo*” (antlion larvae), was presented in 1706 by an anonymous person (probably Monsieur Carré; Anonymous 1706). The text briefly described the observations made by a friend of his on the morphology, trap building and preying of the *Vermilio* larvae based on material collected in the ‘French countryside’. However, it was not until the mid-18<sup>th</sup> century when its entire

life-cycle was discovered. The French scientist René Antoine Ferchault de Réaumur (1683–1757) had searched in vain for wormlion larvae during a long time in the vicinity of Paris, and he had already abandoned hope (Réaumur 1753) when he received a parcel from his friend Reborny (Curé de la Palud, diocèse de Riez en Provence; currently La Palud-sur-Verdon) on 11<sup>th</sup> February 1751 with strange “worms” found on one of his walks. Being intrigued by these little creatures and wondering who else could work on them, Réaumur offered part of the material to the Queen of Sweden Luise Ulrike of Prussia (1720–1782), who was a renowned patron of science and art. Luise Ulrike of Prussia commissioned Carl De Geer (1720–1778) with the study of the larvae. By the time the material arrived to Stockholm from Paris by stagecoach, all of the material had perished except a single larva. Nevertheless, De Geer (1752) was able to rear the insect until the hatching of the imago, closely observing and documenting its development and eventually presenting a detailed and accurate illustrated description of its life-cycle. In the year to follow, Réaumur (1753) published his own detailed account on the matter. English translations of De Geer (1752) and Réaumur (1753) are included in Wheeler (1930).

With the exception of Kehlmaier (2014), taxonomic work on the family has been exclusively based on morphology. The present paper focuses on the molecular variability of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gen, the so-called DNA barcode *sensu*

Hebert et al. (2003a, b), in the wormlion genera *Lampromyia* Macquart, 1835 and *Vermileo*. Whereas the former genus has partly been studied using molecular characters (Kehlmaier 2014), no molecular analyses have been conducted for the latter so far.

## MATERIAL AND METHODS

Larval and adult material was collected and sent in by numerous colleagues, who are mentioned by name in the acknowledgements. Upon reception, living larvae were individualised and placed in film cases half filled with dry sand taken from local forest habitats. The sand was sieved with an ordinary colander and deep-frozen at  $-80^{\circ}\text{C}$  for two weeks beforehand. Larvae were fed once or twice a week with collected ants, mainly *Lasius niger* (Linnaeus, 1758), or flightless *Drosophila hydei* Sturtevant, 1921 obtained from a pet shop. Vermilionidae larvae and adults selected for molecular work were deep frozen and pinned or submerged in pure ethanol, and received a label with a unique DNA voucher number. If not otherwise stated, all material is stored at the Senckenberg Natural History Collections Dresden. All voucher details including ENA accession numbers are included in Table 1.

Morphological species identification were carried out using the works of Stuckenberg (1998) and Kehlmaier (2014) for *Lampromyia*, and Stuckenberg (1965), Nagatomi et al. (1999) and Carles-Tolrá & Cuesta-Segura (2020) for *Vermileo* (also see Discussion below), in combination with the study of the primary type material for *Leptis cylindracea* Costa, 1844 and *Vermileo niloticus* Edwards, 1935. Whereas the latter is deposited at the Natural History Museum (London, United Kingdom) and could be taken on loan, the former is in the collection of the Centro Musei delle Scienze Naturale e Fisiche (Naples, Italy) and two syntypes could only be studied from dorsal habitus photos. Costa (1844a: 85) collected the type material nearby Camaldoli (Naples), and mentioned additional non-type material from the Lago del Bagno on the island of Ischia, about 30 km from the city of Naples (Costa 1844b: 117). Part of the material included in the current study is from the vicinity of these localities and therefore considered conspecific.

Genomic DNA was extracted either from single legs, the apical portion of the abdomen, or from the apical part of larvae using the innuPREP DNA Mini Kit of Analytik Jena AG (Jena, Germany). *COI* sequences were amplified using the primer pair LCO-1490 (5'-GGT-CAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994), which targets a 658 bp long fragment of the 5'-end of the mitochondrial *COI* gen. PCR conditions were an initial  $94^{\circ}\text{C}$  for 4 min, 35 cycles at  $94^{\circ}\text{C}$  for 30 s,  $50^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 45 s, and a final

elongation at  $72^{\circ}\text{C}$  for 10 min. Each PCR was performed with 1–5  $\mu\text{l}$  of DNA extraction in a 20  $\mu\text{l}$  volume (1  $\mu\text{l}$  of each primer at 10 pmol, 1  $\mu\text{l}$  of dNTP-mix at 10 nmol each dNTP, and 1 unit of Taq polymerase (Bioron DFS Taq, Ludwigshafen, Germany), 2  $\mu\text{l}$  PCR buffer 10 $\times$  incl.  $\text{MgCl}_2$ , ultra-pure  $\text{H}_2\text{O}$ ). PCR products were visualised on a 1% agarose gel and purified using the ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems, Foster City, CA, USA; 1:20 dilution; modified protocol: 30 min at  $37^{\circ}\text{C}$  followed by 15 min at  $80^{\circ}\text{C}$ ). For cycle sequencing, the same forward and reverse primers were used in a total reaction volume of 10  $\mu\text{l}$  (2  $\mu\text{l}$  sequencing buffer, 1  $\mu\text{l}$  premix, 1  $\mu\text{l}$  primer at 5 pmol concentration, 0.5–6  $\mu\text{l}$  of DNA template, ultra-pure  $\text{H}_2\text{O}$ ) with 25 cycles at  $96^{\circ}\text{C}$  for 10 s,  $50^{\circ}\text{C}$  for 5 s and  $60^{\circ}\text{C}$  for 4 min using the ABI PRISM Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems). For subsequent purification, Performa DTR V3 96 Well Short Plates (Edge Biosystems, Gaithersburg, MD, USA) were used. Sequencing was done at the Senckenberg Natural History Collections Dresden using an ABI 3130XL or ABI 3730 Genetic Analyser.

Sequences were checked by eye for base-calling errors and aligned in BioEdit 7.0.5.2 (Hall, 1999). No internal stop codons, frame shifts or missing triplets were detected. The final dataset was trimmed to 637 bp and consisted of 69 ingroup and four outgroup sequences. The sequences for the outgroup taxa (three Rhagionidae and one Xylophagidae, which was constrained as the root for the inferred trees) were downloaded from GenBank. Maximum Likelihood and Bayesian Inference were used to infer the evolutionary relationships of these taxa based on *COI* sequences. Best fitting partitioning schemes and models of sequence evolution were obtained with PartitionFinder 2 (Lanfear et al. 2016) and the implemented Bayesian Information Criterion. Maximum Likelihood (ML) analyses were conducted with RAxML (Stamatakis 2014) using the GTR+G model as recommended by Stamatakis (2016: 59f), and a codon-partitioned scheme. As a preliminary step, five independent searches were performed using different starting conditions together with the fast bootstrap algorithm to explore the robustness of the results by comparing the best trees (not figured here). Then, 1,000 non-parametric thorough bootstrap replicates (BP) were calculated and plotted on the cladogram inferred from the Bayesian analysis (Fig. 1). Bayesian analysis (BA) was performed using MrBayes 3.2.6 (Ronquist et al., 2012) with two parallel runs (each with four chains) and default parameters using a codon partitioned data set and the substitution models SYM+G (codon position 1), F81+I (codon position 2), and GTR+G (codon position 3). The chains ran for 10 million generations, with every 1000th generation sampled. Run parameters were checked with Tracer ver. 1.7.1 (Rambaut et al. 2018). Branch support was assessed by the Bayesian posterior probabilities (BPP) and indicated in Fig. 1. Where-

as BP measures the robustness of character support, BPP evaluates the most likely phylogenetic tree for the given data and models of evolution. Although objective thresholds for strong, moderate, and weak support values are a matter of dispute (Zander 2004), BP of 80–89 and BPP of 0.90–0.94 are regarded as moderate, and BP  $\geq$  90 and BPP  $\geq$  0.95 as strong support values in the current study. Uncorrected  $p$  distances were calculated with MEGA ver. 10.0.5 (Kumar et al. 2018).

## RESULTS

New DNA barcodes of Vermileonidae were generated for 52 specimens of *Vermileo*, four specimens of *Lampromyia*, two specimens of *Vermipardus* Stuckenberg, 1960, and one specimen of *Leptynoma* Westwood, 1876. Nine sequences of *Lampromyia* and one of *Vermileo* were added from the International Nucleotide Sequence Database Collaboration (INSDC). No molecular data is currently available for the rest of Vermileonidae genera, namely *Alhajarmyia* Stuckenberg, 2003, *Isalomyia* Stuckenberg, 2002, *Namaquamyia* Stuckenberg, 2002, *Perianthomyia* Stuckenberg, 1996, *Vermilynx* Stuckenberg, 1995, *Vermiophis* Yang, 1979, and *Vermitigris* Wheeler, 1930.

The topologies of both phylogenetic analyses are largely congruent with moderate to strong (BA) or weak to moderate (ML) statistical support values (Fig. 1). The most interesting finding of the current dataset is the discovery of eight putative new species (named sp. A to sp. H) among the Palaearctic members of the genus *Vermileo*. These putative new species show clear differences in the *COI* sequence when compared with the other known *Vermileo* species.

The Nearctic representatives of *Vermileo* were resolved together as the sister group of the remaining wormlion taxa, but not in the same clade as the other *Vermileo* species. Old World *Vermileo* species were placed as the sister lineage to an unsupported clade containing *Lampromyia*, *Leptynoma* and *Vermipardus*. Within this grouping, the Palaearctic *Lampromyia* taxa were resolved together (BPP = 0.99; BP < 70), whereas the single Afrotropical *Lampromyia* species clusters with *Vermipardus* without statistical support. The unnamed *Vermipardus* species from Madagascar represents the first mention of the genus from this island. Old World *Vermileo* members (BPP = 0.96; BP < 70) were resolved into two groups, each comprising several described and putative species from western (Fig. 1 blue colour) and eastern (Fig. 1 green colour) parts of the Palaearctic Region.

Uncorrected  $p$  distances are summarised in Appendix I. Within the Palaearctic *Lampromyia*, the largest intraspecific  $p$  distance was found in *L. canariensis* Macquart, 1838 (0.025), whereas the smallest interspecific  $p$  distance was between *L. pallida* Macquart, 1835 and *L. lecerfi* Séguy, 1930 (0.038) and the largest interspecific

$p$  distance was between *L. iberica* Stuckenberg, 1998 and *L. canariensis* (0.131). The two Nearctic *Vermileo* species have a  $p$  distance from Old World *Vermileo* of 0.141–0.188, with the minimum  $p$  distance between them of 0.129. The western and eastern Old World clades of *Vermileo* are separated by 0.100 to 0.148  $p$  distance. Within the western *Vermileo* clade, the largest interspecific  $p$  distance was found between *V. vermileo* (Linnaeus, 1758) / *Vermileo* sp. G (0.124), and the smallest interspecific  $p$  distance was between *V. immaculatus* Carles-Tolrá & Cuesta-Segura, 2020 and *Vermileo* sp. G (0.050). Within the eastern *Vermileo* clade, the largest interspecific  $p$  distance was between *Vermileo* sp. A and *Vermileo* sp. C (0.121), and the smallest interspecific  $p$  distance was found between *Vermileo* sp. E and *Vermileo* sp. F (0.038). The maximum intraspecific  $p$  distance for an Old World *Vermileo* species does not exceed 0.015, with the exception of *V. ater* Stuckenberg, 1965 (0.036).

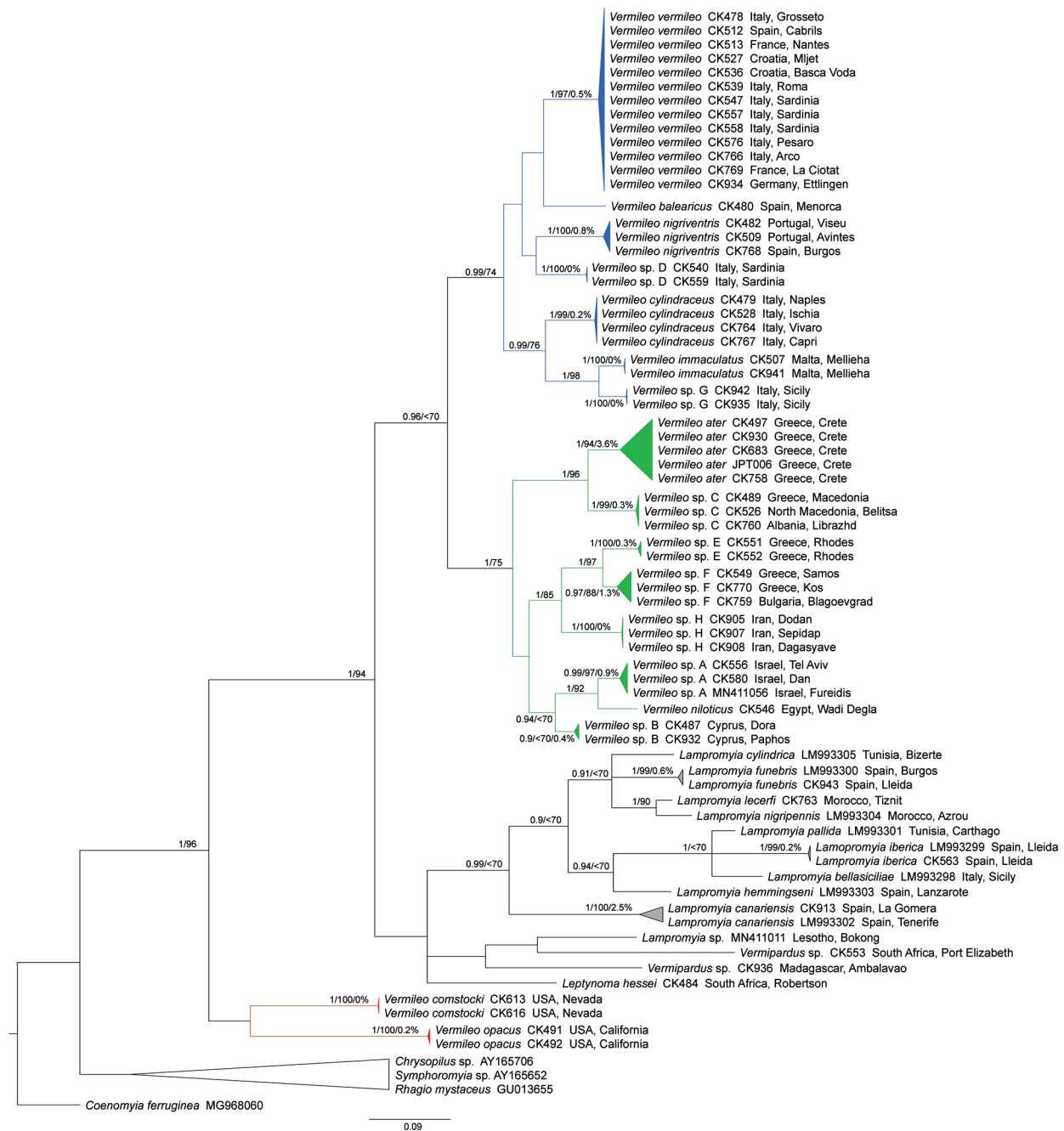
## DISCUSSION

Although phylogenetic reconstruction based on a single mitochondrial gene fragment is not adequate to establish conclusions on evolutionary relationships, it still has the potential to identify aspects in the systematics of a group that need to be scrutinised more thoroughly.

### *Lampromyia*

Stuckenberg (1998) scored 20 morphological characters for his cladistic analysis of *Lampromyia* and identified three species-groups. The *pilosula*-group occurs in southern Africa and comprises *L. flavida* (Engel & Cuthbertson, 1937), *L. pilosula* Engel, 1929 and *L. rebecca* Stuckenberg, 1996. The *canariensis*-group is present on the Canary Islands and has three members, *L. canariensis*, *L. fortunata* Stuckenberg, 1971 and *L. hemmingseni* Stuckenberg, 1971. Finally, the *cylindrica*-group occurs in the West Mediterranean Basin and is further divided into two subgroups, namely the *cylindrica*-subgroup (with *L. cylindrica* (Fabricius, 1749), *L. funebris* Dufour, 1850, *L. lecerfi* and *L. nigripennis* Séguy, 1930) and the *pallida*-subgroup (with *L. iberica* and *L. pallida*). Kehlmaier (2014) described an additional species from the *pallida*-subgroup, *L. bellasiciliae* Kehlmaier, 2014. The current molecular analyses supported the Palaearctic species groups and subgroups (Fig. 1), with the exception that *L. hemmingseni* was resolved with weak to moderate support (BPP = 0.94; BP < 70) as the sister taxon of the *pallida*-subgroup. The future addition of its proposed sister taxon *L. fortunata* to the dataset (the only Palaearctic *Lampromyia* currently missing) might provide additional evidence.

The placement of *Lampromyia* sp. from Lesotho, a member of the *pilosula*-group, with two *Vermipardus* species has no statistical support. A possible reason for



**Fig. 1.** Phylogenetic tree of a Bayesian analysis of *COI* barcoding data of Vermileonidae. Depicted are statistical support values for ingroup nodes of posterior probabilities (BPP) > 0.9 and bootstrap values (BP) > 70. For lineages representing known species or putative species, the maximum intraspecific genetic *p* distance is given in %. DNA voucher numbers follow the species name and more information is provided in Table 1. For those sequences retrieved from GenBank, the GenBank accession number is given instead of the DNA voucher number. The blue colour indicates the western Palaearctic *Vermileo*, the green colour indicates the eastern Palaearctic/Afrotropic *Vermileo*, and the red colour highlights the Nearctic *Vermileo*.

this unexpected placement is the length of the sequence MN411011 (with only 242 base-pairs). A first-hand morphological re-examination of the heavily damaged voucher (head missing, terminalia detached and stored

separately (Torsten Dikow *in litt.*), see habitus photo at [http://v3.boldsystems.org/index.php/Public\\_Record-View?processid=ASILO359-17](http://v3.boldsystems.org/index.php/Public_Record-View?processid=ASILO359-17)) is necessary, but currently not possible. The specimen was last identified by



**Table 1.** Material and sequence accession numbers.

Taxon	Locality	Sex	Field code	DNA voucher	Sequence accession number
Old World <i>Vermileo</i>					
<i>Vermileo ater</i>	GREECE, Crete, Rethymno, Municipal Garden, 35.365628° N, 24.472422° E, leg. M. Auer, 6.vii.2017.	larva	Ret20	CK930	OU230768
<i>Vermileo ater</i>	GREECE, Crete, Maroulas, 4 km SE Perivólia, leg. V. Michelsen, 14.vi.1999, coll. ZMUC.	♂	—	CK497	OU230769
<i>Vermileo ater</i>	GREECE, Crete, Iraklio, at window inside Natural History Museum of Crete, leg. C. Schmidt, v.2001.	♂	—	CK683	OU230770
<i>Vermileo ater</i>	GREECE, Crete, Chania, Thýmia, 35.4106° N 24.0440°E, leg. & coll. J. Pohjoismäki, 9.vi.2019.	♂	JPT006	JPT006	BOLD:ADZ6857
<i>Vermileo ater</i>	GREECE, Crete, Iraklio, Krasi, 35.234241° N 25.467538° E, vii.2013, leg. C. Tümmeler, vii.2013, ex larva 5.iii.2015.	♀	—	CK758	OU230771
<i>Vermileo balearicus</i>	SPAIN, Balear Islands, Menorca, Ferreries, Barranc de Cala Santa Galdana, leg. M.J. Ebejer, 7.VI.2008.	♀	—	CK480	OU230772
<i>Vermileo cylindraceus</i>	ITALY, Ischia, 40°43'56" N, 13°55'15" E, leg. C. Schmidt, 19.V.2011.	larva	Isc1	CK528	OU230773
<i>Vermileo cylindraceus</i>	ITALY, Capri, Anacapri-Faro, 40°32'3" N, 14°12'3" E, leg. M. Mende, 29.ix.2012, ex larva 12.vi.2013.	♂	Cap1	CK767	OU230774
<i>Vermileo cylindraceus</i>	ITALY, Isola di Vivara, 40.743843° N, 13.993472° E, leg. C. Schmidt, ca. 20.iv.2014, ex larva 10.vi.2014	♂	Viv2	CK764	OU230775
<i>Vermileo cylindraceus</i>	ITALY, Napoli, Posillipo, 40°48'24" N, 14°11'31" E, leg. C. Schmidt, 18. iv.2009, ex larva 10.vi.2009.	♂	Nap9	CK479	OU230776
<i>Vermileo immaculatus</i>	MALTA, Mellieha, Santa Marija, ca. 35°57'20" N 14°22'02" E, leg. P. Gatt, 24.vii.2010, ex larva 8.iv.2011.	♀	MT1	CK507	OU230777
<i>Vermileo immaculatus</i>	MALTA, Mellieha, Santa Marija, ca. 35°57'20" N, 14°22'02" E, leg. P. Gatt, 24.vii.2010, ex larva 7.iv.2011.	♂	MT2	CK941	OU230778
<i>Vermileo nigriventris</i>	PORTUGAL, Viseu, Silgueiros, Póvoa Dão, 40°33' N, 7°57' W, leg. J. Almeida, 2.viii.2009, ex larva 8.vi.2010.	♂	Por4	CK482	OU230779
<i>Vermileo nigriventris</i>	PORTUGAL, Avintes, 41°6' N, 8°33' W, leg. R. Andrade, 13.ii.2009.	larva	PT6	CK509	OU230780
<i>Vermileo nigriventris</i>	SPAIN, Burgos, Barbadillo del Mercado, leg. U.R. Lüders, 23.viii.2012, ex larva 22.v.2013.	♀	Urs1	CK768	OU230781
<i>Vermileo niloticus</i>	EGYPT, Cairo, Wadi Degla, leg. leg. H. Badrawy, 6.xi.2010, ex larva 14.iv.2011.	♂	—	CK546	OU230782
<i>Vermileo vermileo</i>	CROATIA, Dubrovnik-Neretva, Mljet, Babino polje (Sutmiholjska beach), leg. J. Podlesnik, 15.vii.2011.	larva	Bab13	CK527	OU230783
<i>Vermileo vermileo</i>	CROATIA, Dalmatia, Baska Voda, leg. D. Devetak, viii.2006, ex larva 10.vi.2007.	♂	—	CK536	OU230784
<i>Vermileo vermileo</i>	FRANCE, Nantes, Faculté des Sciences et des Techniques de Nantes, 47°14'19,62" N, 1°33'17,60" E, leg. A. Lequet, 29.iv.2010, ex larva 12.vi.2010.	♀	F10	CK513	OU230785

Taxon	Locality	Sex	Field code	DNA voucher	Sequence accession number
Old World <i>Vermileo</i>					
<i>Vermileo vermileo</i>	FRANCE, Bouches-du-Rhône, La Ciotat, leg. H. Dumas, 4.viii.2011, ex larva 27.v.2012.	♂	Cio5	CK769	OU230786
<i>Vermileo vermileo</i>	GERMANY, Baden-Württemberg, Ettlingen, 48°57'24.8" N, 8°23'03.1" E (48.956962, 8.384270), leg. K. Grabow, v.2018, ex larva 31.v.2019.	♀	D4	CK934	OU230787
<i>Vermileo vermileo</i>	ITALY, Pesaro & Urbino, Pesaro, Collina Barattoff, leg. M. Paglialunga, 27.vii.2011.	♀	—	CK576	OU230788
<i>Vermileo vermileo</i>	ITALY, Trentino-Alto Adige, Arco, Botanical Garden, x.2013, leg. M. Auer, ex larva 29.iv.2014	♀	Arc4	CK766	OU230789
<i>Vermileo vermileo</i>	ITALY, Toscana, Grosseto, Portiglione, Cala Violina, 10°46'30" E, 42°51'26" N, leg. C. Kehlmaier, 19.iv.2008, ex larva 21.v.2008.	♂	T2A-2	CK478	OU230790
<i>Vermileo vermileo</i>	ITALY, Roma, Lazio, Monte Mario, leg. A. Alfonsi via D. Badano vii.2010, ex larva 14.ii.2011.	♂	LAZ1	CK539	OU230791
<i>Vermileo vermileo</i>	ITALY, Sardinia, Sassari, Alghero, 40°32'17.84" N, 8°19'31.81" E, leg. D. Badano, 28.viii.2010, ex larva 23.i.2011.	♀	Alg1	CK557	OU230792
<i>Vermileo vermileo</i>	ITALY, Sardinia, Sassari, Parco del Monserrato, 40°42'34.59" N, 8°32'54.50" E, leg. D. Badano, 15.x.2010, ex larva 4.iv.2011.	♂	Sar5	CK547	OU230793
<i>Vermileo vermileo</i>	ITALY, Sardinia, Sassari, Platamona, leg. D. Badano, 28.xi.2010, ex larva 21.iii.2011.	♂	Plata2	CK558	OU230794
<i>Vermileo vermileo</i>	SPAIN, Cabrils, leg. M. Carles-Tolrà, 3.v.2009.	larva	SP12C	CK512	OU230795
<i>Vermileo</i> sp. A	ISRAEL, Tel-Aviv, Ramat-Gan, 32°05'00.3" N, 34°48'38.8" E (32.083415 34.810771), leg. A. Nir, 26.vi.2010, ex larva 20.iii.2011.	♂	ISR17	CK556	OU230796
<i>Vermileo</i> sp. A	ISRAEL, Dan, Bet Ussishkin, leg. L. Bodner via A. Freidberg, 22.xi.2011.	larva	Dan5	CK580	OU230797
<i>Vermileo</i> sp. A	ISRAEL, Nahal Tut, 5 km East of Fureidis, leg. W. Mathis & A. Freidberg, 19.v.1980.	?	—	—	MN411056 (as <i>Vermileo vermileo</i> )
<i>Vermileo</i> sp. B	CYPRUS, 6 km N Dora, 34°48'07" N, 32°45'29" E, leg. & coll. M.J. Ebejer, 27.iv.2002, ex larva 18.V.2002.	♂	—	CK487	OU230798
<i>Vermileo</i> sp. B	CYPRUS, Paphos, Apostolou Pavlou Avenue, leg. K. Grabow, 6.iv.2018, ex larva 30.iv.2018.	♂	—	CK932	OU230799
<i>Vermileo</i> sp. C	ALBANIA, Librazhd, Përrenjas, 41.06687° N, 20.54218° E, leg. J. Dils (K15007), 19.vii.2015.	larva	AL12	CK760	OU230800
<i>Vermileo</i> sp. C	GREECE, Macedonia, Lake Kerkini, Sidirochori, 41°14.33' N, 23°01.25' E, leg. & coll. M.J. Ebejer, 7.vi.2010, ex larva 22.vii.2010.	♀	—	CK489	OU230801
<i>Vermileo</i> sp. C	NORTH MACEDONIA, near Belitsa, 41° 40.69' N, 21° 12.54' E, leg. D. Devetak & V. Klokočovník, 16.ix.2011.	larva	Bel18	CK526	OU230802
<i>Vermileo</i> sp. D	ITALY, Sardinia, Sassari, Alghero, Spiaggia del Lazzaretto, leg. D. Badano, 7.xi.2010, ex larva 20.iii.2011.	♂	Lazza1	CK559	OU230803
<i>Vermileo</i> sp. D	ITALY, Sardinia, Sassari, Alghero, 40°32'17.84" N, 8°19'31.81" E, leg. D. Badano, 28.viii.2010, ex larva 27.i.2011.	♀	Alg2	CK540	OU230804

Taxon	Locality	Sex	Field code	DNA voucher	Sequence accession number
Old World <i>Vermileo</i>					
<i>Vermileo</i> sp. E	GREECE, Rhodes Island, Rhodes city, leg. R.A. Pantaleoni, 9.xi.2010, ex larva 17.iii.2011.	♂	Rho2	CK551	OU230805
<i>Vermileo</i> sp. E	GREECE, Rhodes Island, Rhodes city, leg. R.A. Pantaleoni, 9.xi.2010, ex larva 19.iii.2011.	♀	Rho1	CK552	OU230806
<i>Vermileo</i> sp. F	BULGARIA, Blagoevgrad, Campus of the American University in Bulgaria, 42°00'47" N, 23°05'45" E, leg. C. Schmidt, 3.viii.2013, ex larva 28.v.2014.	♂	Bul5	CK759	OU230807
<i>Vermileo</i> sp. F	GREECE, Kos Island, SW Kefalos, leg. M. Auer, vi.2015, ex larva 16.vi.2015.	♂	KOS7	CK770	OU230808
<i>Vermileo</i> sp. F	GREECE, Samos Island, Potamia, 37°47.730 N, 26°40.404 E, leg. G. Stähls, xi.2010, ex larva 3.iv.2011.	♀	SAM1	CK549	OU230809
<i>Vermileo</i> sp. G	ITALY, Sicily, Gorgi Tondi (TR), 37°36'34.82" N, 12°39'24.54" E (37.609672° N, 12.656817° E), leg. R.A. Pantaleoni via D. Badano, 10.IX.2010, ex larva 16.iv.2011.	♀	Sic1	CK942	OU230810
<i>Vermileo</i> sp. G	ITALY, Sicily, Trapani, Mazara del Vallo, Riserva Naturale Lago Preola e Gorgi Tondi, 37.611795° N, 12.654338° E, leg. A. Müller, 8.iii.2016, ex larva 25.v.2016.	♂	Sic11	CK935	OU230811
<i>Vermileo</i> sp. H	IRAN, Kermanshah, Dodan, 34°00' N, 46°12' E, Malaise trap, leg. M. Zardouei, 5.vii.2016.	♂	—	CK905	OU230812
<i>Vermileo</i> sp. H	IRAN, Kermanshah, Sepidap, 34°59' N, 46°14' E, Malaise trap, leg. M. Zardouei, 20.vi.2016.	♀	—	CK907	OU230813
<i>Vermileo</i> sp. H	IRAN, Kermanshah, Dagasyave, 35°02' N, 46°12' E, Malaise trap, leg. M. Zardouei, 20.vi.2016.	♂	—	CK908	OU230814
New World <i>Vermileo</i>					
<i>Vermileo comstocki</i>	USA, Nevada, Zephyr Cove, Camping resort, 39°00'22" N, 119°56'42" W, 1900 m, leg. M. Hauser, 20.viii.2011, ex larva 27.v.2012.	♂	US7	CK613	OU230815
<i>Vermileo comstocki</i>	USA, Nevada, Zephyr Cove, Camping resort, 39°00'22" N, 119°56'42" W, 1900 m, leg. M. Hauser, 20.viii.2011, ex larva 27.v.2012.	♀	US3	CK616	OU230816
<i>Vermileo opacus</i>	USA, California, Los Angeles County, La Crescenta, Eagle Canyon, 34°14'45" N, 118°13'56" W, 2500ft, leg. S.D. Gaimari, M.S. & E.S. Anderson, 10.–17.VI.2007.	♂	US-M	CK491	OU230817
<i>Vermileo opacus</i>	USA, California, Los Angeles County, La Crescenta, Eagle Canyon, 34°14'45" N, 118°13'56" W, 2500ft, leg. S.D. Gaimari, M.S. & E.S. Anderson, 10.–17.VI.2007.	♀	US-W	CK492	OU230818
<i>Lampromyia</i>					
<i>Lampromyia bellasiciliae</i>	ITALY, Sicily, Palermo, 38.148594° N, 13.364795° E, leg. F. Sacco, 19.xi.2012, ex larva 7.vii.2013.	♂	Siz19	CK699	LM993298 (Kehlmaier 2014)
<i>Lampromyia canariensis</i>	SPAIN, Canary Islands, Tenerife, Fasnía, 28.24° N, 16.44° W, leg. G. Peña Tejera, 26.vi.2012.	♂	—	CK635	LM993302 (Kehlmaier 2014)
<i>Lampromyia canariensis</i>	SPAIN, Canary Islands, La Gomera, Hermigua, El Cerrillal, leg. J. Jakobitz, 30.xi.2016, ex larva vi.2017.	♀	LG1	CK913	OU230819

Taxon	Locality	Sex	Field code	DNA voucher	Sequence accession number
<i>Lampromyia</i>					
<i>Lampromyia cylindrica</i>	TUNISIA, 6 km N Bizerte, Cap Blanc, 37.33° N, 9.83° E, leg. M. Hauser, 18.vi.2000.	♀	—	CK494	LM993305 (Kehlmaier 2014)
<i>Lampromyia funebris</i>	SPAIN, Burgos, Ura, 42.014348° N, 3.552813° W, leg. U.R. Lüders, 23.viii.2012, ex larva 12.vi.2013.	♀	Ura2	CK700	LM993300 (Kehlmaier 2014)
<i>Lampromyia funebris</i>	SPAIN, Lleida, Bell-lloc d'Urgell, 41°37'43" N, 0°46'59" E, leg. R.M. Batlle, viii.2012, ex larva 10.vi.2013.	♀	Ram3	CK943	OU230820
<i>Lampromyia hemmingseni</i>	SPAIN, Canary Islands, Lanzarote, Playa de Papagayo, 28.84° N, 13.78° W, leg. C. Schmid-Egger, 5.iii.2008.	♂	—	CK493	LM993303 (Kehlmaier 2014)
<i>Lampromyia iberica</i>	SPAIN, Lleida, Timoneda d'Alfès, 10 km SE Lleida, leg. R.M. Batlle, 4.ix.2010, ex larva 5.iv.2011.	♀	RB7	CK548	LM993299 (Kehlmaier 2014)
<i>Lampromyia iberica</i>	SPAIN, Lleida, Timoneda d'Alfès, 10 km SE Lleida, leg. R.M. Batlle, 4.ix.2010, ex larva 7.iv.2011.	♂	RB1	CK563	OU230821
<i>Lampromyia lecerfi</i>	MOROCCO, Tiznit, Tiznit, 29.69582° N, 9.72542° W, leg. J. Dils (K15002), iv.2015, ex larva 11.vi.2015.	♂	MA10	CK763	OU230822
<i>Lampromyia nigripennis</i>	MOROCCO, South of Azrou, 33.32° N, 5.27° W, leg. A.K. Hundsdörfer, vi.2006.	larva	—	CK504	LM993304 (Kehlmaier 2014)
<i>Lampromyia pallida</i>	TUNISIA, Carthago, 36.86° N, 10.33° E, leg. D. Devetak, 29.v.2010, ex larva 27.x.2010.	♂	TUN1	CK481	LM993301 (Kehlmaier 2014)
<i>Lampromyia</i> sp.	LESOTHO, Bokong, leg. L. Bevis, 26.xii.1946.	♂	—	—	MN411011
<i>Leptynoma</i>					
<i>Leptynoma hessei</i>	SOUTH AFRICA, Western Cape, Riverhuis Farm, 14 km NW Robertson, 33°46'40" S, 19°45'35" E, leg. M. Hauser, 29.ix.2004.	♂	—	CK484	OU230823
<i>Vermipardus</i>					
<i>Vermipardus</i> sp.	MADAGASCAR, Anja Reserve, W Ambalavao, leg. H. Rasolondalao, xii.2017, ex larva 29.iii.2019	♀	Mad11	CK936	OU230824
<i>Vermipardus</i> sp.	SOUTH AFRICA, Eastern Cape Province, 70 km N Port Elizabeth, near Addo Elephant National Park, leg. U. Fritz, 22.xi.2010, ex larva 13.iii.2011.	♂	Addo2	CK553	OU230825
Outgroup					
<i>Chrysophilus</i> sp.	No data available.	?	—	—	AY165706
<i>Rhagio mystaceus</i>	CANADA, Ontario, Pukaskwa, Pukaskwa National Park, Coastal Trail Boreal forest, 48.56°N 86.23°W, leg. J. Cossey <i>et al.</i> , 2.vii.2008.	?	—	—	GU013655
<i>Symphoromyia</i> sp.	No data available.	?	—	—	AY165652
<i>Coenomyia ferruginea</i>	USA, Virginia, Berkeley County, Sleepy Creek Wildlife Area, 39.52° N, 78.1503° W, leg. N.E. Woodley, 22.v.2004.	?	—	—	MG968060

S.W. Bromley before 1955, and *Vermipardus* was erected as a subgenus within *Lampromyia* only some years later by Stuckenberg (1960).

### *Vermileo*

As mentioned earlier, the present study indicates the existence of eight putative new species of the genus

*Vermileo* in the Palaearctic Region. Prior to this study, the genus comprised twelve named species worldwide plus an apparently undescribed species from Costa Rica (Woodley 2009). The known Palaearctic *Vermileo* species are: *V. ater*, *V. balearicus* Wheeler, 1930, *V. immaculatus*, *V. nigriventris* (Strobl, 1906) and *V. vermileo*. In the Afrotropical Region there is only one species, *V. nilot-*



*icus*. Six species are known from the Nearctic: *V. comstocki* Wheeler, 1918, *V. dowi* Wheeler, 1931, *V. fascipennis* (Williston, 1895), *V. opacus* (Coquillett, 1904), *V. tibialis* (Walker, 1852), and *V. willetti* DeLeon, 1938. Based on the current dataset, the species *V. cylindraceus* (Costa, 1844) stat. rev., formerly considered synonymous to *V. vermileo* (see Bezzi 1900), is reinstated as a valid species.

Wheeler (1918, 1930, 1931, 1934), Edwards (1935), Stuckenberg (1965), Nagatomi et al. (1999), and Carles-Tolrá & Cuesta-Segura (2020) where the most recent authors commenting on *Vermileo* taxonomy; the latter describing a *Vermileo* species from Malta and reinstating *V. balearicus* as a valid species – the proposed synonymy of *V. balearicus* and *V. immaculatus* with *V. vermileo* by Ebejer & Gatt (2021) is not corroborated by the molecular data presented in the present study. Reliable identification keys for the Old World fauna of this genus do not exist, neither has there ever existed one. In his Palaearctic revision, Lindner (1925) only included *V. vermileo* into his identification key to the Rhagionidae (wormlions had subfamily status within Rhagionidae at that time) and left *V. balearicus* and *V. nigriventris* as subspecies of *V. vermileo*. Nagatomi et al. (1999) provided the only other identification key of Old World *Vermileo*, which includes *V. ater*, *V. nigriventris*, *V. niloticus*, and *V. vermileo*, using differences of the male postabdomen mentioned by Stuckenberg (1965). Unfortunately, the first couplet of Nagatomi et al. (1999) is already misleading, as the numbers of the couplets to follow on are interchanged. The lack of a proper identification key has repeatedly caused authors to identify their material based on general body coloration. That way, *V. vermileo* has been cited from regions where the genus is represented by different, partly undescribed taxa, e.g., Bulgaria (Popov 1968), Egypt (Hafez et al. 1956a, 1956b), Greece (Papp & Soltész 2019), Malta (Ebejer 1995). Morphological descriptions of the putative new species as well as an identification key will be published elsewhere in the future.

Both analyses resolved the Nearctic species *V. comstocki* and *V. opacus* as the sister group of the remaining studied taxa of the family. Although there is no statistical support, present results agree with the observations made by Nagatomi et al. (1999), who pointed out that the structure of male genitalia of the Palaearctic *Vermileo* species clearly differs from the male genitalia of the Nearctic *V. comstocki*. Earlier, Pechuman (1938) commented on two morphological characters that possibly help to separate New World from Old World species on a generic level. The New World *Vermileo* were last studied by Pechuman (1938) and DeLeon (1938), and there currently are six described species from Cuba, Jamaica, Mexico, and USA. As the Palaearctic *V. vermileo* represents the type species of *Vermileo* Macquart, 1834, and the first species from the New World (*V. tibialis*) was originally described

in the monotypic genus *Pheneus* Walker, 1852, this name would be resurrected from synonymy in case that the phylogenetic placement for the Nearctic *Vermileo* receives further evidence and stronger support.

As highlighted in the present study by the number of putative new species, being apparently undescribed, the species richness of the *Vermileo* in the Old World has been greatly underestimated, especially in the Mediterranean Basin. The group with western *Vermileo* taxa comprises seven species (*V. balearicus*, *V. cylindraceus*, *V. immaculatus*, *V. nigriventris*, *V. vermileo*, *Vermileo* sp. D and *Vermileo* sp. G) and their geographical distribution ranges from Portugal to southern Croatia, whereas the group with eastern *Vermileo* representatives consists of eight species (*V. ater*, *V. niloticus*, *Vermileo* sp. A, *Vermileo* sp. B, *Vermileo* sp. C, *Vermileo* sp. E, *Vermileo* sp. F and *Vermileo* sp. H) and they occur from Albania to western Iran. Preliminary morphological studies of male terminalia confirm this partition. Old World species of *Vermileo* look alike superficially due to the absence of striking coloration and the lack of conspicuous apomorphic features (e.g., on the head, legs or sternites). At the same time, general body coloration and wing venation can vary considerably within a single species. Therefore, stable morphological characters are scarce, especially in females. Promising features being the coloration of the small lateral thoracic sclerites, and the shape of male tergite 9 which displays species specific lateroapical appendages in the eastern Palaearctic/Afrotropic *Vermileo* species group.

The current knowledge does not reflect the true distribution range of the individual species or groups as intensive sampling in the western Palaearctic has not been done, with important geographical gaps in Corsica and Turkey. However, when looking at the distribution of the individual known or putative species it is striking that seven out of 15 (47%) Palaearctic species of *Vermileo* are limited to islands. Keeping in mind the old age of the family and the unsteady geological history of the Mediterranean Basin (e.g., Mediterranean salinity crisis), one tends to ask whether the observed island taxa indeed constitute true endemism, i.e., species that evolved on the island due to reproductive isolation, or might be relicts of formerly much wider distributed species.

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## APPENDIX I

(electronic supplement, available at [www.bonnzoologicalbulletin.de](http://www.bonnzoologicalbulletin.de))

Uncorrected pairwise distance matrix. Blue: western Palaearctic species of *Vermileo*. Green: eastern Palaearctic/Afrotropic species of *Vermileo*. Red: Nearctic species of *Vermileo*.

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