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Genetic data confirm the species status of *Sepsis nigripes* Meigen (Diptera : Sepsidae) and adds one species to the Alpine fauna while questioning the synonymy of *Sepsis helvetica* Munari

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Abstract: Due to their interesting biology, conspicuous sexual dimorphism and the ability to conduct experiments on species that breed under laboratory condition, sepsid flies (Diptera : Sepsidae) are becoming increasingly important model organisms in evolutionary biology. Accurate species boundaries and well supported phylogenetic hypotheses are thus of interest to many biologists. Here we resolve the conflict surrounding the taxonomic status of the European *Sepsis nigripes* Meigen, 1826, which is shown to be a valid species using morphological and molecular data applied to multiple species concepts. The species is also placed onto a phylogenetic tree for the genus *Sepsis* that includes most European and North American species. In addition, we assess the genetic variability between two populations of the Holarctic *Sepsis luteipes* Melander Spuler, 1917 from Europe and North America and find conflicting evidence between morphology and DNA sequences. Different species concepts here yield different inferences, and if two species were to be accepted based on molecular data, *Sepsis helvetica* Munari, 1985 from Europe would have to be resurrected from synonymy. We provide highresolution images for all species in order to aid in accurate identification. Both species are also added to Sepsidnet, the digital reference collection for Sepsidae (<http://sepsidnet-rmbr.nus.edu.sg>). Lastly, we discuss a field site in the Swiss Alps where 12 species of *Sepsis* occur sympatrically on the same pasture.

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Genetic data confirm the species status of *Sepsis nigripes*, Meigen 1826 (Diptera: Sepsidae) and adds one species to the Alpine fauna while questioning the synonymy of *Sepsis helvetica*, Munari 1985

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Abstract

Due to their interesting biology, conspicuous sexual dimorphisms, and the ability to conduct experiments on species that breed under laboratory condition, sepsid flies (Diptera: Sepsidae) are becoming increasingly important model organisms in evolutionary biology. Accurate species boundaries and well-supported phylogenetic hypotheses are thus of interest to many biologists. Here we resolve the conflict surrounding the taxonomic status of the European *Sepsis nigripes* Meigen 1826, which is shown to be a valid species using morphological and molecular data applied to multiple species concepts. The species is also placed onto a phylogenetic tree for the genus *Sepsis* that includes most European and North American *Sepsis* species. In addition, we assess the genetic variability between two populations of the Holarctic *Sepsis luteipes*, Melander and Spuler 1917 from Europe and North America and find conflicting evidence between morphology and DNA sequences. Different species concepts here yield different inferences, and if two species were to be accepted based on molecular data, *Sepsis helvetica*, Munari 1985 would have to be resurrected from synonymy. We provide high-resolution images for all species in order to aid in accurate identification. Both species are also added to Sepsidnet, the digital reference collection for Sepsidae (<http://sepsidnet-rmbr.nus.edu.sg>). Lastly, we discuss a field site in the Swiss Alps where 12 *Sepsis*-species occur sympatrically on the same pasture.

Key words: Sepsidae, *Sepsis nigripes*, *Sepsis luteipes*, species concepts

Introduction

Sepsidae, or black scavenger flies, are a moderately species-rich group of schizophoran flies (>300 species, 37 genera (Ozerov 2005)) that are commonly found on cattle droppings and various decaying organic matter. Male sepsids typically have species-specific spines, bristles and protrusions on their fore femora and tibiae. These sexually dimorphic modifications are used to grasp the female wing base during mating (Ingram *et al.* 2008; Martin *et al.* 2003; Pont and Meier 2002). Over the past few years, Sepsidae have become models in behavioral ecology, sexual selection and speciation studies (Blanckenhorn 1999; Blanckenhorn *et al.* 2000; Eberhard 2001; Martin and Hosken 2003; Puniamoorthy *et al.* 2009; Puniamoorthy *et al.* 2010; Puniamoorthy *et al.* 2012; Puniamoorthy *et al.* 2008; Tan *et al.* 2011; Teuschl and Blanckenhorn 2007), in eco-toxicological surveys (Blanckenhorn *et al.* 2013a; Blanckenhorn

et al. 2013b), and in developmental research (Bowsher *et al.* 2013; Bowsher and Nijhout 2007; Hare *et al.* 2008). This popularity has led to the establishment of steadily growing digital reference collection “Sepsidnet” that facilitates fast and correct identification ((Ang *et al.* 2013), see <http://sepsidnet-rmbr.nus.edu.sg/>).

The phylogenetic relationships within Sepsidae are generally well resolved and supported based on morphological and molecular data (Laamanen *et al.* 2005; Meier 1996; Su *et al.* 2008; Zhao *et al.* 2013). However, a few species remain problematic and others still need to be placed. The latter are either rare or belong to morphologically plastic species complexes consisting of unknown or disputed numbers of species (Tan *et al.* 2010). One of the rare species that has repeatedly been discussed in the literature is the European *Sepsis nigripes*, Meigen 1826, which is not only very small and rare, but also morphologically very similar to closely related species. This has led to disagreements about its species status. While some taxonomists doubted its status as a separate species and often synonymized *S. nigripes* with other species (Collin 1910; Pont 1979; Zuska 1970), other authors disagreed and insisted on its taxonomic validity (Becker 1902; Duda 1926; Hennig 1949; Pont and Meier 2002). However, no molecular evidence was yet available so its placement on the phylogenetic tree remained unclear. In addition, the data were not evaluated based on explicit species concepts. Another European species of dispute that lacks molecular data and belongs to a morphologically plastic species complex is *Sepsis luteipes* Melander & Spuler, 1917. It was described based on Nearctic material. However, Frey (1917) and later Munari (1985) saw European specimens that were morphologically very similar. As discussed in Pont and Meier (2002), Frey (1917) provided a provisional species name that is considered a *nomen nudum* because he failed to provide a species description. Munari later described this species as *Sepsis helvetica* Munari 1985, a species that was subsequently synonymized with *Sepsis luteipes* by Ozerov (1999).

Here, we present evidence for the validity of *S. nigripes* based on morphological and genetic data and incorporate the taxon in a phylogenetic analysis of *Sepsis*. We furthermore describe a surprisingly diverse sepsid community of a field site in the Swiss Alps, thus documenting that *S. nigripes* is part of the Alpine fauna. Lastly, we discuss whether the North American and European populations of *Sepsis luteipes* are conspecific.

Material and methods

Samples

The European specimens used in this study were collected in the Swiss Alps (Lenzerheide, CH: 46.73°N, 9.56° E, 1500 m.a.s.l.) in July 2013. Wild caught male flies were frozen for identification, while females were reared and bred in the laboratory (University of Zurich) on cow dung. Because male characters are important for identifying species, only male offspring of these females were used for species identification. The North American samples for *Sepsis luteipes* were caught with dung traps in 2012 near Lake Tahoe in Nevada, USA (38.93°N, -119.98° E, 1900 m.a.s.l.). *Sepsis thoracica* from South Africa (Coll. Yuchen Ang; Sept. 2013, Cederberg National Park, Western Cape, South Africa: -32°20'35"S, 19° 1'31"E, 360 m.a.s.l.) was also included in this study.

Specimen morphology and imaging

Male specimens were visually documented prior to DNA extraction and sequencing. The lateral habitus (showing leg ornamentation and thoracic pleura) as well as ventral view of the hypopygium were imaged and combined into a single figure, which displays enough species-specific diagnostic characters to reliably differentiate the various species. All specimens were imaged using the Visionary Digital™ BK Plus Lab System, and then focus-stacked using Helicon Focus™ Pro (ver. 5.2.16) and digitally cleaned using Photoshop® to remove image background ‘noise’ and optimize brightness and contrast. Specimen figures are shown in this paper, and also deposited in the digital reference collection “Sepsidnet”.

DNA extraction and sequencing

The molecular dataset consists of the available sequence for 26 *Sepsis* and two outgroup species that were selected from Zhao *et al.* (2013). We here add four new taxa: *Sepsis nigripes*, *Sepsis thoracica* from South Africa, and two populations of *Sepsis luteipes* from Europe and North America (Supplementary Table 1). Genomic DNA of newly added taxa was extracted from whole flies using a CTAB protocol with minor modifications of Shahjahan’s protocol (Shahjahan *et al.* 1995). Sequences for nine gene regions included here were obtained using the PCR and sequencing protocols described in Su *et al.* (2008): the nuclear protein-coding genes Alanine-tRNA synthetase (AATS), Histone H3 (H3) and factor-1a (Ef1a), the mitochondrial protein-coding genes cytochrome c oxidase subunit I (COI), cytochrome c oxidase subunit II (COII) and cytochrome b (CYTB), as well as the ribosomal genes 12s, 16s, 18s, and 28s. A 487bp region of 28s was not included because the pairwise distance analyses of this region suggest that it is uninformative within *Sepsis*. Successfully amplified PCR products

were purified with SureClean before cycle-sequencing with BigDye Terminator v3.1, followed by bidirectional sequencing on an ABI 3100 Genetic Analyser (Perkin Elmer, Waltham, MA).

Raw reads for both directions for each gene were assembled and edited with Sequencher v4.6 (Gene Codes, Ann Arbor, MI, USA) and subsequently checked for contamination with NCBI BLAST before alignment (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Alignments were generated in MAFFT (<http://mafft.cbrc.jp/alignment/server/>, (Kato *et al.* 2005)) with default parameters followed by a check of the protein encoding genes via amino acid translation in Mega 5 (Tamura *et al.* 2011). All aligned genes were concatenated in SequencingMatrix v1.7.8 (Vaidya *et al.* 2011) before phylogenetic analysis.

Sequence analyses

The uncorrected, pairwise distances were computed in SpeciesIdentifier (Meier *et al.* 2006). For previously stated reasons (Srivathsan and Meier 2012), uncorrected distances were preferred over K2P distances. In order to reconstruct the phylogenetic relationships, we performed a maximum parsimony (MP) and two maximum likelihood (ML) analyses for the concatenated dataset. The MP analysis was conducted using TNT v1.0 (Goloboff *et al.* 2008): New Technology Searches were performed with level 100, initial addseqs 5 and by finding the minimum length 10 times). Five-hundred nonparametric bootstrap replicates with same settings were analyzed in order to determine node support for a dataset with indels coded as missing values. ML analyses were performed using Genetic Algorithm for Rapid Likelihood Inference (GARLI 2.01) on XSEDE (Zwickl 2006) via the CIPRES gateway server (Miller *et al.* 2010). We used two partitioning schemes. For the first analysis, we treated the concatenated data as one partition. For the second, the data were first partitioned into nine parts: (1)-(2) mitochondrial protein-encoding genes: 1st and 2nd, 3rd positions; (3)-(4) nuclear protein-encoding genes: 1st and 2nd, 3rd positions; (5) 12S rDNA; (6) 16S rDNA; (7) 18S rDNA; (8)-(9) two regions of 28S rDNA. jModelTest v0.1 (Posada 2008) was used to find the best fit model under the Akaike Information Criterion (AIC). (One partition model: GTP+G; Codon position partition models: 12s: TIM1+I+G, 16s: TIM3+I+G, 18s: F81+I+G, 28ss1: TIM3+G, 28ss2: TPM3uf+G, Mitochondrial protein coding genes 1st and 2nd position: TIM1+G, Mitochondrial protein coding genes 3rd position: TIM2+G, Nuclear protein coding genes 1st and 2nd position: TIM3+G, Nuclear protein coding genes 3rd position: TIM2uf+G). Optimal ML trees were found by running two independent analyses for 20,000 consecutive generations. Support was evaluated using 250 bootstrap replicates with a stop criterion of 10,000 generations.

Results

Relationships among closely related Sepsidae

We obtained sequence data for the newly added four taxa for most of the nine gene regions (see Supplementary Table 1 for details). *COI* distances between *S. nigripes* and *S. flavimana* – the species with the most similar *COI* barcode sequence in Europe (Zhao *et al.* 2013) – is 4.8%, i.e., much higher than normally observed within species in Diptera (Meier *et al.* 2006; Meier *et al.* 2008). On the phylogenetic tree, *S. nigripes* is sistergroup to *S. flavimana*. However, this hypothesis has relatively low bootstrap support in the MP and ML analyses. Inspection of all bootstrap replicate trees reveals that a third, closely related species, *S. pyrrhosoma*, is sistergroup to *S. nigripes*, or all three species are separate branches leading to a clade consisting of the remaining species of the *flavimana* group (*S. biflexuosa*, *S. duplicata*, *S. secunda*). The uncorrected, pairwise distances between the two populations of *Sepsis luteipes* are also large (3.5-3.86%) and more compatible with separate species than intraspecific variability. The populations are well supported as sistergroups and nested within Hennig's *punctum* species group (including *S. orthocnemis*). *Sepsis orthocnemis* lacks the femoral tubercle that is otherwise the distinguishing feature of the species group, but its close relationship to the *punctum* group was already identified in Su *et al.* (2008).

Morphological differentiation

The sepsid species that are morphologically most similarly to *S. nigripes* are *S. pyrrhosoma*, *S. flavimana*, and *S. biflexuosa*; the latter two occur in Europe in sympatry with *S. nigripes* (see Table 1). As discussed in Pont & Meier (2002), *S. flavimana*, *S. biflexuosa* and *S. nigripes* can be distinguished by their male foreleg armature and genital structures, while females cannot be distinguished with certainty. There are several characters that can be used to distinguish male *S. nigripes* from other species. Males usually have slight modifications close to the proximal end of the fore tibia (Fig. 2b). In contrast, ermarginations in *S. biflexuosa* are typically more distal (Fig. 2a), and *S. flavimana* has an shallow notch in the proximal half of the fore tibiae, similar to *S. nigripes*, but much stronger (Fig. 2c). In addition, *Sepsis nigripes* can be distinguished from *S. flavimana* by an additional antero-ventral row of 5-7 spines (Iwasa 1985; Pont and Meier 2002). However, high phenotypic plasticity in fore femoral size can obscure the species differences.

We believe that the most reliable trait for identifying *S. nigripes* is the characteristic blunt-tipped genital surstylus. In contrast, the surstyli of *S. flavimana* are beak-like and the ones of

S. biflexuosa are more slender and slightly more elongated (see in Fig. 2 as well as Pont and Meier 2002).

Biodiversity of Sepsis of a field site in the Swiss Alps

Sepsis nigripes is a very rare species in Central and Northern Europe, with only few, new records from Hungary (Papp 2007), Russia and Japan (Iwasa 1995), whereas its sister taxon *S. flavimana* is very common throughout Europe and beyond (Pont and Meier 2002). *Sepsis nigripes* is here recorded for the first time from the Alps. It was collected as part of a remarkably diverse assemblage of *Sepsis* species at Lenzerheide (Switzerland). The assemblage was dominated by a few common species (Table 1), while most species, including *S. luteipes*, *S. nigripes*, and its sister species *S. flavimana*, were fairly rare.

Discussion

Our phylogenetic analyses of the new sequence data place *S. nigripes* within the *flavimana*-group and *S. luteipes* in the *punctum* group. These placements are congruent with earlier taxonomic work based on morphology (Frey 1925; Iwasa 1985; Pont and Meier 2002). However, the precise relationship of the species within their respective clades was not addressed. One may argue that two additions to the Tree-of-Life for Sepsidae (Zhao *et al.* 2013) are unimportant, but both species occur in Europe and/or North America and they are likely to be included in future sepsid studies focusing on ecology, behavior, and evolution of Holarctic sepsids. In addition, we obtained phylogenetic information in order to be able to discuss the species boundaries based on a range of species concepts (Laamanen *et al.* 2003; Tan *et al.* 2010), and some of these concepts require phylogenetic information (Wheeler & Meier, 2000). Here we were able to place both species on the Tree-of-Life although the bootstrap support for the placement of *S. nigripes* is relatively low (MP: 57; ML 59, 76). Currently, the best support exists for a sistergroup relationship between *S. nigripes* and *S. flavimana* with both species together being sistergroup of the remaining species belonging to Hennig's *flavimana* group [*S. pyrrhosoma*, (*S. biflexuosa*, (*S. duplicata*, *S. secunda*))]. However, there are also two common, conflicting topologies among the bootstrap replicate trees. One supports a sistergroup relationship between *S. nigripes* and *S. pyrrhosoma* (8% of MP bootstrap replicates; 26% of ML_{1 partition} replicates; 17% ML_{10 partitions} replicates) or a "ladderized" *S. flavimana* and *S. nigripes* forming subsequent branches at the base of the *flavimana* species group (20% of MP replicates; 9% of ML_{1 partition} replicates; 6% ML_{10 partitions} replicates). In contrast to the placement

of *S. nigripes*, the position of *S. luteipes* is unambiguous, and it is placed as sistergroup of a clade composed of *S. violacea*, *S. fulgens*, and *S. orthocnemis*.

Species limits for *Sepsis nigripes* and *Sepsis luteipes*

With respect to phylogenetic placement, *Sepsis nigripes* is the difficult case while *S. luteipes* is straightforward. The case is reversed with regard to species limits. *Sepsis nigripes* has subtle, discrete, and fixed morphological differences that distinguish it from all other species in the *flavimana* group (Figure 2a-d). In addition, the DNA barcode for *S. nigripes* differs by >4.8% from all other species that have been discussed as potential synonyms. Fortunately, all these species are sympatric or parapatric with *S. nigripes* so that it is straightforward to decide on species status. The discrete character differences in sympatry make it unlikely that it hybridizes with sympatric congeners; i.e., *S. nigripes* behaves like a reproductively isolated species *sensu* the Biological (Mayr 2000) and Hennigian Species Concept (Meier and Willmann 2000). It has its own independent evolutionary fate as required by the Evolutionary Species Concept (Wiley and Mayden 2000), and it is “diagnosable by a unique combination of character states” so that it is a good species under the Phylogenetic Species Concept *sensu* Wheeler and Platnick (Wheeler and Platnick 2000). As is often the case for rare species (Lim *et al.* 2012), *S. nigripes* is a singleton-species in our analysis because we only have one sequence. Therefore, “monophyly” cannot be tested, but the species is at least not nested within any of the other recognized species with multiple sequences so that there is no reason to reject *S. nigripes* as a phylogenetic species *sensu* Mishler and Theriot (Mishler and Theriot 2000). Overall, all morphological and molecular data presented here support its taxonomic status as a separate species under all species concepts discussed here.

Sepsis nigripes can thus here be added to the Swiss and Alpine fauna of Sepsidae. According to Haenni (1997), all old records of *S. nigripes* in Switzerland were based on misidentifications and the species was therefore not included in the Diptera checklist of Switzerland (Haenni 1998; Merz *et al.* 2001). Only recently, the species was unambiguously identified as belonging to the Swiss fauna based on specimens collected in the vicinity of Geneva, Switzerland, which lies very close to the French border ((Merz 2012); morphological identification verified by Andrey Ozerov). This means that our records are the second for the country, and the first in the higher Alps.

The species boundaries are much less clear for the European and North American populations of *Sepsis luteipes*. The genetic distances for *COI* barcodes are large (3.5-3.9%). Given that

there is no evidence for a widespread role of *COI* in speciation (Kwong *et al.* 2012), such large distances are only *prima facie* evidence for a long time of separation between the populations. Whether it is also evidence for different species depends on the species concept. Proponents of species concepts based on reproductive isolation would be wary of splitting the species because there is a lack of morphological differentiation between the populations, and the barcode distances are consistent with either intra- or interspecific differentiation within sepsids (Puniamoorthy *et al.* 2010; Puniamoorthy *et al.* 2012). Proponents of Evolutionary and Phylogenetic Species Concepts would be more likely to accept that the populations are different species. For example, it can be argued that the populations have their “own independent evolutionary fate and historical tendencies” based on allopatry (Evolutionary Species Concept; Wiley and Mayden, 2000), and that the populations are “diagnosable by a unique combination of character states” (Wheeler and Platnick 2000; see *COI* sequence differences). Proponents of these species concepts would therefore likely resurrect *Sepsis helvetica* from synonymy and add the species to the Swiss fauna. With regard to concepts based on monophyly, we again lack sufficient evidence due to the availability of sequences for only few specimens (Lim *et al.* 2012). Given that we are proponents of species concepts based on reproductive isolation, we prefer to keep the status quo and consider *S. luteipes* as one species until more information regarding reproductive isolation becomes available. However, the two populations can already be regarded as two evolutionarily significant units (Moritz 1994).

Ecology of *Sepsis*

The ecology of sepsid flies remains poorly studied, even though they can be abundant on oviposition sites such as animal dung (Pont and Meier 2002), carcasses (Heo *et al.* 2008), and adult feeding sites such as plants and bushes (Eberhard 1999). The realized niches and ecological functions of the various species within the dung community remain largely unknown (Hammer 1941). All 12 *Sepsis* species (including *S. nigripes*) listed in Table 1 were sympatric on the same alpine cow pasture, and various species were even found on the same cow pat. Unfortunately, there is only limited knowledge about how the different species avoid competition and to what extent niche separation plays a role in speciation. Clearly, there are large differences in abundance among *Sepsis* species on cow pastures. Our observations agree with previous data indicating that *S. cynipsea* is by far the most common species in north-central Europe (Blanckenhorn 1999; Blanckenhorn *et al.* 2000; Parker 1972). Unfortunately, very little is known about what causes this dominance and what factors contribute to the persistence of rarer species.

Conclusion

We here use molecular data to validate the species status of *Sepsis nigripes* under all species concepts while it remains unclear whether *Sepsis luteipes* as currently understood constitutes two different species. In addition, we report unexpectedly high sepsid diversity in a single Swiss habitat, which once more illustrates how little we know about the ecological niches that are occupied by sepsids.

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Fig. 1. Phylogenetic relationships within *Sepsis*: MP (Left) and ML (Right); node values = bootstrap; ML=partitioned analysis above node; one-partition analysis below node; "--" conflicting.

Fig. 2. Morphological differentiation between *Sepsis biflexuosa*, *Sepsis nigripes*, *Sepsis flavimana*, *Sepsis pyrrhosoma* and two intercontinental populations of *Sepsis luteipes*:

- a) *Sepsis biflexuosa* Strobl, 1893: Holarctic distribution. Cuticular protrusion on fore tibia very pronounced, with narrowest part of fore tibia (black arrow) more distal than in *S. flavimana*.
- b) *Sepsis nigripes* Meigen, 1826: Palearctic distribution. Fore femur mostly darkened, with two ventral rows of spines; surstylus with blunt apex. (new specimen from Lenzerheide)
- c) *Sepsis flavimana* Meigen, 1826: Palearctic distribution. Fore femur with one (postero-ventral) row of spines, darkened dorsally; fore tibial cuticular protrusion very slight; surstylus short and beak-like.
- d) *Sepsis pyrrhosoma* Melander & Spuler, 1917: Holarctic distribution. Postgena, lower occiput, pleural thorax (except for band on dorsal katepistenum) and forelegs yellowish-red; abdominal segments also lighter colored than in *S. flavimana*; fore tibial ventral spines weak; yellowish hypopygium; surstylus with medial tooth.
- e) *Sepsis luteipes* Melander & Spuler, 1917 *CH*: Head slightly larger in proportion to thorax than USA specimens; rear tibia darker than USA specimens; medial teeth on surstyli more pronounced than in USA specimens.
- f) *Sepsis luteipes* Melander & Spuler, 1917 *USA*: Head slightly smaller in proportion to thorax than CH specimens; rear tibia lighter in color medially than CH specimens; medial teeth on surstylus more diminutive than in CH specimens.

Table 1: Species distribution of one point sample from Lenzerheide, Switzerland (46.73°N, 9.56° E; 1500 m.a.s.l.), collected in July 2013.

Genus: <i>Sepsis</i> Fallén, 1810	Number of specimen		
Species	male	female	total
<i>S. cynipsea</i> , (Linnaeus 1758)	72	22	94
<i>S. violacea</i> , Meigen 1826	28	25	53
<i>S. duplicata</i> , Haliday 1838	25	1	26
<i>S. thoracica</i> , (Robineau-Desvoidy 1830)	9	17	26
<i>S. fulgens</i> , Meigen 1826	5	5	10
<i>S. flavimana</i> , Meigen 1826	4	2	6
<i>S. biflexuosa</i> , Strobl 1893	3	2	5
<i>S. nigripes</i> , Meigen 1826	5		5
<i>S. luteipes</i> , Melander & Spuler 1917		2	2
<i>S. orthocnemis</i> , Frey 1908		2	2
<i>S. neocynipsea</i> , Melander & Spuler 1917	1		1
<i>S. punctum</i> , (Fabricius 1794)		1	1
	total:		231

References

- Ang, Y., Puniamoorthy, J., Pont, A.C., Bartak, M., Blanckenhorn, W.U., Eberhard, W.G., Puniamoorthy, N., Silva, V.C., Munari, L., and Meier, R. (2013) A plea for digital reference collections and other science-based digitization initiatives in taxonomy: Sepsidnet as exemplar. *Systematic Entomology* **38**(3), 637-644.
- Becker, T. (1902) Die Meigen'schen Typen der sogen. Muscidae acalypterae (Muscaria holometopa) in Paris und Wien. *Z. syst. Hymenopt. Dipterol.* **2**(4), 209-256.
- Blanckenhorn, W.U. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology* **13**(4), 395-409.
- Blanckenhorn, W.U., Muhlhauser, C., Morf, C., Reusch, T., and Reuter, M. (2000) Female choice, female reluctance to mate and sexual selection on body size in the dung fly *Sepsis cynipsea*. *Ethology* **106**(7), 577-593.
- Blanckenhorn, W.U., Puniamoorthy, N., Schafer, M.A., Scheffczyk, A., and Rombke, J. (2013a) Standardized laboratory tests with 21 species of temperate and tropical sepsid flies confirm their suitability as bioassays of pharmaceutical residues (ivermectin) in cattle dung. *Ecotoxicol Environ Saf* **89**, 21-8.

Blanckenhorn, W.U., Puniamoorthy, N., Scheffczyk, A., and Rombke, J. (2013b) Evaluation of ecotoxicological effects of the parasiticide moxidectin in comparison to ivermectin in 11 species of dung flies. *Ecotoxicol Environ Saf* **89**, 15-20.

Bowsher, J.H., Ang, Y., Ferderer, T., and Meier, R. (2013) Deciphering the evolutionary history and developmental mechanisms of a complex sexual ornament: the abdominal appendages of Sepsidae (Diptera). *Evolution* **67**(4), 1069-80.

Bowsher, J.H., and Nijhout, H.F. (2007) Evolution of novel abdominal appendages in a sepsid fly from histoblasts, not imaginal discs. *Evolution & Development* **9**(4), 347-354.

Collin, J.E. (1910) Additions and corrections to the British list of Muscidae Acalyptratae. *Entomologist's mon. Mag.* **46**[= **(2) 21**], 173-178.

Duda, O. (1926) Monographie der Sepsiden (Dipt.). I. *Annln naturh. Mus. Wien* **39** [**1925**], 1-153.

Eberhard, W.G. (1999) Mating systems of sepsid flies and sexual behavior away from oviposition sites by *Sepsis neocynipsea* (Diptera : Sepsidae). *Journal of the Kansas Entomological Society* **72**(1), 129-130.

Eberhard, W.G. (2001) Species-specific genitalic copulatory courtship in sepsid flies (Diptera, Sepsidae, Microsepsis) and theories of genitalic evolution. *Evolution* **55**(1), 93-102.

Frey, R. (1917) Entomologiska anteckningar fran norra Savolaks. *Meddelanden af Societas pro Fauna et Flora Fennica* **43**, 84-97.

Frey, R. (1925) Zur Systematik der Diptera Haplostomata. (II. Fam. Sepsidae). *Not. Ent.* **5**, 69-76.

Goloboff, P.A., Farris, J.S., and Nixon, K.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics* **24**(5), 774-786.

Haenni, J.-P. (1997) Sepsidae (Diptera) nouveaux pour la faune de Suisse. *Bulletin Romand d'Entomologie* **15**, 69-78.

Haenni, J.-P. (1998) Sepsidae. In 'Diptera - Checklist. Fauna Helvetica. Vol. 1.' (Eds. B Merz, G Baechli, J-P Haenni and Y Gonseth) pp. 249-250. (Schweizerische Entomologische Gesellschaft: Neuchâtel)

Hammer, O. (1941) Biological and ecological investigations on flies associated with pasturing cattle and their excrement. *Vidensk. Medd. Dansk Naturhist. Foren.* **105**, 141-394.

Hare, E.E., Peterson, B.K., Iyer, V.N., Meier, R., and Eisen, M.B. (2008) Sepsid even-skipped enhancers are functionally conserved in *Drosophila* despite lack of sequence conservation. *PLoS Genet* **4**(6), e1000106.

Hennig, W. (1949) 39a. Sepsidae. In 'Fliegen palaearkt. Reg.'. (Ed. E Lindner) pp. 1-92. (Schweizerbart: Stuttgart)

Heo, C.C., Mohamad, A.M., Ahmad, F.M., Jeffery, J., Kurahashi, H., and Omar, B. (2008) Study of insect succession and rate of decomposition on a partially burned pig carcass in an oil palm plantation in Malaysia. *Trop Biomed* **25**(3), 202-8.

Ingram, K.K., Laamanen, T., Puniamoorthy, N., and Meier, R. (2008) Lack of morphological coevolution between male forelegs and female wings in *Themira* (Sepsidae : Diptera : Insecta). *Biological Journal of the Linnean Society* **93**(2), 227-238.

Iwasa, M. (1985) Supplementary Notes on the Sepsidae from Japan (Diptera). *Kontyu* **53**(4), 632-638.

Iwasa, M. (1995) Revisional Notes on the Japanese Sepsidae (Diptera). *Jpn. J. Ent.* **63**(4), 781-797.

Katoh, K., Kuma, K., Toh, H., and Miyata, T. (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* **33**(2), 511-8.

Kwong, S., Srivathsan, A., Vaidya, G., and Meier, R. (2012) Is the COI barcoding gene involved in speciation through intergenomic conflict? *Mol Phylogenet Evol* **62**(3), 1009-12.

Laamanen, T.R., Meier, R., Miller, M.A., Hille, A., and Wiegmann, B.M. (2005) Phylogenetic analysis of *Themira* (Sepsidae : Diptera): sensitivity analysis, alignment, and indel treatment in a multigene study. *Cladistics* **21**(3), 258-271.

Laamanen, T.R., Petersen, F.T., and Meier, R. (2003) Kelp flies and species concepts - the case of *Coelopa frigida* (Fabricius, 1805) and *C-nebularum* Aldrich, 1929 (Diptera : Coelopidae). *Journal of Zoological Systematics and Evolutionary Research* **41**(2), 127-136.

Lim, G.S., Balke, M., and Meier, R. (2012) Determining species boundaries in a world full of rarity: singletons, species delimitation methods. *Syst Biol* **61**(1), 165-9.

Martin, O.Y., and Hosken, D.J. (2003) Costs and benefits of evolving under experimentally enforced polyandry or monogamy. *Evolution* **57**(12), 2765-72.

Martin, O.Y., Leugger, R.R., Zeltner, N., and Hosken, D.J. (2003) Male age, mating probability and mating costs in the fly *Sepsis cynipsea*. *Evolutionary Ecology Research* **5**(1), 119-129.

Mayr, E. (2000) The Biological Species Concept. In 'Species Concepts and Phylogenetic Theory: a Debate.' (Eds. QD Wheeler and R Meier) pp. 17-29. (Columbia University Press: New York)

Meier, R. (1996) Larval morphology of the Sepsidae (Diptera: Sciomyzoidea), with a cladistic analysis using adult and larval characters. *Bulletin of the American Museum of Natural History* (228), 3-147.

Meier, R., Shiyang, K., Vaidya, G., and Ng, P.K. (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Syst Biol* **55**(5), 715-28.

Meier, R., and Willmann, R. (2000) The Hennigian species concept. In 'Species Concepts and Phylogenetic Theory: a Debate.' (Eds. QD Wheeler and R Meier) pp. 30-43. (Columbia University Press: New York)

Meier, R., Zhang, G., and Ali, F. (2008) The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification. *Syst Biol* **57**(5), 809-13.

Merz, B. (2012) 'Liste annotée des insectes (Insecta) du canton de Genève ' (Muséum d'histoire naturelle: Genève) 532

Merz, B., Bächli, G., and Haenni, J.-P. (2001) Erster Nachtrag zur Checkliste der Diptera der Schweiz. *Mitteilungen der Entomologischen Gesellschaft Basel* **51**, 110-140.

Miller, M.A., Pfeiffer, W., and Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In 'Proceedings of the Gateway Computing Environments Workshop (GCE)', 14 November 2010, New Orleans,

Mishler, B., and Theriot, E. (2000) The Phylogenetic Species Concept sensu Mishler and Theriot: Monophyly, Apomorphy, and Phylogenetic Species Concepts. In 'Species Concepts and Phylogenetic Theory: a Debate.' (Eds. QD Wheeler and R Meier) pp. 44-54. (Columbia University Press: New York)

Moritz, C. (1994) Defining Evolutionarily-Significant-Units for Conservation. *Trends in Ecology & Evolution* **9**(10), 373-375.

Munari, L. (1985) Una nuova specie di Sepsis del gruppo 'punctum' (Diptera, Sepsidae). *Notulae Sepsidologicae* 9. *Societa Veneziana di Scienze Naturali Lavori* **10**, 51-57.

Ozerov, A.L. (1999) 88. Fam. Sepsidae. In 'Key to the insects of the Russian Far East. 6, Diptera and Siphonaptera, part 1.' (Ed. LP Ler) pp. 556-570. (Dal'nauka: Vladivostok)

Ozerov, A.L. (2005) World catalogue of the family Sepsidae (Insecta: Diptera). *Zoologicheskie issledovania (Zoological Studies)* **8**, 1-74.

Papp, L. (2007) A study of the cow pat Diptera on the Hortobágy, Hungary. *Folia ent. hung.* **68**, 123-135.

Parker, G.A. (1972) Reproductive Behavior of *Sepsis-Cynipsea* (L) (Diptera-Sepsidae) .1. Preliminary Analysis of Reproductive Strategy and Its Associated Behavior Patterns. *Behaviour* **41**, 172-&.

Pont, A.C. (1979) Sepsidae. Diptera Cyclorrhapha, Acalyptrata. *Handbk Ident. Br. Insects* **10**(5), 1-35.

Pont, A.C., and Meier, R. (2002) The Sepsidae (Diptera) of Europe. *Fauna Entomologica Scandinavica* **37**, 1-221.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Mol Biol Evol* **25**(7), 1253-6.

Puniamoorthy, N., Ismail, M.R., Tan, D.S., and Meier, R. (2009) From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae). *J Evol Biol* **22**(11), 2146-56.

Puniamoorthy, N., Kotrba, M., and Meier, R. (2010) Unlocking the "Black box": internal female genitalia in Sepsidae (Diptera) evolve fast and are species-specific. *Bmc Evolutionary Biology* **10**.

Puniamoorthy, N., Schafer, M.A., and Blanckenhorn, W.U. (2012) Sexual selection accounts for the geographic reversal of sexual size dimorphism in the dung fly, *sepsis punctum* (Diptera: Sepsidae). *Evolution* **66**(7), 2117-26.

Puniamoorthy, N., Su, K.F., and Meier, R. (2008) Bending for love: losses and gains of sexual dimorphisms are strictly correlated with changes in the mounting position of sepsid flies (Sepsidae: Diptera). *BMC Evol Biol* **8**, 155.

Shahjahan, R.M., Hughes, K.J., Leopold, R.A., and DeVault, J.D. (1995) Lower incubation temperature increases yield of insect genomic DNA isolated by the CTAB method. *Biotechniques* **19**(3), 332-4.

Srivathsan, A., and Meier, R. (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. *Cladistics* **28**(2), 190-194.

Su, K.F.Y., Kutty, S.N., and Meier, R. (2008) Morphology versus molecules: the phylogenetic relationships of Sepsidae (Diptera: Cyclorrhapha) based on morphology and DNA sequence data from ten genes. *Cladistics* **24**(6), 902-916.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**(10), 2731-9.

Tan, D.S.H., Ang, Y., Lim, G.S., Ismail, M.R.B., and Meier, R. (2010) From 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection of *Sepsis pyrrhosoma* (Sepsidae: Diptera). *Zoologica Scripta* **39**(1), 51-61.

Tan, D.S.H., Ng, S.R., and Meier, R. (2011) New information on the evolution of mating behaviour in Sepsidae (Diptera) and the cost of male copulations in *Saltella sphondylii*. *Organisms Diversity & Evolution* **11**(4), 253-261.

Teuschl, Y., and Blanckenhorn, W. (2007) The reluctant fly: what makes *Sepsis cynipsea* females willing to copulate? *Animal Behaviour* **73**(1), 85-97.

Vaidya, G., Lohman, D.J., and Meier, R. (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27**(2), 171-180.

Wheeler, Q.D., and Platnick, N.I. (2000) The Phylogenetic Species Concept sensu Wheeler and Platnick. In 'Species Concepts and Phylogenetic Theory: a Debate.' (Eds. QD Wheeler and R Meier) pp. 55-69. (Columbia University Press: New York)

Wiley, E.O., and Mayden, R.L. (2000) The Evolutionary Species Concept. In 'Species Concepts and Phylogenetic Theory: a Debate.' (Eds. QD Wheeler and R Meier) pp. 70-89. (Columbia University Press: New York)

Zhao, L., Annie, A.S., Amrita, S., Yi, S.K., and Rudolf, M. (2013) Does better taxon sampling help? A new phylogenetic hypothesis for Sepsidae (Diptera: Cyclorrhapha) based on 50 new taxa and the same old mitochondrial and nuclear markers. *Mol Phylogenet Evol* **69**(1), 153-64.

Zuska, J. (1970) Zoogeographic aspects of the European fauna of the family Sepsidae (Diptera). *Polskie Pismo ent.* **40**(3), 605-610.

Zwickl, D.J. (2006) Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. The University of Texas at Austin, Austin