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 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 species of *Sepsis* occur sympatrically on the same pasture.

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 (Pont and Meier 2002; Martin *et al*. 2003; Ingram *et al*. 2008). Over the past few years, species of Sepsidae have become models in behavioural ecology, sexual selection and speciation studies (Blanckenhorn 1999; Blanckenhorn *et al*. 2000; Eberhard 2001; Martin and Hosken 2003; Teuschl and Blanckenhorn 2007; Puniamoorthy *et al*. 2008, 2009, 2010, 2012; Tan *et al*. 2011), in eco-toxicological surveys (Blanckenhorn *et al*. 2013a,

2013b), and in developmental research (Bowsher and Nijhout 2007; Hare *et al*. 2008; Bowsher *et al*. 2013). This popularity has led to the establishment of a 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 (Meier 1996; Laamanen *et al*. 2005; 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 synonymised *S. nigripes* with other species (Collin 1910; Zuska 1970; Pont 1979), other authors disagreed and insisted on its taxonomic validity (Becker 1902; Duda 1926; Hennig 1949; Pont and Meier 2002). However, no molecular evidence was 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 has not been studied with molecular data and belongs to a morphologically plastic species complex is *Sepsis luteipes* Melander & Spuler, 1917. It was initially 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 synonymised 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.

Materials 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 whereas 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* (Robineau-Desvoidy, 1830) 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. The total numbers of specimens were: *Sepsis nigripes*: 1 specimen; *S. luteipes* (Switzerland): 4 specimens; *S. luteipes* (USA): 3 specimens; *S. thoracica* (South Africa): 1 specimen.

Specimen morphology and imaging

Male specimens were digitally imaged before 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 species. All specimens were imaged using the Visionary Digital™ BK Plus Laboratory System, and then focus-stacked using Helicon Focus™ Pro (ver. 5.2.16) and digitally cleaned using Photoshop® to remove image background 'noise' and optimise brightness and contrast. Specimen figures are shown in this paper, and also deposited in the digital reference collection 'Sepsidnet' (http://sepsidnet-rmbr.nus.edu.sg/).

DNA extraction and sequencing

The molecular dataset consists of the available sequences 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 (Table 1). Genomic DNA was extracted using a CTAB protocol with minor modifications of Shahjahan's protocol (Shahjahan *et al.* 1995). Sequences for ten gene regions included here were obtained using the PCR and sequencing protocols described in Su *et al.* (2008): the nuclear protein-coding genes Alanyl-tRNA synthetase (AATS), Histone H3 (H3) and Elongation factor-1α (EF1α), 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 487 bp 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/; Katoh *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 addsEqns 5 and by finding the minimum length 10 times. Five hundred nonparametric bootstrap replicates with the same settings were analysed 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

Table 1. List of all taxa and genes in current study

New additions are marked with an asterisk. The remaining taxa stem from Zhao et al. (2013). Outgroups are indicated with '†'

Taxon	Author name	Locality	12S	16S	COII	COI	CYTB	18S	28S	AATS	Ef1a	H3
Allosepsis sp.1†	—	Malaysia. Malacca	EU435559	EU435607	EU435886	EU435811	EU435947	EU435667	EU435742	EU436065	EU436011	EU436115
Dicranosepsis distincta†	Iwasa & Tewari, 1990	Asian	EU435531	EU435584	EU435859	EU435784	EU435917	EU435637	EU435710	EU436041	EU435984	EU436094
Sepsis arotrolabis	Duda, 1926	South Africa	KF199480	KF199526	KF199669	KF199844	KF199716	KF199573	KF199620	KF199797	(N/A)	KF199741
Sepsis biflexuosa	Strobl, 1893	Turkey	EU435551	EU435601	EU435878	EU435804	EU435939	EU435660	EU435734	EU436060	(N/A)	(N/A)
Sepsis coprophila	de Meijere, 1906	China	EU435552	(N/A)	EU435879	EU435830	EU435940	(N/A)	EU435735	EU436061	EU436004	(N/A)
Sepsis cynipsea	(Linnaeus, 1758)	Switzerland	EU435553	EU435602	EU435880	EU435805	EU435941	EU435661	EU435736	(N/A)	EU436005	EU436111
Sepsis dissimilis	Brunetti, 1909	Egypt	EU435554	EU435603	EU435881	EU435806	EU435942	EU435662	EU435737	(N/A)	EU436006	(N/A)
Sepsis duplicata	Haliday, 1838	Denmark	EU435555	EU435604	EU435882	EU435807	EU435943	EU435663	EU435738	EU436062	EU436007	EU436112
Sepsis fissa	Becker, 1903	Turkey	EU435556	EU435605	EU435883	EU435808	EU435944	EU435664	EU435739	(N/A)	EU436008	(N/A)
Sepsis flavimana	Meigen, 1826	Germany	EU435557	(N/A)	EU435884	EU435809	EU435945	EU435665	EU435740	EU436063	EU436009	EU436113
Sepsis frontalis	(Walker, 1860)	Asian	EU435525	EU435579	EU435853	EU435778	EU435911	EU435631	EU435704	EU436035	EU435978	EU436090
Sepsis fulgens	Meigen, 1826	Germany	EU435558	EU435606	EU435885	EU435810	EU435946	EU435666	EU435741	EU436064	EU436010	EU436114
Sepsis hirsuta	(de Meijere, 1906)	Australia	EU435538	EU435591	EU435866	EU435791	(N/A)	EU435644	EU435717	EU436048	EU435991	(N/A)
Sepsis lateralis	Wiedemann, 1830	Indonesia	EU435560	EU435608	EU435887	EU435812	EU435948	EU435668	EU435743	EU436066	EU436012	EU436116
Sepsis luteipes*	Melander & Spuler, 1917	Switzerland	KJ766337	KJ766341	KJ766325	KJ766321	KJ766329	KJ766313	KJ766317	(N/A)	KJ766333	(N/A)
Sepsis luteipes*	Melander & Spuler, 1917	Switzerland				KM102542 KM102543 KM102544						
Sepsis luteipes*	Melander & Spuler, 1917	USA	KJ766338	KJ766342	KJ766326	KJ766322	KJ766330	KJ766314	KJ766318	(N/A)	KJ766334	(N/A)
Sepsis luteipes*	Melander & Spuler, 1917	USA				KM102545 KM102546						
Sepsis monostigma	Thomson, 1869	China	EU435561	EU435609	EU435888	EU435813	EU435949	EU435669	EU435744	(N/A)	EU436013	(N/A)
Sepsis neglecta	Ozerov, 1986	USA	EU435562	EU435610	EU435889	EU435814	EU435950	EU435670	EU435745	(N/A)	EU436014	(N/A)
Sepsis neocynipsea	Melander & Spuler, 1917	USA	EU435563	EU435611	EU435890	EU435815	EU435951	EU435671	EU435746	EU436067	EU436015	EU436117
Sepsis nigripes*	Meigen, 1826	Switzerland	KJ766339	KJ766343	KJ766327	KJ766323	KJ766331	KJ766315	KJ766319	(N/A)	(N/A)	KJ766336
Sepsis nitens	Wiedemann, 1824	Pakistan	EU435564	EU435612	EU435891	EU435816	EU435952	EU435672	EU435747	(N/A)	EU436016	EU436118
Sepsis niveipennis	(Becker, 1903)	African	KF199481	KF199527	KF199670	KF199845	KF199717	KF199574	KF199621	KF199798	KF199765	(N/A)
Sepsis niveipennis	(Becker, 1903)	Asian	EU435526	EU435580	EU435854	EU435779	EU435912	EU435632	EU435705	EU436036	EU435979	EU436091
Sepsis orthocnemis	Frey, 1908	Denmark	EU435565	EU435613	EU435892	EU435817	EU435953	EU435673	EU435748	EU436068	EU436017	EU436119
Sepsis punctum	(Fabricius, 1794)	USA	EU435566	EU435614	EU435893	EU435831	EU435954	EU435674	EU435749	EU436069	EU436018	EU436120
Sepsis pyrrhosoma	Melander & Spuler, 1917	USA	KF199482	KF199528	(N/A)	KF199846	KF199718	(N/A)	KF199622	KF199799	KF199766	(N/A)
Sepsis secunda	Melander & Spuler, 1917	USA	EU435567	EU435615	EU435894	EU435818	EU435955	EU435675	EU435750	(N/A)	EU436019	EU436121
Sepsis sepsi	Ozerov, 2003	Vietnam	KF199483	KF199529	KF199671	KF199847	KF199719	KF199575	KF199623	(N/A)	KF199767	(N/A)
Sepsis sp. (Isangi)	—	Congo DRC	KF199484	KF199530	KF199672	KF199848	KF199720	KF199576	KF199624	KF199800	(N/A)	(N/A)
Sepsis thoracica	(Robineau-Desvoidy, 1830)	Germany	EU435568	EU435616	EU435895	EU435819	EU435956	EU435676	EU435751	EU436070	EU436020	EU436122
Sepsis thoracica*	(Robineau-Desvoidy, 1830)	South Africa	KJ766340	KJ766344	KJ766328	KJ766324	KJ766332	KJ766316	KJ766320	(N/A)	KJ766335	(N/A)
Sepsis violacea	Meigen, 1826	Switzerland	EU435569	EU435617	EU435896	EU435820	EU435957	EU435677	EU435752	EU436071	EU436021	EU436123

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 taxa for most of the nine gene regions, which were submitted to GenBank (Table 1). The smallest COI distance 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). The parsimony analysis found one tree (5365 steps) while the Garli scores for the partitioned analysis were –33833.9562 and –37038.4688 for the unpartitioned

analysis. On the phylogenetic tree (Fig. 1 and Supplementary Figs S1–S3), *S. nigripes* is sistergroup to *S. flavimana*. However, this hypothesis has 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* (8% of MP bootstrap replicates; 26% of $ML_{1\ partition}$ replicates; 17% $ML_{10\ partitions}$ replicates), or all three species are related as specified here: (*S. flavimana* (*S. nigripes* (*S. pyrrhosoma* (*S. biflexuosa*, (*S. duplicata*, *S. secunda*))))) (20% of MP replicates; 9% of $ML_{1\ partition}$ replicates; 6% $ML_{10\ partitions}$ replicates). The uncorrected, pairwise distances between the two populations of *Sepsis luteipes* are also large (3.5–3.9%) and more compatible with interspecific than intraspecific variability. Intraspecific variability is low within the North American population (<0.48% for three specimens) and the Swiss population (<0.14% for three specimens). The populations are well supported as sistergroups and nested within Hennig's *S. punctum* species group (including *S. orthocnemis*, see Fig. 1 for detail). *Sepsis orthocnemis* lacks the femoral tubercle that is otherwise the distinguishing feature of the species group, but its close relationship to the *S. punctum* species group was already identified in Su *et al.* (2008).

Morphological differentiation

The sepsid species that are morphologically most similar to *S. nigripes* are *S. pyrrhosoma, S. flavimana*, and *S. biflexuosa*, of which the latter two occur in Europe in sympatry with *S. nigripes* (Table 2). As discussed in Pont and Meier (2002), *S. flavimana, S. biflexuosa* and *S. nigripes* can be distinguished
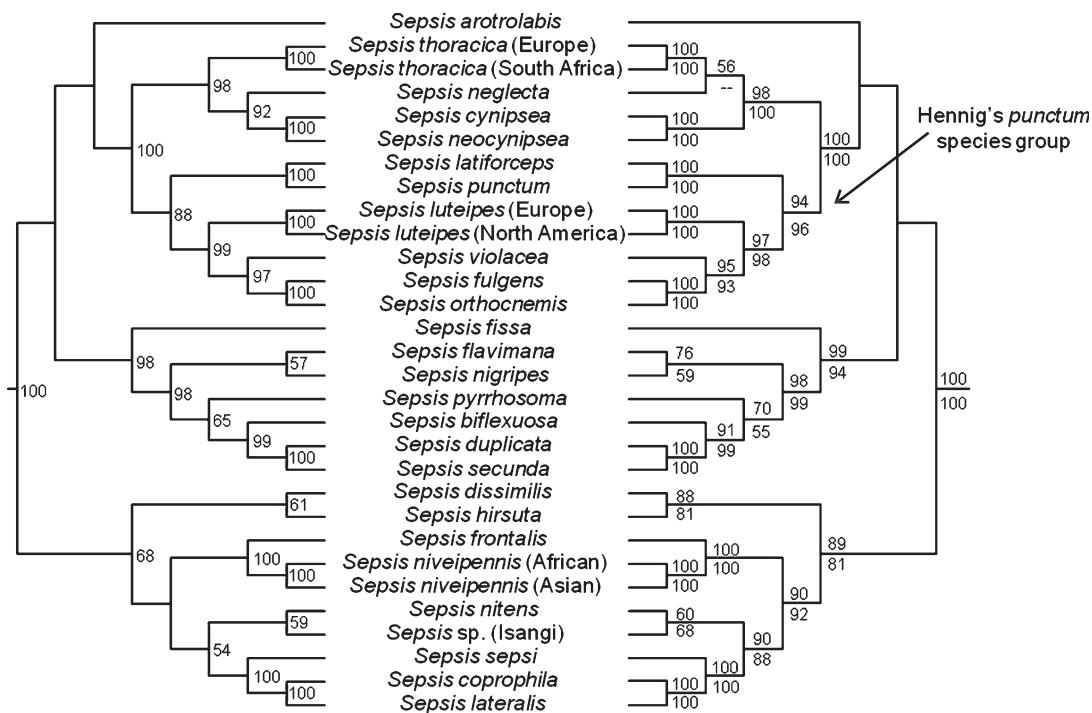
Fig. 1. Phylogenetic relationships within *Sepsis*: MP (Left) and ML (Right); node values = bootstrap; ML = partitioned analysis above node, one-partition analysis below node, '–' conflicting; no value = bootstrap value <50, see Supplementary Figs S1–S3 for details.
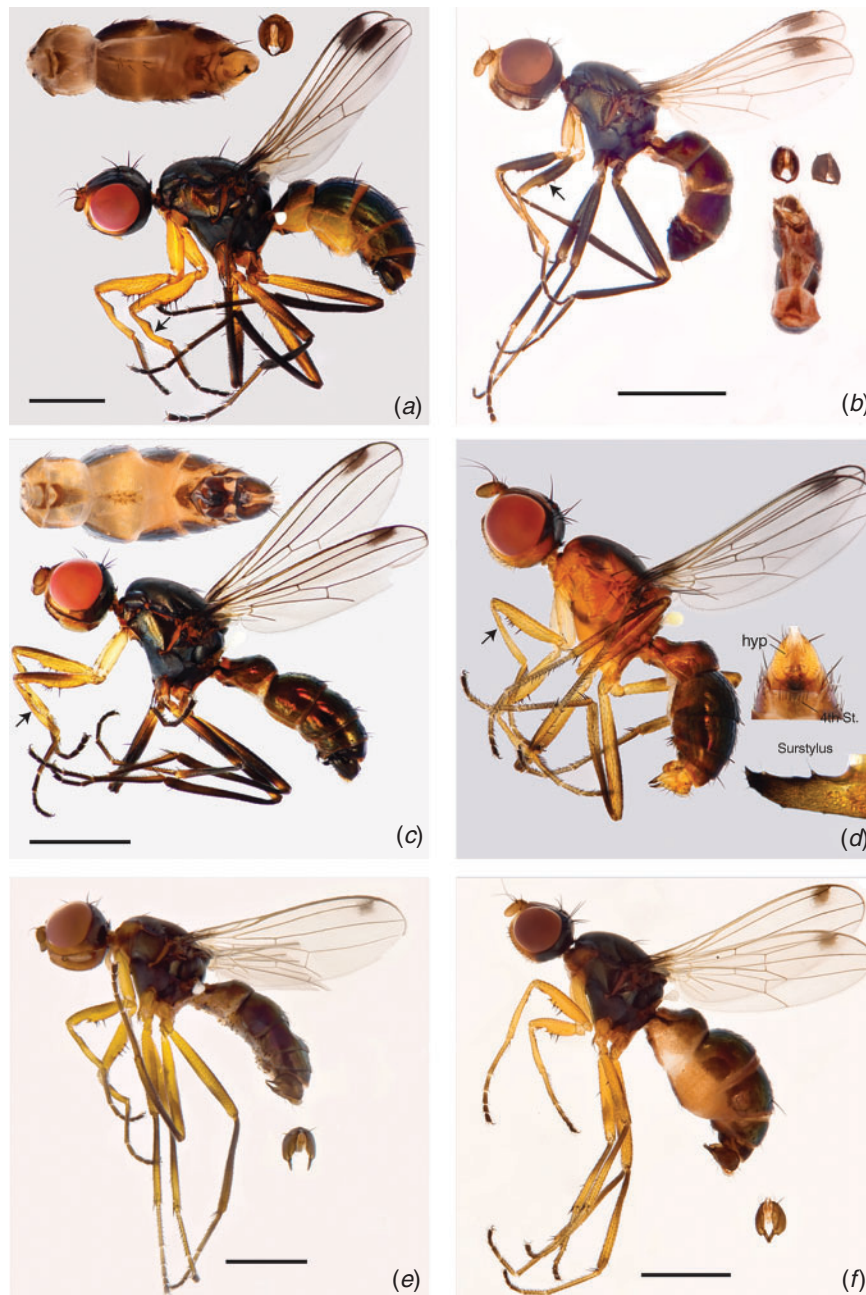
Fig. 2. Morphological differentiation between *Sepsis biflexuosa*, *Sepsis nigripes*, *Sepsis flavimana*, *Sepsis pyrrhosoma* and two intercontinental populations of *Sepsis luteipes*. (*a*) *Sepsis biflexuosa* Strobl, 1893: Holarctic distribution. Cuticular protrusion on fore tibia very pronounced, 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 (posterio-ventral) row of spines, darkened dorsally; fore tibial cuticular protrusion very slight; surstylus short and beak-like. (*d*) *Sepsis pyrrhosoma* Melander & Spuler, 1917: Nearctic distribution. Postgena, lower occiput, pleural thorax (except for band on dorsal katepistenum) and forelegs yellowish-red; abdominal segments also lighter coloured 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 colour medially than CH specimens; medial teeth on surstylus more diminutive than in CH specimens.

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

by their male foreleg armature and genital structures, whereas 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. 2*b*). In contrast, emarginations in *S. biflexuosa* are typically more distal (Fig. 2*a*), and *S. flavimana* has a shallow notch in the proximal half of the fore tibiae, similar to *S. nigripes*, but much stronger (Fig. 2*c*). In addition, *S. 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 sometimes 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 those of *S. biflexuosa* are more slender and slightly more elongated (see Fig. 2 and Pont and Meier 2002).

Biodiversity of Sepsis *in 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 2), while most species, including *S. luteipes*, *S. nigripes*, and its sister species *S. flavimana*, were fairly rare.

Discussion

All three phylogenetic analyses carried out using MP and ML analyses with different partitioning strategies obtained largely congruent and well supported relationships for most nodes. Conflict between MP and ML trees concerned clades with low support. Our phylogenetic analyses of the new sequence data place *S. nigripes* within the *flavimana*-group and *S. luteipes* in

the *S. punctum* species group. These placements are congruent with earlier taxonomic work based on morphology (Frey 1925; Iwasa 1985; Pont and Meier 2002). However, in those publications, 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, behaviour, 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 and 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 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 *S. flavimana* species 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* or a 'ladderised' *S. flavimana* and *S. nigripes* forming subsequent branches sister to the *S. flavimana* species group. 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 a difficult case while *S. luteipes* is more 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 *S. flavimana* species group (Fig. 2*a*–*d*). In addition, the COI 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*. These discrete morphological and molecular character differences in sympatry make it unlikely that it hybridises with sympatric congeners; i.e. *S. nigripes* behaves like a reproductively isolated species *sensu* the Biological (Mayr 2000) and Hennigian Species Concept *sensu* 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), we only have specimens from one collecting event (Table 2). Therefore, 'monophyly' cannot be tested rigorously but the species is, at least, not nested within any of the other recognised species with data for multiple populations 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 mentioned.

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, 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 probably resurrect *Sepsis helvetica* from synonymy for the European population, and add species to the Swiss fauna. With regard to concepts based on monophyly, we again have little evidence due to the availability of sequences for only one population (Lim *et al*. 2012), but the presence of reciprocal monophyly would likely lead to the provisional acceptance of two species by some authors. 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 realised 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 2 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 (Parker 1972; Blanckenhorn 1999; Blanckenhorn *et al*. 2000). 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.

Acknowledgements

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