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Systematics of Pipizini and taxonomy of European *Pipiza* Fallén: molecular and morphological evidence (Diptera, Syrphidae)

ANTE VUJIĆ, GUNILLA STÅHLS, JELENA AČANSKI, HANS BARTSCH†, RUNE BYGEBJERG & ANA STEFANOVIĆ

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In the present work the monophyly and molecular phylogenetic relationships of the genera of tribe Pipizini (Syrphidae) were investigated based on mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 28S rDNA sequences, and the relationships among species of genus *Pipiza* Fallén, 1810 based on mtDNA COI sequences. Molecular phylogenetic analyses of Pipizini supported *Pipiza* as monophyletic and as sister group to all other Pipizini, and resolved other Pipizini genera as monophyletic lineages except for genus *Heringia* Rondani, 1856. To recognize the distinctness and maintain the monophyly the genus *Heringia* was redefined, generic rank was assigned to *Neocnemodon* Goffe, 1944 stat. n., and the genus *Claussenia* Vujić & Ståhls gen. n., type-species *Claussenia hispanica* (Strobl, 1909), was described. A revision of the European *Pipiza* species, including a discussion of taxonomic characters and a morphological redefinition of all included species, is presented. One new species, *Pipiza laurusi* Vujić & Ståhls sp. n. was described. The taxa *Pipiza carbonaria* Meigen, 1822; *Pipiza fasciata*, Meigen 1822; *Pipiza lugubris* (Fabricius, 1775), *Pipiza noctiluca* (Linneaues, 1758), *Pipiza notata* Meigen, 1822 were redefined. Lectotypes are designated for 17 taxa, and neotypes were designated for seven taxa. Fourteen new synonymies were proposed. Male genitalia were illustrated for all the species, and a key of the 12 European species for males and females was provided. Geometric morphometrics of wing landmarks and extended sampling of mtDNA COI sequences was employed to delimitate taxa of the *P. noctiluca* and *P. lugubris* complexes. Despite subtle morphological differences, wing geometric morphometrics variables of wing size and shape showed highly significant differences among species within *P. noctiluca* and *P. lugubris* complexes, which were supported by the molecular data.

Corresponding author: *Ante Vujić, Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia. E-mail: ante.vujic@dbe.uns.ac.rs*

Gunilla Ståhls, Entomology Department, Finnish Museum of Natural History, PO Box 17, FI-00014 University of Helsinki, Finland. E-mail: gunilla.stahls@helsinki.fi

Jelena Ačanski, Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia. E-mail: jelena.acanski@dbe.uns.ac.rs

Rune Bygebjerg, Museum of Zoology, Lund University, Helgonavägen 3, SE-223 62 Lund, Sweden. E-mail: Rune.Bygebjerg@biol.lu.se

Ana Stefanović, Department of Biology and Ecology, Faculty of Science, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia. E-mail: ana.grkovic@dbe.uns.ac.rs

†Järfälla, Sweden. Deceased 4 April, 2011.

Introduction

The monophyletic tribe Pipizini (Diptera, Syrphidae) is a sister group to subfamily Syrphinae (Ståhls *et al.* 2003). All Pipizini are small to medium sized, dark coloured, but some species have spots on the abdomen. A pilose postpronotum, a flat face lacking a facial knob and entirely covered in long pilosity, and wing vein R4 + 5 straight, not sinuate, and crossvein R-M perpendicular ending before

middle of discal cell readily separates this taxon from all other hoverflies. The family-group name Pipizini was first introduced by Williston (1885) (as Pipizinae). *Pipiza* is the type genus of the tribe. The tribe comprises six genera, *Cryptopipiza* Mutin, 1998, *Heringia* Rondani, 1856, *Pipiza* Fallén, 1810, *Pipizella* Rondani, 1856, *Trichopsomyia* Williston, 1888 and *Triglyphus* Loew, 1840, with altogether approx. There were 63 species in Europe. *Cryptopipiza* is an Old World monotypic genus, and with *Pipiza*, *Pipizella* and *Heringia* sensu lato are confined to the Holarctic region. *Triglyphus* and *Trichopsomyia* comprise only few species in the Old World, but are speciose in the New World and/or Australian regions. *Neocnemodon* Goffe, 1944 was originally described as the genus *Cnemodon* Egger, 1865 preoccupied by *Cnemodon* Schoenherr, 1823 (synonym of *Phytoscaphus* Schoenherr), but subsequently treated as subgenus of *Heringia* (Rotheray & Gilbert 1989). Recent taxonomic revisions or reviews including European Pipizini taxa are those of Claussen *et al.* (1994) on the genus *Heringia* s.l., Vujić (1999) on the *Heringia* subgenus *Neocnemodon*, Vujić *et al.* (2008) on the *Pipiza luteitarsis* group and van Steenis & Lucas (2011) on the genus *Pipizella*. The generic relationships of Pipizini remain, however, unstudied.

Species of genus *Pipiza* are medium-sized, blackish hoverflies, although some species have one or two pairs of pale spots on the abdomen. Adults prefer habitats on the forest edge and larvae are predators of gall-forming aphids (Speight 2010). Diagnostic characters for this genus are the following: anterior anepisternum and katepisternum bare or with very short pilosity, katepimeron with short hairs, male with distinct conically produced frontal prominence, hind trochanter of male simple, without ventral spur; male genitalia with two clear apomorphies: surstylus with well-developed dorsal semicircular lobe, lower gonocercus sickle-cell like shape (adapted based on Thompson & Rotheray 1998).

The catalogue of Palaearctic Diptera (Peck 1988) listed 17 species for the region, of which 12 were distributed in Europe. Violovitsh (1988) revised the Palaearctic *Pipiza* species including 15 species, but the identity of most European *Pipiza* still remained convoluted. Hence, the genus was still in need of a revision, as pointed out by many syrphid workers (e.g. Speight 2010). The only recent taxonomic treatment of *Pipiza* taxa is Vujić *et al.*'s (2008) review of the *P. luteitarsis* group, which comprises but three easily diagnosed species. Speight (2010) included eight species for Europe in his Species Accounts of European Syrphidae. These were *Pipiza accola* Violovitsh, 1985; *Pipiza austriaca* Meigen, 1822; *Pipiza bimaculata* Meigen, 1822; *Pipiza festiva* Meigen, 1822; *Pipiza luteibarba* Vujić, Radenković & Polić, 2008, *P. luteitarsis* Zetterstedt, 1843; *Pipiza noctiluca* (Linnaeus, 1758) and *Pipiza quadrimaculata* (Panzer, 1802). In The Biosystematic Database of World Diptera (Pape & Thompson 2012), available names were listed for 15 species occurring in Europe.

Molecular phylogenetic studies of Syrphidae taxa have frequently employed parsimony analyses of the mitochondrial cytochrome c oxidase subunit I (COI) and nuclear 28S ribosomal RNA (28S) gene regions, and these gene regions have shown levels of variability informative for both generic level and species level phylogenies (e.g. Ståhls *et al.* 2004; Mengual *et al.* 2008a; Vujić *et al.* 2012). The mtDNA COI gene was informative in several studies of hoverfly species level molecular phylogenetic relationships, but has showed low and/or overlapping uncorrected pairwise sequence divergences between closely related taxa (e.g. Claussen & Ståhls 2007; Mengual *et al.* 2008b; Milankov *et al.* 2008; Ståhls *et al.* 2008).

Geometric morphometrics is a useful tool to detect minimal morphological variations, which often are undetectable by classical morphometrical studies (Zelditch *et al.* 2004; Vilemant *et al.* 2007). Geometric morphometrics has mostly been used to discriminate between groups defined a priori using morphological or biological information (Baylac *et al.* 2003). Wing shape in insects (e.g. Diptera, Hymenoptera and Odonata) has been shown to be highly heritable and less sensitive to environmental changes than the wing size (Birdsall *et al.* 2000; Moraes *et al.* 2004; Mezey & Houle 2005; Dworkin & Gibson 2006; Yeaman *et al.* 2010). Wings of flies usually have strong and clear veins and vein junctions, with very little positional variation. Geometric morphometrics applied to wing vein junctions was successfully used to resolve taxonomic uncertainties in species-pairs of the family Drosophilidae (Moraes *et al.* 2004), of closely related the family Syrphidae (Ludoški *et al.* 2008; Francuski *et al.* 2011) and for other insects, for example, Hymenoptera (Baracchi *et al.* 2011; Kandemir *et al.* 2011). In our study, wing morphometry was used to discern between and delimit species of the *Pipiza lugubris* and *P. noctiluca* complexes.

The aims of the present study are fourfold, to (i) elucidate the molecular phylogenetic relationships of the Pipizini genera; (ii) to assess the monophyly of the Pipizini taxa based on mtDNA COI and nuclear 28S rDNA gene regions and the classificatory implications thereof; further to (iii) clarify the long-standing issue of unclear species concepts of all European *Pipiza* taxa including solving the nomenclatural issues and providing redefinitions and a morphological identification key to all European species; and finally to (iv) use both wing geometric morphometrics and increased COI sequence sampling (of both sexes and multiple geographic locations) to evaluate the utility of

these data for delimitation the species of the *P. lugubris* and *P. noctiluca* complexes.

Material and methods

Specimens

For the molecular phylogenetic analyses elucidating the relationships among Pipizini genera multiple representatives of all Pipizini genera were included. Species were mainly acquired from the Palaearctic area, but a few representatives of Pipizini taxa from the New World were available for DNA work (Table S1). For the analysis of phylogenetic relationships among taxa of genus *Pipiza* all European *Pipiza* species were included except for the uncommon taxa *Pipiza carbonaria* (Meigen, 1822) and *P. festiva*, for which suitable specimens for DNA analyses were not available. To support species delimitation of the taxa of the *P. noctiluca* and *P. lugubris* complexes multiple individuals were used for mtDNA COI sequencing and a large sample of the same individuals were also used for wing morphometric analysis. Specimens used for DNA work are listed in Table S1 including GenBank accession numbers, and specimens used for wing analysis are listed in Table S2. Specimens with a labcode including the acronym MZH are deposited as DNA voucher specimens in the Zoological Museum of the Finnish Museum of Natural History, Helsinki, Finland and labelled accordingly, while the remaining specimens are deposited in collections of University of Novi Sad, Serbia (FSUNS). Morphological studies were based on more than 2000 specimens of *Pipiza* spp. from European collections, including museums housing *Pipiza* types and other depositories.

Molecular work

Laboratory procedures. DNA was extracted from 1 to 3 legs using the Nucleospin Tissue DNA extraction kit (Machery-Nagel, Düren, Germany) following the manufacturer's protocols and then resuspended in 50 μL of ultrapure water. PCRs were carried out in 25-μL reaction aliquots containing 2–4 μL of DNA extract, 1 μL of each primer (at 10 pmol/μL) and ultrapure water, using Illustra PuReTaq™ Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, UK). Thermocycler conditions were initial denaturing at 95 °C 2 min, 29 cycles of 30-s denaturing at 94 °C, 30-s annealing at 49 °C, 2-min extension at 72 °C, followed by a final extension of 8 min at 72 °C. The universally conserved primers used for amplifying and sequencing the COI fragment were the forward primer C1-J-2183 (5′-CAA CAT TTA TTT TGA TTT TTT GG-3′) (alias JERRY) and reverse primer TL2-N-3014 (5′-TCC AAT GCA CTA ATC TGC CAT ATT A-3′) (alias PAT) (Simon *et al.* 1994). The D2-3 region of the nuclear 28S rRNA gene was amplified with the forward primer F2 (5′-AGA GAG AGT TCA AGA GTA CGT G-3′) and reverse primer 3DR (5′-TAG TTC ACC ATC TTT CGG GTC-3′).

Amplified DNA was electrophoresed on 1.5% agarose gels for visual inspection of amplified products. PCR products were enzymatically treated with ExoSap-IT (USB, Cleveland, OH, USA) and then sequenced (using the PCR primers) in both directions using the Big Dye Terminator Cycle Sequencing Kit (version 3.1) (Applied Biosystems, Foster City, CA, USA) on a ABI 3730 (Applied Biosystems) DNA analyzer at the Sequencing Service laboratory of the Finnish Institute for Molecular Medicine (http://www.fimm.fi). The sequences were edited for base-calling errors and assembled using Sequence Navigator™ (version 1.01) (Applied Biosystems). All new sequences were submitted to GenBank (see Table S1 for accession numbers).

Sequence alignment. The sequences of the protein-coding COI gene were aligned manually, and it was not necessary to include gaps in this alignment. The alignment of the 28S rDNA fragment was carried out using the E-INS-I strategy as implemented in MAFFT (Katoh *et al.* 2005, 2009).

Phylogenetic analyses. Phylogenetic relationships of the genera of the tribe Pipizini and the monophyly of genus *Pipiza* was investigated using combined mitochondrial COI and nuclear 28S rDNA sequences. A total of 42 terminal taxa were included in the parsimony and maximum likelihood (ML) analyses. The analyses included 11 *Pipiza* taxa, 28 other Pipizini taxa and the outgroup taxa, *Microdon bidens* (Fabricius, 1805) (Microdontinae), *Merodon equestris* (Fabricius, 1794) (Eristalinae) and *Syrphus vitripennis* (Meigen, 1822) (Syrphinae). The trees were rooted on *M. bidens.* For assessing the relationships among *Pipiza* species altogether 45 *Pipiza* spp. samples were included in parsimony analysis of COI gene, using three pipizines as outgroups (*Triglyphus fulvicornis Bigot, 1884*, *Cryptopipiza notabila* (Violovitsh, 1985) and *Heringia heringi* (Zetterstedt, 1843), with trees rooted on *T. fulvicornis*). And finally, an extended sampling of COI sequences was carried out for the *P. noctiluca* complex. This matrix included 39 *P. noctiluca* complex samples (males, females and larvae) and used *P. lugubris* to root the tree and other *Pipiza* spp. as outgroups.

Parsimony analysis was performed using NONA (Goloboff 1999) and spawn with the aid of Winclada (Nixon 2002), using heuristic search algorithm with 1000 random addition replicates (mult × 1000); holding 100 trees per round (hold/100), max trees set to 100 000; and applying TBR branch swapping. All base positions were

treated as equally weighted characters, and gaps were treated as fifth state. Nodal support was assessed with bootstrap resampling (1000 replicates) using Winclada.

Best-fitting evolutionary models following the Bayesian Information Criteria (BIC) scores for ML analysis were calculated using modeltesting as implemented in MEGA5 (Tamura *et al.* 2011). RAxML likelihood analysis (Stamatakis 2006) was performed using the RAxML version 7.3.1 at the CIPRES portal (Miller *et al.* 2010; http://www.phylog.org), with nodal support estimated with 100 bootstrap replicates.

Type studies

The study and revision of old-type material of European species described within the genus commenced in 2010. The main depositories for *Pipiza* types are Naturhistoriches Museum, Wien (NHWM), Austria (Meigen and Mik types), Muséum national d'Histoire naturelle, Paris (MNHN), France (Meigen and Macquart), Zoologiska Museet, Lund (ZMUL), Sweden (Zetterstedt), Zoologisk Museum, Copenhagen (ZMUC), Denmark (Fabricius), The Linnaean Collections (LC), London, UK, and University of Novi Sad, Department of Biology and Ecology (FSUNS). In most collections the relevant specimens were not labelled as putative type specimens, but had to be identified given the scarce locality information given in the publications and the labels. A strong attempt was made to locate all true syntype specimens in the collections. In case this was impossible because of missing material, a neotype was designated as appropriate. Meigen described species from material of 'Hrn. Megerle von Mühlfeld' and from 'Kais. Königl. Museum'. These type specimens were apparently destroyed by fire in the Naturhistorisches Museum in 1848. Some Meigen-type specimens were apparently held in the Wiedemann and Winthem collections that arrived at the museum after 1848, and are indicated by a label 'Alte Sammlung' (see Thompson 1988 and references therein).

The *Pipiza* materials housed in the above–mentioned, and other collections were identified and accordingly labelled, and a database of European records (http://www.dbe.uns.ac.rs/o_departmanu/laboratorije/laboratorija_za_istrazivanje_i_zastitu_biodiverziteta/prilog) including distributional maps of species of the genus will be published separately.

Figures of male genitalia have been prepared from macerated material, and drawings of all morphological features have been made using drawing tube attached to a binocular microscope. The morphological terminology followed is that of Thompson (1999) except terms for the male genital parts which are those of Verlinden (1999).

Wing morphometry

Wing morphometric measurements were made for the right wing which was removed from the body and mounted in Hoyer's Medium between a microscope slide and a cover slip. Wings are archived and labelled using unique codes saved in our local database (http://www.dbe.uns.ac.rs/o_departmanu/laboratorije/laboratorija_za_istrazivanje_i_zastitu_biodiverziteta/prilog) with other data relevant to the specimens. High-resolution wing images were captured using a digital camera (Leica DFC 320, Wetzlar, Germany) mounted on a stereomicroscope (Leica MZ16). Fifteen homologous landmarks that could be reliably identified and representing wing shape were chosen. The landmarks are positioned at vein intersections or terminations (Fig. 1). Their Cartesian coordinates were digitized using the software tpsDIG 2.05 (Rohlf 2006), and the work was done by a single experimenter. All wings were digitized two times for the *P. noctiluca* complex as a higher number of individuals were available for digitization, and three times for *P. lugubris* complex, to reduce the measurement error (Arnqvist & Mårtensson 1998). For the *P. noctiluca* complex we did separate analyses for males and females and used *P. carbonaria* as out group.

Generalized least squares Procrustes superimposition was first applied to the landmark data to remove non-shape variations in location, scale and orientation, and also to superimpose the objects in a common coordinate system (Rohlf & Slice 1990; Zelditch *et al.* 2004). Centroid size, an isometric estimator of size, was derived from the square root of the summation of the squared distances between the centre of the objects and each landmark (Zelditch *et al.* 2004). One-way analysis of variance (ANOVA) was used to test differences in centroid size between sexes and/or species. For the wing shape analysis we calculated partial warp scores (thin-plate spline coefficients) (Zelditch *et al.* 2004) and analysed wing shape variation within and between species using multivariate analysis of variance (MANOVA), canonical variates analysis (CVA) and discriminant function analysis (DA). Procrustes superimposition, centroid size and partial warps were

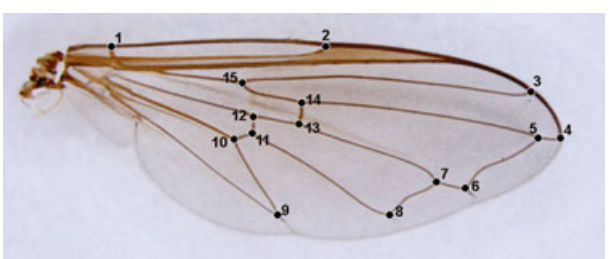

Fig. 1 The location of 15 landmarks positioned at vein junctions on the right wing, used in geometric morphometrics analysis.

computed using the free IMP software's CoordGen6f and CVAgen6j (Sheets 2001), and for the thin-plate spline deformation visualization we used free software MorphoJ v.2. 0. (Klingenberg 2011), and all statistical analyses were calculated using Statistica for Windows (StatSoft 2011: version 10.0).

Results

Sequences

The COI sequence fragment was obtained for all included taxa (Table S1). The pruned COI data matrix used for analyses contained a total of 731 nucleotide characters, and the full fragment was obtained for all included taxa. The average A + T content was 74.2%. The interspecific uncorrected pairwise divergences between Pipizini taxa ranged between 0.2% [*Pipizella viduata* (Linnaeus, 1758) vs. *Pipizella virens* (Fabricius, 1805)] and 12.8% [*Claussenia hispanica* (Strobl, 1909) vs. *P. luteibarba*], and varied among *Pipiza* spp. between 0.4 (*P. noctiluca* vs. *Pipiza notata* Meigen, 1822) and 4.5% (*P. quadrimaculata* vs. *P. noctiluca*).

The pruned fragment of the D2-3 domain of the 28S ribosomal gene was obtained for all taxa but *Trichopsomyia flavitarsis* (Meigen, 1822), and the length of the pruned fragment ranged 566–76 nucleotides among Pipizini taxa. The aligned 28S data set for outgroup and ingroup taxa comprised 621 nucleotide positions. The average A + T content was 74.4%.

The combined matrix used in phylogenetic analyses comprised 1352 nucleotide positions.

Phylogenetic analyses

For assessing the generic relationships the sequence data (COI+ aligned 28S) were combined in one matrix. The numbers of parsimony informative sites for the COI and 28S data sets were 179 and 127, respectively. The parsimony analysis resulted in 12 equally parsimonious trees of length 1212 steps (Consistency Index = 0.49, Retention Index = 0.71), and the strict consensus (length = 1240 steps) is shown in Fig. 2 with bootstrap support values above branches. All Pipizini genera were resolved as independent lineages in their present sense, except for genus

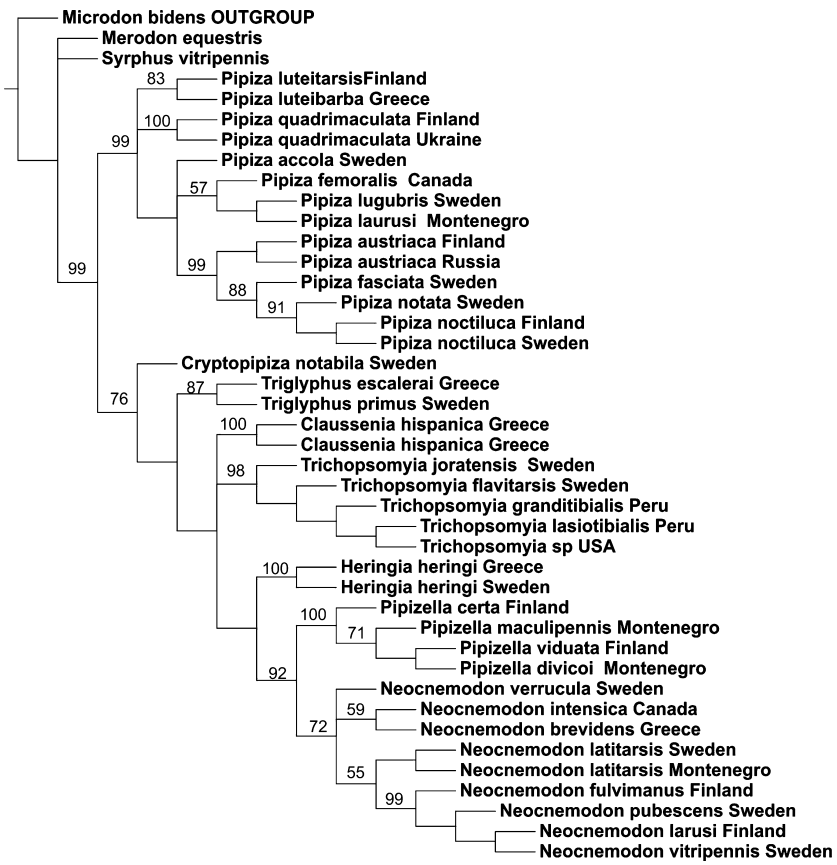

Fig. 2 Strict consensus tree of parsimony analysis of combined cytochrome c oxidase subunit I and 28S sequence data.

Heringia. The monophyly of the genus *Pipiza* was well supported (99%), and the genus was resolved as sister taxon to remaining Pipizini. *Cryptopipiza* and *Triglyphus* were resolved as sisters to {*C. hispanica* + *Trichopsomyia* + [*H. heringi* + (*Pipizella* + *Neocnemodon*)]}. High bootstrap support was also found for the *Neocnemodon* + *Pipizella* grouping (92%), and the other Pipizini genera also received high bootstrap support, except for genus *Trichopsomyia* (Fig. 2).

The general time reversible model with gamma parameter (GTR + G) was the best-fitting substitution model according to the BIC criterion for both COI and 28S sequence matrices. The BIC scores were 10290.761 and 6489.791 for the COI and 28S data sets, respectively. The log likelihood score for the best RAxML tree of the combined gene regions was −7519.70. The ML tree resolved the same lineages as the parsimony tree and again the genus *Pipiza* as sister to rest of Pipizini, but the arrangement of the *Cryptopipiza*, *C. hispanica*, *Triglyphus* and *Trichopsomyia* lineages is different (Fig. 3). *Heringia* was resolved in three lineages, *H. heringi* and *C. hispanica* as separate lineages (*Heringia hispanica*), and the members of subgenus *Neocnemodon* as separate clade. High bootstrap support (99%) was again found for the monophyly of *Pipiza*, and the *Neocnemodon* + *Pipizella* grouping (96%).

Fig. 3 Maximum likelihood (RAxML) tree of the combined cytochrome c oxidase subunit I and 28S gene regions.

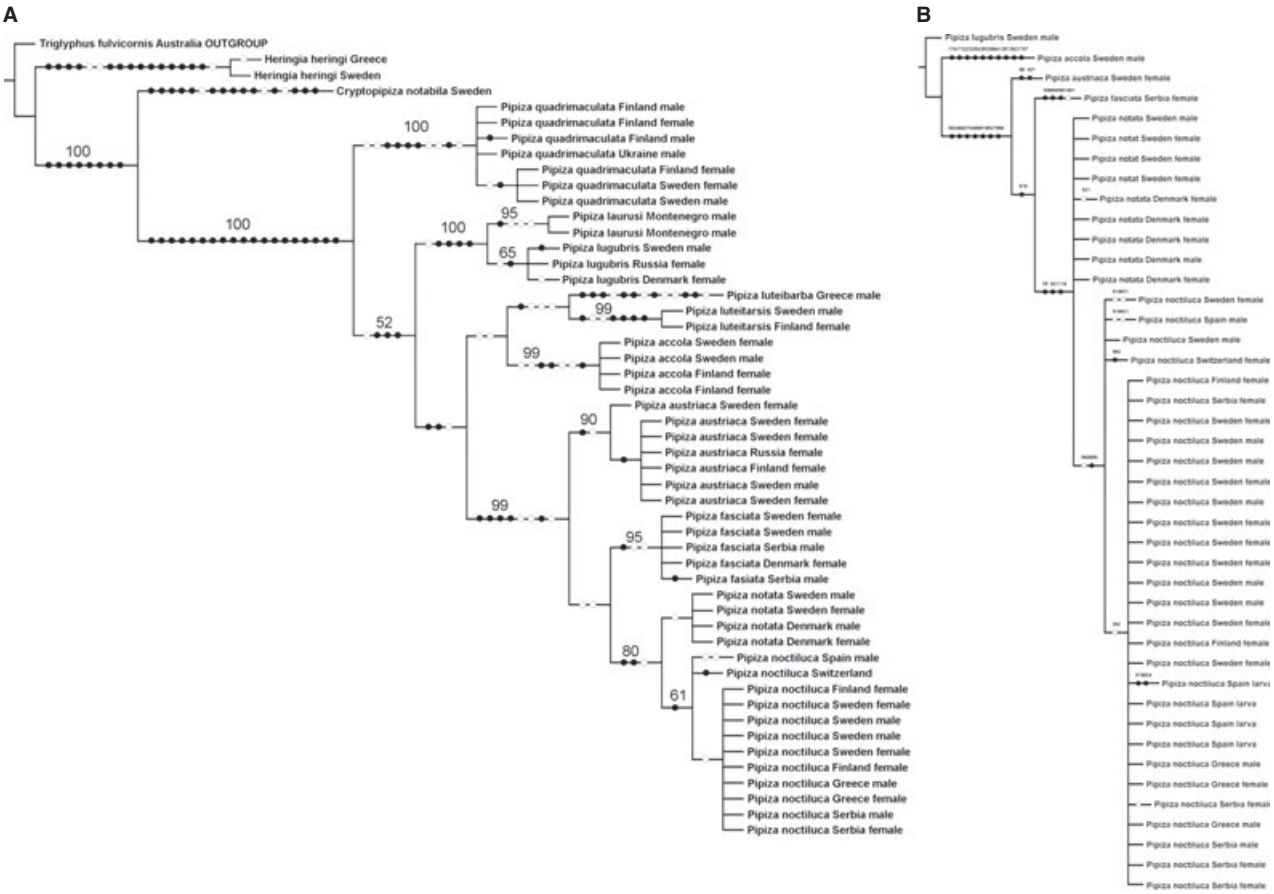

Fig. 4 A. Strict consensus of parsimony analysis of cytochrome c oxidase subunit I gene sequences of *Pipiza* spp. —B. Single most parsimonious tree for the *Pipiza noctiluca* and *Pipiza notata*.

The parsimony analysis of COI gene sequences for assessing the relationships among *Pipiza* species resulted in four equally parsimonious trees of length 243 steps (CI = 0.72, RI = 0.90), and the strict consensus is shown in Fig. 4A with bootstrap support values above branches. The strict consensus resolved *P. quadrimaculata* as sister group to remaining *Pipiza* spp. The members of *P. luteitarsis* group (sensu Vujić *et al.* 2008) were not resolved as a monophyletic clade, but *P. luteibarba* and *P. luteitarsis* were resolved as sister taxa. The *P. lugubris* complex was resolved as sister to trichotomy [((*P. luteibarba* + *P. luteitarsis*) + (*P. accola*)) + ((*P. austriaca*) + (*Pipiza fasciata* Meigen, 1822) + (*P. notata*) + (*P. noctiluca*))]. Figure 4B shows the most parsimonious topology for the extended set of samples of the *P. noctiluca* complex.

Classification

Pipizini systematics. Rotheray & Gilbert (1989) placed *Neocnemodon* as a subgenus of *Heringia* based on the analysis of immature characters. Later, Rotheray & Gilbert (1999) in their generic phylogeny of Palaearctic Syrphidae recovered *Neocnemodon* and *Heringia* as separate lineages in the successive weighting analysis {*Pipizella* + (*Neocnemodon* + [*Trichopsomyia* + (*Pipiza* + *Heringia*)]}, but the included Pipizini were recovered as unresolved clade in the consensus tree. Claussen *et al.* (1994) reviewed the genus *Heringia* s. str. and defined the borders between the nominal subgenus and sg. *Neocnemodon*. They placed the taxon *H. hispanica* in the subgenus *Neocnemodon*. Our molecular results revealed several well-supported clades in conflict with previous generic classification of *Heringia* as developed in Claussen *et al.* (1994). As our results were stable and supported by important morphological characters, we are confident to propose classificatory changes. The diagnostic characters of *Heringia* group of genera are as follows: *Neocnemodon* (male coxae and trochanters with calcars, basoflagellomere short, as long as broad, male genitalia (Fig. S1B) with surstylus narrowed on apical half, aedeagus two-segmented and gonocercus oval), *Heringia* (basoflagellomere elongated, male genitalia (Fig. S1C) with surstylus rectangular, aedeagus non-segmented

and gonocercus plate-like without ventral toothed prolongation), *Claussenia* [basoflagellomere elongated, male genitalia (Fig. S1A) with surstylus toothed basally, aedeagus two-segmented and gonocercus oval with ventral toothed prolongation].Thus there is good support from both morphological and molecular characters to split *Heringia* into three genera, *Heringia* sensu stricto with type-species *H. heringi*, *Neocnemodon* Goffe stat. n. with type-species *Neocnemodon latitarsis* (Egger, 1865), and *Claussenia* Vujić & Ståhls gen. n., with type-species *Claussenia hispanica*. This recognizes the distinctness of the constituent lineages and maintains the monophyly and the systematic utility of the remaining genera. *Claussenia* gen. n. is a monotypic genus, *Heringia* comprises species *heringi* and *adpropinquans* (Becker, 1908), and *Neocnemodon* comprises presently 16 Palaearctic and 23 Nearctic species. The present molecular study includes mainly Palaearctic representatives of the genera *Pipiza* and *Neocnemodon*, but the Nearctic taxa are morphologically similar to their Palaearctic congeners, and we are confident that the addition of additional taxa will not change the topology of the trees and thus not alter our classification.

***Key to genera of tribe Pipizini with bare anterior anepisternum (adapted based on Thompson & Rotheray 1998; Claussen et al.* 1994)**

1 Vein M1 joining vein R4 + 5 perpendicularly (cell r4 + 5 truncate apically)…… *Pipizella* Rondani
-Vein M1 progressive apically, cell r4 + 5 acute apically…… 2
2 Frontal lunule of female without semi-adpressed hairs on the medial prong, male with distinct conically produced frontal prominence (Fig. S29…… 3
-Frontal lunule of female with semi-adpressed hairs on the medial prong (a few to many), male with frontal prominence very short…… 4
3 Katepimeron without hairs…… *Pipiza* Fallen
-Katepimeron with hairs (at least dorsally)…… *Cryptopipiza* Mutin
4 Male coxae and trochanters with calcars. Female basoflagellomere not, or only slightly longer than wide; frons with very small and inconspicuous pollinose spots…… *Neocnemodon* Goffe
-Male coxae and trochanters without calcars. Female basoflagellomere much longer than wide; frons with lateral pollinose spots…… 5
5 Aedeagus non-segmented (Fig. S1C). Surstylus elongated, oval (Fig. S1C). In females scutellar margin without long black bristles…… *Heringia* Rondani
-Aedeagus two-segmented (Fig. S1A). Surstylus with basal prong (toothead basally) (Fig. S1A). In females scutellar margin with long black bristles…… *Claussenia* Vujić & Ståhls, gen. n.

Claussenia Vujić & Ståhls, gen. n.

Type. Pipizella heringii (Zetterstedt) var. *hispanica* Strobl in Czerny & Strobl 1909: 208.

Heringia (*Neocnemodon*) *hispanica*: Claussen *et al.* 1994: 312 (des. female lectotype, male paralectotype, description).

Diagnosis. Male with basoflagellomere 1.5–2 times longer than wide; mid tibia without central dilatation and bulge; two-segmented aedeagus and surstylus with basal prong; female with basoflagellomere 2–2.5 times longer than wide; frons with pollinose spots; occiput and scutellar margin with long black bristles.

Etymology. The new genus is named after Claus Claussen, German entomologist, dedicated to his master work on hoverflies.

European species of the genus Pipiza with synonymies

To account for morphological character variation we studied specimens from different countries across Europe. All available European types of *Pipiza* taxa were revised. Five *Pipiza* species are described from the Eastern Palaearctic region, but not treated here (but see Vujić *et al.* 2008). Our morphological, morphometric and molecular analyses have shown the presence of 12 species in Europe. The species biology is presented for *Pipiza* spp. in Speight (2010), and for species not covered by Speight we present here a brief account on biology. Given distributional ranges are adapted from Speight (2010) incorporating data from this study.

Pipiza accola Violovitsh, 1985 (Figs S2 and S20E–F; Appendix S1)

Types. No types studied. Type-locality: Russian Far East, South Primorie, nr Vladivostok (Violovitsh 1985).

Distribution. Southern Fennoscandia; Germany (Lower Saxony, Baden-Württemberg) and Russia (Siberia).

Pipiza austriaca Meigen,1822 (Figs S4, S5B and S32; Appendix S2)

syn n. *albipila* Meigen, 1830

Types. Pipiza austriaca (Meigen 1822: 252). Type-locality: Austria. Holotype: male, 'Kais. Köningl. Museum', presumably lost. Neotype (NHWM, designated here): male, 'Austria', 'Alte Sammlung' ('*funebris* det. Egger').

Pipiza albipila (Meigen 1830: 350). Type-locality: Europe. Type: unspecified number of females (Wiedemann collection, presumably lost). Neotype (NHWM, designated here): Wiedemann coll. (2 females det. as *funebris* and *lugubris*), first specimen designated as neotype (label: '*funebris* coll. Wiedem.').

Distribution. Distributional range is still uncertain, owing to confusion with related species, but apparently occurs in much of the Atlantic zone and southern parts of Scandinavia (but not recorded in Denmark since 1962). Present revision of European collections indicates distribution through Central Europe, until Southern-East Europe (Aegean island, Lesvos).

Pipiza carbonaria Meigen, 1822 (Figs S5C, S6, S30I, S31G, S33A–B and S34; Appendix S3)

Types. *Pipiza carbonaria* (Meigen 1822:251). Described from male, type-locality not given. Type not studied, presumably lost as type not present in the NHWM collection, but identification was based on male genitalia of holotype figured by Goeldlin de Tiefenau (1997: 193).

Distribution. Balkan Peninsula, Austria.

Pipiza fasciata Meigen, 1822 (Figs S7, S10A, S25A–B, S26A, S31B–C, S33E, S35G–J,N–O and S37B; Appendix S4)
fenestrata Meigen, 1822 of recent authors

Types. *Pipiza fasciata* (Meigen 1822: 242). Type-locality: Austria. Described from one female of Hrn. Megerle von Mühlfeld (considered as lost). Neotype female (NHWM, designated here): 'M.5(V).70. Bgst', '*fasciata* det. Bergenst.'.

Distribution. Based on material from European collections range is wide, but still uncertain. From Fennoscandia south to France; from Germany eastwards through central Europe to the Balkan Peninsula.

Pipiza festiva Meigen, 1822 (Figs S3B, S8, S31A, S35A–E and S37A; Appendix S5)
syn. *artemis* Meigen, 1822
syn. *lunata* Meigen, 1822
syn. *ornata* Meigen, 1822

Types. *Pipiza festiva* (Meigen 1822: 243). Type-locality: Germany (?Stolberg near Aachen). Described based on six females. In MNHN, Meigen coll: '1374 40', '*Pipiza festiva*', two female syntypes, one designated as lectotype, second as paralectotype.

Pipiza artemis (Meigen 1822: 244). Type-locality: Austria. Holotype: female (presumably lost). Neotype (NHWM, designated here: 'Austria') conspecific with *P. festiva*.

Pipiza lunata (Meigen 1822: 243). Type-locality: Austria. Described based on one female (presumably lost). Three female specimens in NHWM with labels: 'au (an) *lunata* (Sch…)' were found and one chosen and designated as neotype. *Pipiza lunata* should be regarded as a junior synonym of *P. festiva*.

Pipiza ornata (Meigen 1822: 243). Type-locality: Austria. Holotype: female, from Kais. Köningl. Museum (considered as lost). Neotype (NHWM, designated here: 'Austria') conspecific with *P. festiva*.

Distribution. From Belgium and the Netherlands south to the France, eastwards through southern and central Europe.

Pipiza laurusi Vujić & Ståhls, sp. n. (Figs S9, S10B–D, S14, S15 and S16A–C)

Holotype. m, MONTENEGRO, Boka Kotorska, Morinj, 31 April 2011, leg. A. Vujić (UNS).

Paratypes. MONTENEGRO, Boka Kotorska, Morinj, 2 mm, 7 May 1994, leg. A. Vujić; 1 f *idem* 4 May 2000, leg. S. Radenković; 2 mm *idem* 31 April 2011, leg. A. Vujić. FYR MACEDONIA, s. Vratnica, 1 m, 21 July 1921, leg. Čingovski. GREECE, Corfu, Barbati, 1 f, 16–24 July 2000, leg. V. Vrabec (all in UNS).

The known distributional range of *P. lugubris* is in North-West Europe. The new taxon is described from one isolated population in Montenegro bay, Boka Kotorska, locality Morinj, but additional specimens were found in two other Mediterranean and Submediterranean localities on the Balkan Peninsula: Greece (Corfu) and FYR Macedonia (Vratnica).

Etymology. Name is derived from Latin name of evergreen tree of genus *Laurus* refer to the habitat on type-localities with forest of *Laurus nobilis*.

Diagnosis. Hind femora with pair of ventral longitudinal ridges at the distal end (as on Fig. S3B); basoflagellomere elongated (Fig. S16A–C); wing with very distinct dark area along central parts (as on Fig. S32); tergite 2 usually and tergite 3 always without pale spots (Fig. S10B–D); in male tergites shiny, without pollinosity; face, mesonotum and lateral side of tergites 1–2 predominantly pale haired; male genitalia on Fig. S9; in female sternite 3 predominantly dark; tergite 4 predominantly pale haired, at least in posterior half. *Pipiza laurusi* sp. n. is first detected by mtDNA COI data (Fig. 4A). It is morphologically 'identical' with *P. lugubris*, but clearly differs in wing morphometrics. These two taxa together with *P. carbonaria* belong to a morphologically isolated group characterized by slightly elongated basoflagellomere and shiny tergites in males.

Geometric morphometric evidence: wing size and shape

ANOVA test and Tukey's post hoc test showed highly significant difference of wing size between *P. carbonaria* and *P. lugubris* ($F_{1,115} = 39.66$; $P < 0.00000$, Tukey post hoc $P < 0.000104$) and between *P. carbonaria* and *P. laurusi* sp. n. ($F_{1,94} = 15.52$; $P < 0.000156$, Tukey post hoc $P < 0.000262$). The ANOVA showed no wing size differences between *P. lugubris* and *P. laurusi* sp. n. species ($F_{1,61} = 0.08$, $P = 0.77$). *Pipiza carbonaria* specimens have larger wings than those belonging to *P. lugubris* and *P. laurusi* sp. n. (Fig. S11).

Multivariate analysis of variance showed highly significant differences in wing shape between species *P. carbonaria*, *P. laurusi* sp. n. and *P. lugubris* (Wilks' Lambda = 0.08; $F_{26,110} = 45.91$; $P < 0.00000$). Discriminant analysis successfully classified species with overall classification of 99.25% (one specimen of *P. lugubris* was misclassified as *P. carbonaria*) (Table S3).

Canonical variate analysis produced two highly significant axes. First CV axes with 81.85% (Wilks' Lambda = 0.04; $\chi^2 = 390.93$; $P < 0.00000$) of total variation clearly separated *P. carbonaria* from *P. laurusi* sp. n. and *P. lugubris*, and second CV axes with 18.15% (Wilks' Lambda = 0.34; $\chi^2 = 125.30$; $P < 0.00000$) clearly separated *P. lugubris* from *P. laurusi* sp. n. (Fig. S12).

Thin-plate spline deformation grid allows recognition of wing regions that are contributing to the discrimination. The major wing deformations between *P. laurusi* sp. n. and *P. lugubris* occur in central part of wing and are associated with position of landmarks 2, 8 and 9; they indicate that the shape difference between this two species is because of wing width (Fig. S13A).

The major wing deformation between *P. carbonaria* and *P. lugubris* and between *P. carbonaria* and *P. laurusi* sp. n are in central and distal part of wing for both. The deformations of these parts are mainly because of the major relative displacements of the landmarks (vein junctions) 2, 13 and 14, and 3–5 indicating differences in wing length (Fig. S13B–C).

Description

Male. Head (Fig. S14): Face black, dark-grey pollinose, covered with whitish hairs mixed with some black ones. Frons heavily dark-grey pollinose, black haired, except pale hairs posteriorly; angle of eye approximation about 100–110°; height of frons 1.2 times longer than eye suture. Vertex dark pollinose, covered with mixed pale and dark long hairs; occelar triangle equilateral. Occiput silver pollinose, pale haired, except few longer black hairs. Eyes uniformly covered with long grey hairs. Antennae dark, except dark-brown ventral area on basoflagellomere; arista dark; basoflagellomere elongated (Fig. S16A).

Thorax: Mesoscutum shiny, moderately punctured, completely pale haired, except few black hairs on lateral sides; scutellum with pale hairs. Pleurae slightly pollinose, pale haired, except mixed pale and black hairs on upper half of anepisternum; katepisternum with upper and lower hair patches separated, and shiny central area; metasternum bare. Femora dark, except pale apices; fore tibiae pale with dark submedian area; middle tibiae pale with dark submedian ring; hind tibiae dark, except pale basal 1/4 and top 1/5; tarsi pale, except dark apical 2 (3) tarsomere and dorsal surface of hind basitarsus; legs almost completely pale haired. Squamae whitish; halteres yellowish. Wing with dark-brown to yellow-brown veins and with darkened area (as on Fig. S32).

Abdomen (Fig. S10B–C): Entirely black, with strong dark lustrous; pale haired except shorter, black hairs on posterior margin of tergite 3, and anterior margin of tergites 3 and 4; tergite 2 in some specimens with pair of narrow, yellow spots (Fig. S10C). Sternites covered with long pale-yellow hairs; sternite 4 with mixed pale and black hairs.

Genitalia (Fig. S9): Theca of hypandrium and basale of epandrium short. Surstylus with small semicircular lobe. Lower gonocercus elongated, broad in basal 1/4, and very narrow in upper part.

Size: Body length, 9.1 mm; wing length, 8.0 mm.

Variability: face from pale haired to mixed pale and many black hairs; short hairs on mesoscutum can be pale, but also all black; tergite 2 black or with pair of pale spots (Fig. S10B–C); the stripes or areas of black hairs on tergites 2–4 can be variable; the arrangement of black and pale hairs on other part of body (legs, pleurae, sternites) can also be variable.

Female (Figs S10D, S15 and S16B,C). Differs from the male in the following characters: Frons and vertex shiny black except pollinose lateral spots that occupy about one-third of frons width (Fig. S15B). Frons, vertex and occiput predominantly yellow haired except black hairs above and laterally of antennae and along eye margin, above pollinose lateral spots. Hairs on mesoscutum shorter than in male, all yellow. Microtrichia on wing more reduced than in male: basal 1/3 of cell CuP, basal 1/2 of cell br bare. Tergite 2 with two lateral yellow spots (Fig. S10D).

Pipiza lugubris (Fabricius, 1775) (*Syrphus*) (Figs S16D–F and S39; Appendix S8)

syn. *funebris* Meigen, 1822
syn. n. *geniculata* Meigen, 1822
syn. n. *signata* Meigen, 1822
syn. n. *jablonskii* Mik, 1867

Types. Pipiza lugubris (Fabricius 1775: 770). Type-locality: 'Dania' (Denmark). Syntypes presumably lost. Neotype:

male, '11/8/09 Ermelund', 'coll. W. Lundbeck', 'lugubris F.', here designated, in ZMUC.

Pipiza funebris (Meigen 1822: 250). Type-locality: not given. Two syntypes in MNHN, Meigen coll: '1386 40', '*Pipiza funebris*', male designated here as lectotype and female as paralectotype.

Pipiza geniculata (Meigen 1822: 245). Type-locality: Germany (?Stolberg near Aachen). Two syntypes in MNHN, Meigen coll: '1377 40', '*Pipiza geniculata*', male designated here as lectotype and female as paralectotype.

Pipiza signata (Meigen 1822: 246). Type-locality: not given. Two syntypes in MNHN, Meigen coll: '1378 40', '*Pipiza signata*', male desiganted here as lectotype and female as paralectotype.

Pipiza jablonskii (Mik 1867: 417). Type-locality: Austria. Syntypes in NHWM: male 'Schneeberg, Alte Sammlung'; '*Pipiza jablonskii* type'; 'Jablonskii det. Egger', here designated as lectotype; three male paralectotypes: one with same labels and two labelled as 'Austria, Alte Sammlung; *Pipiza jablonskii*'.

Distribution. From Fennoscandia south to Belgium and Germany; numerous populations in Denmark.

Pipiza luteibarba Vujić, Radenković & Polić, 2008 (Figs S3A, S17, S19A,B, S20A,B and S40B; Appendix S6)

Distribution. Austria, Serbia, Greece (Samos island). Vujić *et al.* (2008) suggested that this European localized endemic should be regarded as a threatened species.

Pipiza luteitarsis Zetterstedt, 1843 (Figs S5A, S18, S19C, S20C,D, S30D–F and S40A; Appendix S7)

Types. Pipiza luteitarsis (Zetterstedt 1843: 828). Type-localities: 'in Suecia: in Scania ad Lund e Glogavia' (Sweden). Lectotype designated here: '*P. luteitarsis* male. Lund', in ZMUL.

Distribution. From Fennoscandia south to Belgium and France; from Ireland eastwards through central Europe (Alps) into European parts of Russia.

Pipiza noctiluca species complex

The *P. noctiluca* species complex includes two closely related taxa which concepts appear nearly always mixed, as we have observed in most faunistic papers and among specimens in museum collections all around Europe. Typically, specimens were identified as taxa *P. bimaculata*, *P. noctiluca* and *P. notata*, but for a high percentage of specimens, and also many other names of different *Pipiza* taxa were used. All collections (e.g. NHWM, MNHN), need additional revision based on results presented in this paper.

Based on our preliminary morphological analysis the presence of two taxa was established. As described above, for these taxa we apply names *P. noctiluca* and *P. notata*. The morphological study was based on material from the Balkan Peninsula from which observation of small, but stable morphological differences were made (used in the identification key), mainly of male genitalia and female wing characteristics. To support these morphological results we included additional DNA analysis of mitochondrial COI gene of specimens from wide distributional range, and wing geometric morphometric analysis.

Molecular evidence. The molecular COI characters allow the separation of *P. noctiluca* and *P. notata* taxa (Fig. 4B). The sequence divergence between *P. noctiluca* and *P. notata* is low, involving 1–4 nucleotide differences, pointing to recent divergence. *Pipiza noctiluca* presented seven COI haplotypes and two *P. notata* haplotypes, but the taxa do not share haplotypes. A few nuclear ITS2 sequences were generated for the taxa, but were invariant among *P. noctiluca* and *P. notata* samples.

Geometric morphometric evidence: wing size and shape. The wing geometric morphometrics analysis on size and shape variables showed a clear separation between *P. noctiluca* and *P. notata*.

The ANOVA of wing centroid size showed highly significant difference between *P. notata* and *P. noctiluca* sexes (males: $F_{2,131} = 26.19$; $P < 0.00000$, post-hoc Tukey test $P < 0.000022$; females: $F_{2,255} = 108.34$; $P < 0.00000$, post-hoc Tukey test $P < 0.000022$). Specimens belonging to *P. noctiluca* have larger wings than those of *P. notata* (Fig. S21).

Wing shape between *P. notata* and *P. noctiluca* differed highly significantly using MANOVA, for males (Wilks' Lambda = 0.06; $F_{26,106} = 63.12$; $P < 0.00000$), and for females (Wilks' Lambda = 0.25; $F_{26,230} = 25.96$; $P < 0.00000$). Discriminant analysis applied on the wing shape variables correctly classified species with overall classification success 99.25% for males, and 98.84% for females indicating that wing shape has an important interspecific discrimination power (Table S4). Of the 392 digitizations, only four were misclassified, one *P. notata* male as *P. noctiluca* male, one *P. notata* female as *P. carbonaria* female, and two *P. noctiluca* females, one as *P. notata* female and one as *P. carbonaria* female.

Canonical variate analysis conducted on shape variables (PW scores) of males and females separately gave two highly significant axes. The first canonical axis (CV1) with 56.1% (Wilks' Lambda = 0.03; $\chi^2 = 413.64$; $P < 0.00000$) of total variation clearly separated males of *P. notata* and *P. carbonaria*. The second canonical axis (CV2) with 43.9%

(Wilks' Lambda = 0.19; χ^2 = 194.86; $P < 0.00000$) of total variation clearly separated males of *P. noctiluca* from *P. notata* and *P. carbonaria* (Fig. S22). Among females, CV1 with 76.6% of total variation clearly separated *P. notata* from *P. noctiluca* and *P. carbonaria*. Second canonical axis (CV2) separated *P. noctiluca* and *P. carbonaria* with 23.4% of total variation (Fig. S23).

The thin-plate spline visualizations showing major wing deformations between *P. notata* and *P. noctiluca* females occur in central part of wing and are associated with landmarks 2, 13 and14, and in distal part of wing associated with landmarks 3–5 (Fig. S24A). The shape differences between the males are associated with differences in the relative positions of landmarks 8, 13 and 14 (Fig. S24B). The deformation grid indicates that the shape difference between *P. noctiluca* and *P. notata* is because of wing length.

Pipiza noctiluca (Linnaeus, 1758) (*Musca*) (Figs S16G–I, S25C–G, S26C–D, S31D,E, S33C,D, S36C,I–K, S38 and S41B; Appendix S9)

syn. n. *calceata* Meigen, 1822
syn. n. *fenestrata* Meigen, 1822
syn. n. *guttata* Meigen, 1822
syn. *albitarsis* Meigen, 1830
syn. *rufithorax* Meigen, 1830
syn. *obsoleta* Zetterstedt, 1838
syn. *vana* Zetterstedt, 1843

Types. Pipiza noctiluca (Linnaeus 1758: 593). Type-locality: Europa. Holotype in Linneaeus collection: studied.

Pipiza calceata (Meigen 1822: 251). Type-locality: Germany (?Stolberg near Aachen). Two male syntypes in MNHN, Meigen coll: '1388 40', '*Pipiza calceata*', one designated here as lectotype, second as paralectotype.

Pipiza fenestrata (Meigen 1822: 248). Type-locality: Germany (?Stolberg near Aachen). Described based on two female specimens. One syntype found in MNHN, Meigen coll: '1382 40', '*Pipiza fenestrata*' designated here as lectotype.

Pipiza guttata (Meigen 1822: 247). Type-locality: Germany (?Stolberg near Aachen). Described based on unspecified number of males and females. Syntypes in MNHN, Meigen coll: '1381 40', '*Pipiza guttata*' male designated here as lectotype, female as paralectotype.

Pipiza albitarsis (Meigen 1830: 350). Type-locality: Europe. Described based on unspecified number of males from Wiedemann collection (presumably lost). Neotype male, in NHWM: Winthem collection, '*notata* coll. Winthem', designated here.

Pipiza rufithorax (Meigen 1830: 350). Type-locality: Europe. Described by unspecified number of males from Wiedemann collection (presumably lost). Neotype male, in NHWM: Wiedemann collection '*signata* coll. Wiedem.', designated here.

Pipiza obsoleta (Zetterstedt 1838: 616). Type-locality: In Lapponia meridionali, ad Lycksele (Sweden). Holotype ZMUL 019 female: '*P. obsoleta* female. Lycksele'.

Pipiza vana (Zetterstedt 1843: 835). Type-locality: In Scania circa Lund, in Paradislyckan (Sweden). Type ZMUL 012 male designated here as lectotype: '*P. vana* male. Lund'.

Distribution. All Europe, Russia and Turkey.

Pipiza notata Meigen, 1822 (Figs S16J–L, S26B, S27, S31F, S36L–T and S41A; Appendix S10)

syn. n. *anthracina* Meigen, 1822
syn. n. *bimaculata* Meigen, 1822
syn. n. *biguttula* Zetterstedt, 1838
syn. n. *binotata* Zetterstedt, 1838
syn. n. *hyalipennis* Zetterstedt, 1838
syn. n. *morionella* Zetterstedt, 1843
syn. n. *stigmatica* Zetterstedt, 1859

Types. Pipiza notata (Meigen 1822: 246). Type-locality: Germany (?Stolberg near Aachen). Holotype, female in MNHN, Meigen coll: '1379 40', '*Pipiza notata*', two females, one designated here as lectotype, second as paralectotype.

Pipiza anthracina (Meigen 1822: 253). Type-locality: Germany (?Stolberg near Aachen). Two male syntypes in MNHN, Meigen coll: '1399 40', '*Pipiza anthracina*', one designated here as lectotype, second as paralectotype.

Pipiza bimaculata (Meigen 1822: 246). Type-locality: Germany (?Stolberg near Aachen). Described based on unspecified number of males and females. Two syntypes in MNHN, Meigen coll: '1375 40', '*Pipiza bimaculata*', the male designated here as lectotype and the female as paralectotype.

Pipiza biguttula (Zetterstedt 1838: 616). Type-locality: In Lapponia, ad Lycksele Lapponiae Umensis, ad Evenas Nordlandiae (Sweden). Holotype in ZMUL (female). Label 1: '*P. binotata*'. Label 2: '*P. biguttula* female Lycksel.'

Pipiza binotata (Zetterstedt 1838: 616). Type-locality: In Lapponia Umensi ad Lycksele (Sweden). Female syntype in ZMUL, '016', designated here as lectotype '*P. binotata* female Lycksel.'

Pipiza hyalipennis (Zetterstedt 1838: 616). Type-locality: In Lapponia Umensi ad Lycksele (Sweden). Female syntype in ZMUL, '017', designated here as lectotype '*P. hyalipennis* male Lycks.'

Pipiza morionella (Zetterstedt 1843: 837). Type-locality: Dania (Denmark). In Peck (1988) this name was cited under genus *Neocnemodon*, but in Biosystematic Database of World Diptera (Pape & Thompson 2012) this taxon is considered as member of genus *Pipiza*. Holotype in ZMUL (male with-

out genitalia), '*P. morionella* male (*carbonaria* Meig. a Staeger)'. The holotype is from Denmark, and the specimen was sent to Zetterstedt by Staeger. The genitalia of the holotype is illustrated in Goeldlin de Tiefenau (1997).

Pipiza stigmatica (Zetterstedt 1859: 6029). Type-locality: In Scania ad Stödhaf (Sweden). Holotype in ZMUL ('023' male). Label 1: '*Pip. stigmatica* Zett. n.sp. male.'. Lable 2: 'coll C. Roth.'

Note: Among three potential Meigen names for this taxon (*P. anthracina*, *P. notata*, *P. bimaculata*), we accepted and chose *P. notata*, because in many collections specimens belonging to this taxon have this identification label. The name *P. bimaculata* was in collections used predominantly for specimens belonging to *P. noctiluca* in the sense of this revision. In recent identification keys the identity of *P. bimaculata* is either *P. notata* or *P. noctiluca* in the sense of this revision. In our opinion our interpretation of the names diminishes taxonomic confusion concerning the usage of these names hereafter.

Distribution. Distributional range of *P. notata* is narrower than that of *P. noctiluca*, from Fennoscandia south to France, and from Ireland eastwards through central Europe (Alps) into European parts of Russia and until south-east Greece. *Pipiza notata* nearly always occur sympatrically with *P. noctiluca*.

Pipiza quadrimaculata (Panzer, 1804):19 (*Syrphus*) (Figs S19D,E, S28, S29, S30A–C and S40C; Appendix S11)

syn. *quadriguttata* Macquart, 1829: 178

syn. *quadrimaculata* var. *bipunctata* Strobl, 1898: 230

syn. *quadrimaculata* var. *immaculata* Strobl, 1898: 230

Distribution. From Fennoscandia south to the Pyrenees; through northern, central and southeast Europe into Russia.

Doubtful names for European species of Pipiza – type not found or too damaged

These types cannot change the names of presented 12 taxa and they can only be regarded as *nomina dubia*. Continued inclusion of these in lists of recognized European species is meaningless.

Meigen

Pipiza leucopeza Meigen, 1838

Type: unspecified number of females presumably lost.

Macquart

Pipiza luctuosa Macquart, 1829

Holotype severely damaged only wing and part of scutum in MNHN.

Pipiza obscura Macquart, 1834

Holotype not present in MNHN, instead of specimen there is label 'Kassebeer loan'.

Rondani

Pipiza excalceata Rondani, 1857

Pipiza plana Rondani, 1857

Pipiza vidua Rondani, 1857

Szilady

Pipiza festiva var. *zonata* Szilady, 1935

Type presumably lost.

Key to European species of genus Pipiza

1 Antennae inserted just below middle of head (profile) (Fig. S29A,B). Abdomen broadly ovoid, usually with two pairs of yellow spots (one or both occasionally missing) (Fig. S30A–C). Male: sides of ocellar triangle distinctly longer than base. Transversely oval, backward pointing lobe of surstyli virtually bare on upper side (Fig. S28). Female: Vertex with at least predominately black hairs. Most hairs on sternite 2 shorter than thickness of hind tibia…… ……. *Pipiza quadrimaculata* (Panzer)
Antennae inserted in upper half of head (profile) (Figs S14A and S15A). Abdomen more elongate, with or without one or two pairs of yellow spots... 2

2 Hind femora without a pair of apico-ventral ridges (Fig. S3A); ventral part of basoflagellomere reddish; male genitalia: hypandrium with short lower gonocercus (about 1/3 length of theca) (Figs S2, S17 and S18)..... 3
Hind femora with a pair of apico-ventral longitudinal ridges (Fig. S3B); basoflagellomere dark; male genitalia: hypandrium with long lower gonocercus, in lateral view (about 3/4 length of theca) (as on Fig. S4)…..... 8

3 Holoptic: males…….....…… 4
Dichoptic: females.....…… 6

4 Face pale haired; basoflagellomere elongated (almost 1.5 times longer than wide) (Fig. S20A); tarsi yellow, only metatarsus of hind legs darkened; tergite 2 with long sticking out hairs; male genitalia: basal part of surstyli with well-developed semicircular lobe (Fig. S17)…… ……*Pipiza luteibarba* Vujić, Radenković & Polić
Face predominantly dark haired; basoflagellomere short, oval (wider than long) (Fig. S20C,E); tarsi of all legs partly darkened or at least of middle and hind legs; tergite 2 without long sticking out hairs; male genitalia: surstyli with reduced or with small basal semicircular lobe (Figs S2 and S18)…..…… 5

5 Male genitalia: surstyli with small basal semicircular lobe surpassing epandrium; inner part of basal semicircular lobe with dense pale spines (Fig. S2)…….....…… *Pipiza accola* Violovitsh
Male genitalia: surstyli with reduced basal semicircular lobe not surpassing epandrium and without inner spines (Fig. S18)…… ……*Pipiza luteitarsis* Zetterstedt

6 Tergite 5 longer than wide (Fig. S19C)...…… *Pipiza luteitarsis* Zetterstedt
Tergite 5 wider than long (Fig. S19A).....…… 7

7 Basoflagellomere short, oval (Fig. S20F); frons at the level of anterior margin of pollinose lateral spots broader than width of eye (dorsal view)……..…..……*Pipiza accola* Violovitsh
Basoflagellomere elongated (Fig. S20B); frons at the level of anterior margin of pollinose lateral spots narrower than width of eye (dorsal view)……..…… *Pipiza luteibarba* Vujić, Radenković & Polić

8 Tarsi completely pale.
Male: male genitalia (Fig. S8). Female: frons with larger pollinose spots (distance between same or narrower than lateral pollinose spots) (Fig. S37A), sternite 3 usually dark or only half pale; tergite 4 predominantly pale haired, wing with distinct spot (as on Fig. S32).....…… *Pipiza festiva* Meigen
Apical tarsal segments darkened……..…… 9

9 Hind femur with distinct apico-ventral ridge (Fig. S5B); male genitalia (Fig. S4). Tergite 1 in male on lateral sides pale haired…… *Pipiza austriaca* Meigen
Hind femur without or with less distinct apico-ventral ridge……..… 10

10 Males…… 11
Females…… 15

11 Surstylus elongated (Fig. S6)..…….…… *Pipiza carbonaria* (Meigen)
Surstylus not so elongated…… 12

12 Male genitalia large, epandrium and hypandrium long and narrow, length of basale of epandrium 1.0–1.2 mm. (Figs S7, S25A,B and S26A); tergites 2 and 3 usually with pale spots or markings (Fig. S35F,K–M), face usually pale haired…… *Pipiza fasciata* Meigen
Male genitalia: epandrium and hypandrium shorter and broader, length of basale of epandrium 0.5–0.9 mm (as on Fig. S26B–D)…..…… 13

13 Male genitalia smaller (Fig. S26B), length of basale of epandrium 0.5–0.65 mm, relation between length of surstylus and its ventral extension 3.2–3.5 (Fig. S31F); wing without distinct dark area along central part……..… *Pipiza notata* Meigen
Male genitalia bigger (as on Fig. S26C–D), length of basale of epandrium 0.7–0.9 mm, relation between length of surstylus and its ventral extension 2.4–2.8 (Fig. S31D–E)…… 14

14 Tergites shiny, non-pollinose, lateral sides of tergites 1–2 pale haired, male genitalia on Figs S9 and S39): lower gonocercus broad in basal 1/3 and narrow in apical 2/3; basoflagellomere elongated (Fig. S16A,D); wing usually with very distinct dark area along central part (as on Fig. S32)…….*Pipiza lugubris* Fabricius (and *laurusi* Vujić & Ståhls sp. n.)
Tergites pollinose on central parts (at least tergite 2), lateral side of tergites 1–2 predominately black haired (in central European populations), wing usually with less distinct dark area along central part, male genitalia (Fig. S38): lower gonocercus without clearly broader basal 1/3……..… *Pipiza noctiluca* (Linnaeus)

15 Wing clear, without a dark area along central part, or only slightly darkened (Fig. S41A)…...…….…… *Pipiza notata* Meigen
Wing with darkened area in central part, and with a clear border between pale and dark area on cell br (Fig. S41B)…….…….…… 16

16 Sternite 3 mostly pale, tergite 2 and usually 3 with pale spots (Fig. S35N,O); tergite 4 predominantly pale haired; metatarsus of hind leg darkened basodorsally, frons with smaller pollinose spots (distance between more than width of spot) (Fig. S37B), basoflagellomere usually rounded dorso-apically, wing spot not so obvious.……..…… *Pipiza fasciata* Meigen
Sternite 3 predominantly dark; tergite 3 without pale spots……..…… 17

17 Sternite 3 with long erect hairs, as long as on sternite 2. Body hairs longer (pale hairs usually whitish), frons pale haired (only few black ones), basoflagellomere not so elongated (Fig. S34B), anterior 1/5–1/6 of tergite 4 with stripe of black hairs centrally……..……. *Pipiza carbonaria* Meigen
Sternite 3 with shorter medially adpressed hairs……...…… 18

18 Tergite 4 often predominantly black haired (or sometimes with broad band of black hairs along anterior margin); tergite 2 with pale spots (Fig. S24C,I–K); basoflagellomere shorter (Fig. S16H–I); wing spot less obvious; microtrichia less reduced in basal cells; alula usually with reduced microtrichia……*Pipiza noctiluca* (Linneaues)
Tergite 4 predominantly pale haired, at least in posterior half; tergite 2 usually without pale spots; basoflagellomere elongated (Fig. S16B,C,E,F); wing spot obvious; microtrichia more reduced in basal cells; all alula usually covered with microtrichia…….. *Pipiza lugubris* Fabricius (and *laurusi* Vujić & Ståhls sp. n.)

Variability of Pipiza species

Except variability mentioned in the species accounts in the group of species with very similar morphological features and male genitalia three species can be separated:

A. Species with large genitalia, length of basale of epandrium 1.0–1.2 mm: *P. fasciata* (Fig. S25A,B);

B. Species with medium-sized genitalia, length of basale of epandrium 0.7–0.9 mm: *P. noctiluca* (Fig. S25C–G);

C. Species with small genitalia, length of basale of epandrium 0.5–0.65 mm: *P. notata* (Fig. S27) (Fig. S27A presents smaller specimen, 5.5 mm, and Fig. S27D bigger specimen, 8.5 mm).

The specimens of these three species figured on Fig. S26 are of the same length (7.5 mm); the male genitalia are very similar, but very different in size.

Traditional morphological characters used in keys for *Pipiza* taxa are very variable in almost all species, especially colour and arrangement of hairs, presence or absence of pale spots on tergites 2 and 3 (see under variability in the list of species). Also, some of characters (darkened area on wings, colour of antennae, colour of tarsi, hairs on face and mesoscutum, shape of spots on tergites) are more indications than diagnostic characters. The male genitalia, pollinosity of tergites and shape of hind femora are the most stable features.

The species *P. accola*, *P. austriaca*, *P. luteibarba*, *P. luteitarsis*, *P. festiva*, and *P. quadrimaculata*, can be easily separated using presented key. For delimitation of taxa *P. noctiluca* and *P. lugubris* complexes we successfully used an integrative taxonomy approach, but *P. lugubris* and *P. laurusi* sp.n. are not presently separable using traditional characters.

Discussion

We here present a resolution for the longstanding confusion on taxonomy and nomenclature of European *Pipiza*, and clarify and stabilize the status of taxa of *Heringia* sensu Claussen *et al.* (1994) as monophyletic lineages. These results will be useful for both taxonomy and ecology, but previous species identifications and reports on, for example, larval biology of *Pipiza* spp. should be reviewed in the light of the present results. The molecular data of the COI and 28S genes were informative for resolving the relationships among Pipizini genera. The two gene regions resolved the taxa almost in the same way, and the independence of the lineages is supported by critical morphological characters of male genitalia.

Sibling species are expected to show high morphological similarity. Some differences in morphology that allow for the discrimination of morphologically close taxa can be detected with the application of morphometric approaches (Moraes *et al.* 2004). During last decade mtDNA genes have been extensively used for species level studies in insect molecular taxonomy, with the COI gene region as the most commonly applied gene region. Low mtDNA COI sequence divergences between closely

related species-pairs of hoverfly taxa have also been registered, for example, between *Cheilosia* species (Ståhls *et al.* 2008). The low sequence divergence as such does not hamper the use of the informative nucleotide changes as supporting characters. Integrating and contrasting these character sources for the questions asked in this study was informative. Our results showed good agreement between geometric morphometrics of wings and the mitochondrial COI characters, and these data could discern and delimitate the taxa of the *P. noctiluca* and *P. lugubris* complexes. We found that geometric morphometrics of wings made a crucial contribution to this study, as traditional morphological differences between the taxa are recognizable but very subtle and thus in traditional sense do not appear as conclusive characteristics. We registered an unusually high correct classification rate for the taxa subjected to wing morphometric analyses. We conclude that our findings indicate that wings carry sufficient information to distinguish the taxa of the *Pipiza* complexes that we examined.

Synopsis of systematic and taxonomic changes of European *Pipiza* species

1. *Pipiza accola* Violovitsh, 1985
2. *Pipiza austriaca* Meigen, 1822
3. *Pipiza notata* Meigen, 1822
4. *Pipiza carbonaria* Meigen, 1822
5. *Pipiza fasciata* Meigen, 1822
6. *Pipiza festiva* Meigen, 1822
7. *Pipiza larusi* Vujić & Stahls sp. n.
8. *Pipiza luteibarba* Vujić, Radenković & Polić, 2008
9. *Pipiza luteitarsis* Zetterstedt, 1843
10. *Pipiza lugubris* Fabricius, 1775
11. *Pipiza noctiluca* (Linnaeus, 1758)
12. *Pipiza quadrimaculata* (Panzer, 1804)

Lectotypes are designated for the following 17 taxa: *P. anthracina* Meigen, 1822; *P. bimaculata* Meigen, 1822; *P. binotata* Zetterstedt, 1838; *P. calceata* Meigen, 1822; *P. fenestrata* Meigen, 1822; *P. festiva* Meigen, 1822; *P. funebris* Meigen, 1822; *P. geniculata* Meigen, 1822; *P. guttata* Meigen, 1822; *P. hyalipennis* Zetterstedt, 1838; *P. jablonskii* Mik, 1867; *P. lunata* Meigen, 1822; *P. lugubris*, *P. luteitarsis* Zetterstedt, 1843; *P. notata*, *P. signata* Meigen, 1822; and *P. vana* Zetterstedt, 1843.

Neotypes are designated for seven taxa: *P. albipila* Meigen, 1830; *P. albitarsis* Meigen, 1830; *P. artemis* Meigen, 1822; *P. austriaca* Meigen, 1822; *P. fasciata*, *P. ornata* Meigen, 1822; *P. rufithorax* Meigen, 1830.

The following 14 new synonymies are proposed: *P. albipila* syn.n. (=junior synonym of *P. austriaca*); *P. anthracina* syn.n. (=junior synonym of *P. notata*); *P. biguttula* Zetterstedt, 1838 syn.n. (=junior synonym of *P. notata*); *P. bimaculata* syn.n. (=junior synonym of *P. notata*); *P. binotata* syn.n. (=junior synonym of *P. notata*); *P. calceata* syn.n. (=junior synonym of *P. noctiluca*); *P. fenestrata* syn.n. (=junior synonym of *P. noctiluca*); *P. geniculata* syn.n. (=junior synonym of *P. lugubris*); *P. guttata* syn.n. (=junior synonym of *P. noctiluca*); *P. hyalipennis* syn.n. (=junior synonym of *P. notata*); *P. jablonskii* syn.n. (=junior synonym of *P. lugubris*); *morionella* Zetterstedt, 1843 syn.n. (=junior synonym of *P. notata*); *P. signata* syn.n. (=junior synonym of *P. lugubris*); *P. stigmatica* Zetterstedt, 1859 syn.n. (=junior synonym of *P. notata*).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Male genitalia, lateral view.

Fig. S2 Male genitalia of *Pipiza accola*.

Fig. S3 Hind femur of male, ventral view.

Fig. S4 Male genitalia of *Pipiza austriaca*.

Fig. S5 Hind leg of male, lateral view.

Fig. S6 Male genitalia of *Pipiza carbonaria*.

Fig. S7 Male genitalia of *Pipiza fasciata*.

Fig. S8 Male genitalia of *Pipiza festiva*.

Fig. S9 Male genitalia of *Pipiza laurusi* sp. n.

Fig. S10 Tergite 2.

Fig. S11 Boxplot of mean centroid size showing differences in the wing size between species *Pipiza carbonaria*, *P. lugubris* and *P. laurusi* sp. n.

Fig. S12 Scatter plot of individual scores of the two-first canonical axes showing shape differentiation between species *Pipiza carbonaria*, *P. lugubris* and *P. laurusi* sp. n.

Fig. S13 Thin-plate-spline deformation grids shape differences.

Fig. S14 Head of male of *Pipiza laurusi* sp. n.

Fig. S15 Head of female of *Pipiza laurusi* sp. n.

Fig. S16 Antenna, lateral view.

Fig. S17 Male genitalia of *Pipiza luteibarba*.

Fig. S18 Male genitalia of *Pipiza luteitarsis*.

Fig. S19 Abdomen, dorsal view.

Fig. S20 Antenna, lateral view.

Fig. S21 Boxplot of mean centroid size showing differences in the wing size between species of the *Pipiza noctiluca* complex.

Fig. S22 Scatter plot of individual scores of the two-first canonical axes showing shape differentiation between males of *Pipiza notata*, *P. noctiluca* and *P. carbonaria* species.

Fig. S23 Scatter plot of individual scores of the two-first canonical axes showing shape differentiation between females of *Pipiza notata*, *P. noctiluca* and *P. carbonaria* species.

Fig. S24 Thin-plate-spline deformation grids shape differences.

Fig. S25 Male genitalia.

Fig. S26 Male genitalia, lateral view (specimens with same size 7.5 mm).

Fig. S27 Male genitalia of *Pipiza notata*.

Fig. S28 Male genitalia of *Pipiza quadrimaculata*.

Fig. S29 Head of of *Pipiza quadrimaculata* lateral view.

Fig. S30 Abdomen, dorsal view.

Fig. S31 Surstylus, lateral view.

Fig. S32 Wing of male of *Pipiza austriaca*.

Fig. S33 Mesonotum, lateral view.

Fig. S34 *Pipiza carbonaria*, antenna, lateral view.

Fig. S35 Tergites 2 and 3, dorsal view.

Fig. S36 Tergite 2, dorsal view.

Fig. S37 Head of female, dorsal view.

Fig. S38 Male genitalia of *Pipiza noctiluca*.

Fig. S39 Male genitalia of *Pipiza lugubris*.

Fig. S40 Frons of female, dorsal view.

Fig. S41 Wing of female.

Table S1 List of specimens used for molecular analyses.

Table S2 List of specimens used for geometric morphometric analysis, by geographical area and species.

Table S3 Percent of correctly classified specimens of *Pipiza lugubris* complex.

Table S4 Percent of correctly classified specimens of *Pipiza notata* complex with outgroup species *P. carbonaria.*

Appendix S1 Additional information for species *Pipiza accola* Violovitsh, 1985.

Appendix S2 Additional information for species *Pipiza austriaca* Meigen, 1822: 252.

Appendix S3 Additional information for species *Pipiza carbonaria* Meigen, 1822.

Appendix S4 Additional information for species *Pipiza fasciata* Meigen, 1822: 242.

Appendix S5 Additional information for species *Pipiza festiva* Meigen, 1822: 243.

Appendix S6 Additional information for species *Pipiza luteibarba* Vujić, Radenković & Polić, 2008.

Appendix S7 Additional information for species *Pipiza luteitarsis* Zetterstedt, 1843: 828.

Appendix S8 Additional information for species *Pipiza lugubris* (Fabricius, 1775): 770 (*Syrphus*).

Appendix S9 Additional information for species *Pipiza noctiluca* (Linnaeus, 1758): 593 (*Musca*).

Appendix S10 Additional information for species *Pipiza notata* Meigen, 1822: 246.

Appendix S11 Additional information for species *Pipiza quadrimaculata* (Panzer, 1804): 19.