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Phylogenetics and morphological study of the Iberian flightless Tachydromiini (Diptera: Hybotidae) unveil overlooked diversity

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Resumo

Os Diptera (moscas e mosquitos) são uma ordem incrivelmente diversa, mas muito pouco estudada, cuja maioria das espécies não está ainda descoberta. É também um grupo de insectos que normalmente é visto como nocivo e desagradável o que, a par com a sua diminuta dimensão, reduz o interesse no seu estudo. De resto, o estudo da sua taxonomia, biologia, ecologia e evolução, são ferramentas basilares para contrariar este cenário e conhecer o estado de conservação destes animais. Não é possível conservar qualquer ser vivo sem que antes se produza conhecimento fundamental.

Entre os dípteros, na família Hybotidae, existe um género com mais de 100 espécies de distribuição mundial, as *Tachydromia* Meigen, 1803. São pequenas, escuras, mas predadoras vorazes de outras moscas. Existem sobretudo em zonas temperadas e têm normalmente hábitos cursoriais. A nível taxonómico foram classificadas em nove grupos de espécies com base em características morfológicas.

Os hábitos cursoriais foram levados ao extremo em quatro espécies de *Tachydromia*, cujas asas se reduziram ao ponto de não permitirem o voo. O nível de redução da asa é variável nestas espécies de distribuição disjunta, no entanto, uma delas, *Tachydromia apterygon* Plant & Deeming, 2006 (Itália), tem asas reduzidas a simples escamas e perdeu também os halteres. De resto, a perda de asas é um fenómeno comum na família Hybotidae, sendo que diversas hipóteses para a sua origem têm sido sugeridas, tais como a estabilidade do habitat.

Existe um grupo de espécies não-voadoras que se assemelha bastante às *Tachydromia* em termos morfológicos. São um endemismo da Península Ibérica e dos Pirenéus e ocorrem na manta morta de florestas de folha caduca, em regiões temperadas. Cinco espécies foram anteriormente descritas, tendo levado à criação de dois géneros: *Pieltainia* Arias, 1919 (uma espécie) e *Ariasella* Gil, 1923 (quatro espécies). Estes géneros foram erguidos para acomodar estas espécies simplesmente com base no facto de não possuírem nem asas funcionais - ou de serem totalmente ápteras no caso de *Pieltainia* - nem halteres.

As asas não funcionais destas moscas, que se assemelham na forma e comportamento a formigas, reduziram-se a um filamento com um lobo na parte distal, estando este lobo ausente nas fêmeas (que podem também ter as asas reduzidas a uma escama). Pelo menos no caso de uma das espécies, *Ariasella lusitanica* Grootaert et al., 2009 as asas têm uma função sinalizadora durante o acasalamento, em que são abanadas em frente aos olhos da fêmea.

Com alguma exceção no caso de *A. lusitanica*, estas espécies endémicas não-voadoras estavam muito pouco estudadas a praticamente todos os níveis de conhecimento (e.g. morfologia, comportamento, ecologia, sistemática). Em concreto, as descrições originais são muito superficiais e não incluem caracteres importantes para a taxonomia, como a terminália masculina. Também a distribuição geográfica se conhecia praticamente apenas da localidade tipo e os holótipos de algumas espécies encontram-se provavelmente perdidos ou não foram sequer designados.

Recentemente, foi proposta a sinonímia destes dois géneros com *Tachydromia*, contudo, apenas uma espécie foi estudada a nível molecular, onde não se incluiu *Pieltainia*.

O presente estudo tem por objectivo contrariar a falta de conhecimento atual sobre estas moscas endémicas não-voadoras. É, portanto, uma revisão da posição sistemática, biologia e comportamento destas espécies, tendo por base técnicas como: observação dos comportamentos de espécies chave, inferência molecular multilocus, descrições morfológicas detalhadas e prospeção de habitats.

As observações comportamentais basearam-se em espécimes (10 machos, 10 fêmeas) mantidos em cativeiro, pertencentes a duas espécies, uma áptera (originalmente descrita como *Pieltainia iberica* Arias, 1919) e outra com asas filiformes (originalmente *Ariasella semiaptera* Gil, 1923). Foram alimentados com Sciaridae, uma das suas presas naturais.

Para a análise e descrição morfológica, obtiveram-se ilustrações da terminália masculina e asas filiformes, assim como imagens de microscopia de varrimento das asas reduzidas a escamas e dos esporões presentes na tíbia do macho. Também se obtiveram fotografias de todas as espécies (macho e fêmea) tanto vivas, como montadas em coleção. O macho de *Tachydromia pieltaini* (Gil, 1936) e a fêmea de *Tachydromia apterygon* foram descritos pela primeira vez. Um lectótipo foi designado para *Tachydromia pandellei* (Séguy, 1941). Além disso, foi elaborada uma chave taxonómica para todas as espécies não-voadoras Ibéricas (e dos Pirenéus) e para *T. apterygon*.

Em concreto, na análise molecular, utilizaram-se cinco marcadores mitocondriais e nucleares (28S, 12S, PGD, AATS, COI) e recorreram-se a dois métodos de análise filogenética, nomeadamente à máxima verossimilhança e à inferência Bayesiana. Incluíram-se 31 espécies separadas em 35 espécimes, das quais fazem parte todas as espécies endémicas não-voadoras, a italiana *Tachydromia apterygon*, além de quatro *Tachydromia* com asas funcionais. Como *outgroup*, incluíram-se representantes das subfamílias de Hybotidae e um Empididae (família filogeneticamente muito relacionada).

Em resultado das análises efectuadas, descreveram-se quatro espécies anteriormente desconhecidas. O resultado da inferência filogenética demonstra uma sinonímia óbvia entre *Pieltainia*, *Ariasella* e *Tachydromia*. A falta de valor filogenético da asa é evidenciada pelo posicionamento da espécie tipo de *Ariasella* como táxon irmão das *Pieltainia* e das restantes *Ariasella*. As espécies endémicas não-voadoras formam um grupo evolucionariamente relacionado, em que as relações filogenéticas observadas são congruentes com os caracteres morfológicos.

As espécies aqui descritas (*T. ebejeri* sp. nov., *T. cantabrica* sp. nov., *T. stenoptera* sp. nov. e *T. nigrohirta* sp. nov) estão agrupadas no conjunto do qual *T. lusitanica* faz parte. São espécies morfologicamente similares, quase todas com asas filamentares no macho à exceção de *T. ebejeri* sp. nov., em que ambos os sexos são micrópteros.

Tachydromia semiaptera - anteriormente classificada no género *Ariasella* da qual era a espécie tipo - é sem dúvida a espécie menos relacionada deste grupo, tal como a sua morfologia sugere. Entre as características que lhe são únicas, encontra-se a asa muito maleável (em oposição à asa filamentar rígida das outras espécies) e o facto de o macho possuir duas projeções na tíbia média, em vez de uma única como nas restantes espécies. Este é o único caso conhecido no género. Além disso, é uma estrutura com provável relevância evolutiva, uma vez que o macho segura as fêmeas durante a cópula com recurso às patas médias.

A espécie tipo do género *Pieltainia*, agora classificada como *Tachydromia iberica*, não possui a maioria dos caracteres morfológicos de relevância taxonómica encontrados nas espécies relacionadas. As diferenças encontradas entre populações ao nível da terminália masculina são pouco relevantes a nível taxonómico e os espécimes sequenciados são muito próximos filogeneticamente, tal como inferido a partir da análise molecular.

Finalmente, as espécies irmãs, *T. pieltaini* e *T. pandellei*, apesar de serem morfologicamente muito similares, possuem características óbvias que permitem uma identificação fácil. Estas diferenças estão de acordo com a inferência filogenética, pois encontram-se separadas por ramos relativamente longos.

Não é possível associar facilmente as espécies endémicas não-voadoras aos grupos de espécies definidos para *Tachydromia*, apesar de se aproximarem do grupo *arrogans*. A posição de *T. apterygon* no grupo *terricola* não está de acordo com a inferência filogenética, estando filogeneticamente mais próxima das espécies ibéricas.

A evolução das asas nas *Tachydromia* não-voadoras, originalmente *Ariasella* e *Pieltainia*, parece estar relacionada com pressões tanto ecológicas como, pelo menos em um caso, sexuais. As observações comportamentais revelaram que *T. semiaptera* não usa as asas filamentares durante a cópula, apesar de serem superficialmente similares às de *T. lusitanica*. Em vez disso, usa as patas anteriores, cuja tíbia é caracteristicamente inflada e escura, da mesma forma que *T. lusitanica* usa as asas (abanando-as em frente aos olhos da fêmea). *T. iberica* também recorre às patas anteriores, amarelas e pretas, dando-lhes o mesmo uso.

As espécies ibéricas, de acordo com a prospeção efetuada, parecem ocorrer em habitats relativamente similares, tendo requerimentos ecológicos provavelmente próximos. É possível que tenham evoluído paralelamente em habitats similares, na sequência de processos de isolamento geográfico. Sendo que a existência de habitats estáveis como montanhas e florestas favorecem a perda de asas, é possível que estes factores ecológicos tenham tido importância na perda de asas funcionais destas espécies. Contudo, pelo menos no caso de *T. lusitanica*, as asas não só se reduziram como, sob pressão sexual, passaram a ter uma função de sinalização sexual durante a cópula.

Com base no conhecimento obtido, pode formular-se uma hipótese relativa à evolução deste grupo. Os nós curtos da árvore filogenética podem indicar uma rápida sucessão de eventos de divergência. Provavelmente, uma linhagem ficou separada geograficamente e a partir daí evoluiu de diferentes modos (e.g. asas), o que impede que as diferentes entidades resultantes se cruzem atualmente mesmo na presença de contacto secundário.

O trabalho de campo levou a um aumento significativo do número de localidades conhecidas da maioria destas espécies e, além disso, revelou um padrão relativo ao bioclima em que existem. Assim, parecem estar associadas a habitats sob influência supra-mediterrânica e temperada, ocorrendo sobretudo em florestas de folha caduca e marcescente, em montanhas. Estes habitats encontram-se frequentemente fragmentados pelas mais diversas atividades de origem antrópica. Em conjugação com o aquecimento global previsto, são possivelmente ameaças importantes à conservação deste grupo de espécies endémicas.

Palavras-chave: Península Ibérica, Endemismo, Hybotidae, não-voador, Filogenia

Abstract

Phylogenetic inference, based on five molecular markers (COI, 28S, ATTS, 12S, PGD), provides further evidence to confirm the synonymy of the flightless genera *Pieltainia* Arias, 1919 and *Ariasella* Gil, 1923 with the genus *Tachydromia* Meigen, 1803. Molecular and morphological data are largely congruent for all known species of flightless *Tachydromia* and four new species for science are described: *T. ebejeri* sp. nov., *T. stenoptera* sp. nov., *T. cantabrica* sp. nov. and *T. nigrohirta* sp. nov. Species habitus (live and mounted), terminalia of the male, spur-like structures of male mid tibia, and wings are illustrated/photographed for all species (nine Iberian and one Italian species), most of them for the first time. The male of *Tachydromia pieltaini* Gil, 1936 and the female of *Tachydromia apterygon* Plant & Deeming, 2006 are described for the first time, while a lectotype is assigned to *Tachydromia pandellei* Séguy, 1941. A key to all Iberian flightless *Tachydromia* and the Italian *T. apterygon* is supplied. Knowledge on the geographic distribution of most species is considerably enhanced. These species tend to occur in temperate and supra-mediterranean regions, mostly in deciduous/ marcescent forests, which are habitats that are often fragmented. Coupled with global warming, this likely has a relevant impact on the Conservation of these endemic species. The mating behaviour of *Tachydromia semiaptera* Gil, 1923 and *Tachydromia iberica* Arias, 1919 is documented for the first time. It is likely that vicariance processes took place in the evolution of the Iberian ant-like flies, which evolved different sexual signalling strategies under similar ecological conditions but without gene flow between them. The ecological conditions may have helped drive the loss of functional wings, while, at least one species, evolved to use the reduced wing as a structure with sexual signalling function. We propose a change in the definition of terms apterous and micropterous to properly accommodate the diversity of wing states in this cluster of species.

Keywords: Iberian Peninsula, Endemic, Hybotidae, Flightless, Phylogeny

List of Abbreviations and Symbols

♀	Female
♂	Male
12S	12S small ribosomal subunit
28S	28S ribosomal RNA
AATS	Alanyl-tRNA synthetase
COI	Cytochrome c oxidase subunit I
DNA	Deoxyribonucleic Acid
e.g.	"for example"
GBOL	German Barcode of Life
i.e.	"that is"
KOH	Potassium hydroxide
PCR	Polymerase chain reaction
PGD	6-phosphogluconate dehydrogenase
sp.	Singular species
spp.	Plural of species (sp.)

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1. Introduction

1.1. Diptera: a poorly known, megadiverse order

Diptera (flies and mosquitoes) is a good example of an extremely diverse group of organisms that is generally disliked and often perceived as being unpleasant and noxious to humans. However, the species that are agricultural pests or carry diseases are just a very small fraction of the overall diversity (McLean 2000). They occur in practically all terrestrial and aquatic environments, occupy a great variety of trophic levels, and have a great range of interactions with other species. Furthermore, they possess an impressive morphological diversity, with its taxa presenting very distinct and, often, unique phenotypes. However, these animals are usually small and, as most inconspicuous species of any order, tend to be neglected even by entomologists. Hence, with approximately 160.000 described species worldwide, Diptera comprises one of the extant megadiverse orders of insects but the knowledge regarding its diversity remains very incomplete, with most species awaiting description (Marshall 2012).

The mentioned general lack of fundamental knowledge coupled with societal dislike of Diptera (and of invertebrates in general) has important consequences, being, for example, an enormous obstacle to establish proper conservation efforts (Samways 1993). To tackle this issue, the process of categorizing and describing diversity is, without a doubt, a vital step to gather wider information, including on ecology and evolutionary history (Troudet et al. 2017). Hence, taxonomy as well as information on life history, ecology and evolution, are essential tools to conservation itself because it is not possible to conserve organisms without adequate knowledge and description (Mace 2004).

1.2. Scope taxa: Iberian ant-like *Tachydromia*

The genus *Tachydromia* Meigen, 1803 (Diptera, Hybotidae) includes over 100 species distributed worldwide, most of them occurring in the temperate regions of the Holarctic (Shamshev & Grootaert 2018). These are small-bodied flies (1-3.5 mm) usually shining black to blackish brown and with dark banded or clouded wings (Chvála 1975). This genus is currently divided into nine species-groups based on morphological characters, hence Chvála (1970) and Chvála (1975) defined eight groups of species (*terricola*, *ornatipes*, *arrogans*, *interrupta*, *connexa*, *calcanea*, *annulimana*, *punctifera*) and Stark & Doczkal (2017) added the *acklandi* group.

There is no published information on the immature stages, but adults are predators of small flies, mostly nematocerans. They are often encountered in large numbers, swiftly running either on vertical or horizontal surfaces such as tree trunks, logs, low vegetation and river margins (Chvála 1970). These frequent cursorial habits are taken to an extreme in four species of *Tachydromia*, which have evolved to become flightless. *Tachydromia rossica* Shamshev, 1994 (Russian Far East and Mongolia) presents a stalk-like wing, without the lobed tip, but preserves the halteres. *Tachydromia microptera* (Loew, 1864) and *Tachydromia schnitteri* Stark, 1996 (central Europe) are brachypterous and have halteres (venation and shape similar to macropterous *Tachydromia*). *Tachydromia apterygon* Plant & Deeming, 2006 (Italy) has wings reduced to a small scale and is the only species without halteres. Wing reduction is present in at least 20 Diptera families (Hackman 1964) and it is frequent in Hybotidae (Grootaert & Shamshev 2008). It seems to be explained by several factors, including the stability of the habitat and cryptic behaviour (Hackman 1964).

A morphologically related cluster of flightless species endemic to the Iberian Peninsula and the Pyrenees closely resembles these flightless *Tachydromia*. These are minute ant-like flies, *ca.* 2.5 mm, which occupy specific microhabitats among the leaf litter of deciduous forests. They were originally placed in

the genera *Pieltainia* Arias, 1919 and *Ariasella* Gil, 1923 (Tachydromiini). Five species of *Ariasella* and *Pieltainia* have been described: *Pieltainia iberica* Arias, 1919, *Ariasella semiaptera* Gil, 1923, *Ariasella pieltaini* Gil, 1936, *Ariasella pandellei* Séguy, 1941 and, more recently, *Ariasella lusitanica* Grootaert, Shamshev & Andrade, 2009. Despite presenting similarities with *Tachydromia*, they were originally assigned to the above-mentioned genera solely based on the complete absence of halteres and on the extremely reduced or absent wings. Hence, the monospecific *Pieltainia* is characterized by aptery in both sexes, while in *Ariasella* spp. males are stenopterous – i.e. very narrow but long wings (complete loss of flight ability) (Figs 20E, 21A–B, D). *Ariasella* males present tubular, stalk-like wings with a lobed tip, which, at least in *A. lusitanica*, are waved during copulation (Andrade 2011). In females, both *A. semiaptera* and *A. lusitanica* are micropterous (Figs 20F, 21E) – wings reduced to a minute bilobed scale – while females of *A. pandellei* and *A. pieltaini* are stenopterous but lack the developed lobed tip present in males (Fig. 21C).

Probably due to a combination of their small size, cryptic habitat preferences and relatively short period of activity, most Iberian ant-like species were poorly studied at the morphological, behavioural, systematic and ecological level. The original descriptions are generally superficial and lack information on important taxonomic characters, such as the male terminalia. The species distribution was also only known from either the type locality or a very few more regions, generally based on few specimens, and in the case of *A. pieltaini*, only the female was known and described. Additionally, the types of *P. iberica*, *A. semiaptera*, and *A. pieltaini* seem to be lost and no holotype was assigned to *A. pandellei*. There is, however, a noteworthy exception to this scenario which is the case of *A. lusitanica*, which description includes the male terminalia and other important details of taxonomic relevance. Additionally, several important observations on its behaviour were published by Andrade (2011). Other than that, the only other published observations on behaviour or habitat preferences were the brief ones provided by Arias (1919) on *Pieltainia iberica*, describing its great voracity by preying on Sciaridae (Diptera, Nematocera) and agility, resembling small ants quickly running about on the ground.

A recent work (Shamshev & Grootaert, 2018) proposed *Ariasella* and *Pieltainia* as synonyms to *Tachydromia*, based on a perceived lack of morphological differences with taxonomic value between the genera, as well as on previous reconstructions of phylogenetic relationships (J. Mortelmans, unpublished data; Nagy *et al.* 2013). However, within this work, the Iberian species were again underrepresented, and as such important questions on their evolutionary history remain unanswered.

1.3. Aims of the study and its relevance

This research study has the overall aims of **i)** inferring the phylogenetic relationship between *Pieltainia* and *Ariasella*, **ii)** and how differentiated are the species, **iii)** providing further evidence on the synonymy of *Ariasella* and *Pieltainia* concerning *Tachydromia*, **iv)** shedding light on the wing evolution of the Iberian species and, finally, **v)** obtaining information on habitat preferences and distribution patterns with importance on species Conservation.

This study is a comprehensive overview of the systematics and biology of the overlooked diversity of Iberian *Tachydromia*-like flightless flies. A combination of multilocus sequence data, detailed morphological descriptions and behavioural observations is employed to unravel the previously neglected diversity of this remarkable group of species. Using this data, an updated proposal for the systematics of the group, including the description of four new species, is provided. Additionally, a

considerable knowledge improvement on the geographic distribution of these species is obtained, as well as new observations on mating behaviour.

2. Material and Methods

2.1. Sampling, species distribution and specimens' deposition

Specimens were manually collected with a vial after direct observation. Afterwards, they were preserved either dry-mounted, in 70% ethanol or absolute ethanol (for molecular analysis). Distribution data was compiled from published records and based on recent sampling efforts. Several sampling expeditions took place along the Iberian Peninsula and the Pyrenees, mostly from January to July. Historical and new occurrence records were plotted using QGIS v. 2.18.16, with the coordinates projected in the reference system Madrid 1870 (Madrid), code 4903. The raster map dataset (Natural Earth II with Shaded Relief, Water, and Drainages) was obtained from https://www.naturalearthdata.com.

The abbreviations used for deposit sites are given below:

LNHM: London Natural History Museum, United Kingdom.

NMGW: National Museum Wales, United Kingdom.

RBINS: Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

In the absence of mention to a deposit site, the specimen is deposited in the author's collection.

2.2. Morphological analysis

Terms used for adult structures primarily follow those of McAlpine (1981), although the terminology for the antenna and for the male terminalia follows Stuckenberg (1999) and Sinclair & Cumming (2006), respectively. Male terminalia were macerated in cold 10% KOH and hot 85% lactic acid and immersed in glycerine. Drawings of the terminalia were made with a *camera lucida* attached to a compound microscope. Drawings of the wing were made based on direct observation by using a stereomicroscope, SteREO Lumar.V12 (Carl Zeiss MicroImaging). Images of minute wings and spurs were obtained using a scanning electron microscope JSM-5200LV (JEOL, Ltd.). Photographs of pinned specimens were taken with a Canon EOS 7D mounted on a P–51 Cam-Lift (Dun Inc.) and with the help of Adobe Lightroom (version 5.6). Afterwards, the images were stacked using the software Zerene Stacker 1.04. Photographs of live specimens were taken with a Fujifilm FinePix HS10, with a Raynox DCR-250 macro lens.

Body length was measured from frons to end of terminalia in lateral view. Wing length was measured from the wingtip to its base. Measurements were taken in dry specimens. In descriptions, the right and left side of the male terminalia are based on the unrotated position viewed posteriorly, such that in the illustrations the right surstylus appears on the reader's left side and vice versa. All male terminalia are figured in their unrotated position.

2.2.1. Wing reduction terminology in Diptera

Hackman (1964) proposed the terminology regarding the degree of wing reduction in Diptera, as follows: **brachypterous**: wing reduced, shorter than abdomen, broad and more or less blunt, not permitting flight, with at least radial veins distinct; **stenopterous**: wing very narrow but sometimes long, not permitting flight, with at least radial veins distinct; **micropterous**: wing reduced to small appendage

of varying shape with at most traces of the radial vein; **apterous**: wing at most represented by a minute scale, at most carrying some setae or totally absent. Recently, Roháček (2012) added the term **submacropterous**: wing similarly shaped, including venation, as in normal macropterous specimens but distinctly shorter, darker, about as long as abdomen. Here we propose a modification to this terminology where the term **micropterous** assumes a more inclusive definition: wing reduced to small appendage, stub or minute scale, of varying shape with at most traces of the radial vein present; furthermore we alter the term **apterous**, with its definition being restricted to wing totally absent, without any traces of it present.

2.3. Observations on mating behaviour

Observations in captivity were made for *Tachydromia semiaptera* (population of Spain, Cáceres, Las Villuercas) and *Tachydromia iberica* (population of Portugal, Leiria, Arrimal). These species were selected due to their level of wing reduction: the first species is stenopterous, with very malleable stalk-like wings (in opposition to the rigid wings of the other stenopterous Iberian ant- like species); and the second species is apterous. The mating behaviour of a brachypterous species with rigid stalk-like wings, *T. lusitanica*, was described by Andrade (2011). Specimens were kept in a small terrarium (27x15x17 cm), with a section of 4cm deep soil, including leaf litter. In two separated instances, each species (10♂♂, 10♀♀) was kept and observed for five consecutive days. Observations were made using a Fujifilm FinePix HS20 EXR, with a Raynox DCR-250 macro lens. Specimens of the nematoceran Sciaridae were offered as food resource. To test intraspecific interactions with pre-defined conditions (e.g. quantity of specimens, sex), specimens were temporarily taken from the terrarium and put inside transparent plastic vials. Various types of interactions were observed but here only the mating behaviour is described due to its relevance to help understand the evolutionary importance of wing reduction/modification. These non-systematic observations were aimed at obtaining qualitative descriptions of behaviour.

2.4. Molecular analysis

2.4.1. Taxon sampling

The taxon sampling included 35 specimens belonging to 31 different species: 30 Hybotidae and one Empididae. We sequenced and analysed for the first time nine taxa, namely eight Iberian ant-like species (described and undescribed species) as well as *Tachydromia apterygon*. Additionally, it included four macropterous and one apterous species of *Tachydromia* assigned to three species-groups *sensu* Chvála (1970): *terricola*, *connexa* and *arrogans*. The abovementioned taxa constitute the ingroup. Representatives of the subfamilies of Hybotidae were included as outgroups, as well as the tribes within Tachydromiinae *sensu* Sinclair & Cumming (2006). *Empis tessellata* Fabricius, 1794 (Empididae) was used to root the tree. Table 1 lists the species included in the analysis, as well as the collection data. The samples were made available either by obtaining freshly collected specimens or from the GBOL DNA collection preserved in the ZFMK BioBank.

2.4.2. Molecular marker selection

Three nuclear genes (28S, PGD, AATS) and two mitochondrial genes (COI and 12S) were selected to be sequenced. The choice of these genes is based on their different evolutionary rates and on the existence of primers (Table 2) used in groups of insects, including some specific for Diptera. Genes that often have higher evolutionary rates (mitochondrial) are useful for lower-level systematics, while genes with lower evolutionary rates (nuclear) are useful for higher-level systematics.

2.4.3. DNA extraction and sequencing

Entire specimens, ethanol preserved and stored at -20º C, were used for DNA extraction. Extractions were carried out using the Dneasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) to extract total nucleic acids following the manufacturer's instructions; samples were resuspended in 50– 100 µl ultra-pure water. Entire specimens were preserved and labelled as DNA voucher specimens for the purpose of morphological studies and deposited at the ZFMK. The Qiagen Multiplex PCR Kit (Qiagen, Valencia, CA, USA) was used to carry out the PCRs. PCRs (20 µl) included 2.5 µl DNA extract, 1.6 µl of each primer (at 10 pmol/µl), 2 µl of Q-solution, 10 µl of Qiagen Multiplex-Mix and 2.3 µl of ultra-pure water. PCR amplifications were carried out with a Biometra PCR Thermal Cycler. PCR "touchdown" programs are listed in Table 3, primers used for amplifying and sequencing the molecular markers are listed in Table 4. Amplified DNA was electrophoresed on 1.5% agarose gels for visual inspection of amplified products. PCR products were enzymatically treated with gel purification using the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). Sequencing reactions were carried out by Macrogen Inc. using the same primer pairs as those used for PCR reaction.

2.4.4. Sequence alignment

The sequences were edited for base-calling errors and assembled using Geneious R7 (version 7.1.3, Biomatters Ltd.). To detect contamination, sequences were compared with known sequences of relatives as well as the GenBank database using Blast Madden (2013). The alignment of the protein-coding COI gene was done manually, and it was not necessary to include gaps in this alignment. The genes 12S, AATS and PDG were aligned by multiple alignment using fast Fourier transform (MAFFT) program (Katoh *et al*. 2005, 2009) version 7, which implements iterative refinement methods (Katoh and Standley 2013). The E-INS-I algorithm was selected as it is optimized for small-scale alignments and is recommended for sequences with multiple conserved domains and long gaps, such as rRNA genes (Katoh *et al*. 2009). The AATS sequenced contained an intron consisting of 206 nucleotides, which were deleted as they were not useful due to ambiguous alignment. The alignment of 28S gene was done by following its secondary structure, as explained by Kjer (1995) and two versions of the sequence were produced. The REC and RSC regions, which can't be aligned, were kept in one version of the sequence (non-trimmed) and deleted from another (trimmed).

2.4.5. Sequence analysis

Pair-wise evolutionary distance among the Iberian ant-like species (11 nucleotide sequences belonging to 9 taxa) was determined by using the p-distance substitution model in the software program MEGA7 (Kumar et al. 2016).

2.4.6. Phylogenetic analyses

For the combined dataset, two different methods of phylogenetic analysis, maximum-likelihood (ML) and Bayesian inference (BI), were performed. For both analyses, the molecular data set was divided into 11 partitions: first, second and third codon positions of COI, AATS and PGD, the genes 28S and 12S. The best choice of model was determined for each partition using jModelTest 2.1.5 (Darriba *et al*. 2012) under the Akaike Information Criterion (AIC), as recommended by Posada & Buckley (2004), and the data was analysed under the recommended models (see Table 4).

ML analyses were performed using the Genetic Algorithm for Rapid Likelihood Inference, Garli v2.01 (Zwickl 2006). Forty-six independent runs (46 different runs with the command searchreps = 1) were conducted using the scorethreshforterm = 0.05 and significanttopochange = 0.001 settings and using the automated stopping criterion, terminating the search when the ln score remained constant for 50, 000

consecutive generations. Bootstrap support values (BS) were estimated from 1000 replicates using the same independent models in Garli.

Phylogenetic estimation using the Markov Chain Monte Carlo algorithm as implemented in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was performed using a parallelized version of the software (XSEDE in CIPRES Science Gateway). Priors were applied with default values. Six runs, with four chains each (one "cold" chain and three heated chains; temp = 0.2), were performed simultaneously for 20,000,000 generations which were sufficient to bring the convergence (average standard deviation) to a value of < 0.003, sampling trees every 2,500 generations. The initial 2,000 trees (25%) were discarded as burn-in, and Bayesian posterior probabilities (PP) were calculated using a 50% majority-rule consensus tree inferred from the data. Analytical runs were performed on CIPRES Science Gateway (Miller et al. 2010) and all trees were drawn with the aid of FigTree 1.4.2 (Rambaut 2009) and Adobe Illustrator cc 19.2.1.

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3. Results

3.1. Sequence characteristics

Aligned nucleotide lengths of gene fragments were of 1,504 for COI, 518 for AATS, 382 for 12S, 766 for PGD, 838 for non-trimmed 28S and 791 for trimmed 28S. The total datasets included 4008 nucleotide characters (non-trimmed 28S) and 3961 nucleotide characters (trimmed 28S). Of the 35 specimens included in the analysis, sequences were obtained from 35 individuals, representing the selected 31 taxa, for COI (100% of the data), 19 for 12S (54%), 33 for 28S (94%), 23 for AATS (66%) and 25 for PGD (71%). *Tachydromia* and ant-like species (i.e. ingroup) were overall successfully sequenced and the amplification success only failed regarding PGD in *T. nigrohirta* sp. nov. and in *T. cantabrica* sp. nov., AATS and 28S in *T. apterygon*. Among the Iberian ant-like species, the uncorrected pairwise sequence divergences (*p*) for the COI gene (Table 5) varied from 8% between *Tachydromia lusitanica* and *T. nigrohirta* sp. nov., to 12% between *T. semiaptera* and *T. pandellei*, and *T. semiaptera* and *T. pieltaini*. The COI *p*-distance at intraspecific (interpopulational) level regarding *T. iberica* varied from 4% (between Leiria and Madrid) to 5% (between Huelva and Madrid, Huelva and Leiria). A nexus file with the final alignment is provided as Supporting Information and two additional text files are also given in the Supporting Information with the original structural alignment for 28S.

3.2. Phylogenetic relationships reconstruction

The ML tree, non-trimmed 28S version, with the best likelihood score (ln L = -29397.646621) is presented in Fig. 1. Although the taxonomic sampling was mainly focused on the diversity and phylogenetic relationships within the Iberian flightless ant-like species (originally placed in genera *Pieltainia* and *Ariasella*) and their systematic position related to *Tachydromia*, the Hybotidae subfamilies and respective tribes within Tachydromiinae were included in our analyses, hence, we would as well like to comment on the topology of these taxa. The relationships between the subfamilies of Hybotidae received low support, thus it is not possible to draw any conclusion regarding their affinities, however, the evolutionary relationships within the subfamily Tachydromiinae generally

received strong support. This subfamily was recovered as monophyletic, with the tribe Symballophthalmini as a sister group to Drapetidini [*Drapetis ephippiata* + *Crossopalpus* sp.) + *Stilpon* sp.] and to Tachydromiini (BS= 100; PP= 1). The relationship between Drapetidini and Tachydromiini was resolved with a high posterior probability of 0.97 and a moderate bootstrap value of 71. The phylogenetic relationships between the Tachydromiini genera [(*Platypalpus* + (*Tachypeza* + *Tachydromia*)] were overall inferred with very high support. Regarding the ingroup, which the present analysis was designed to study, *Tachydromia* genus was recovered as paraphyletic concerning the species originally assigned to genera *Ariasella* and *Pieltainia*. The evolutionary relationships within the ingroup (*Tachydromia* + "*Ariasella*" + "*Pieltainia*") depicted in our results are of a very well-supported grouping with many internal nodes with high to moderate support. (*Tachydromia connexa* + *T. terricola*) - comprising the single species sampled for *connexa* and *terricola* species-groups, respectively - was resolved as a sister clade to the group containing the

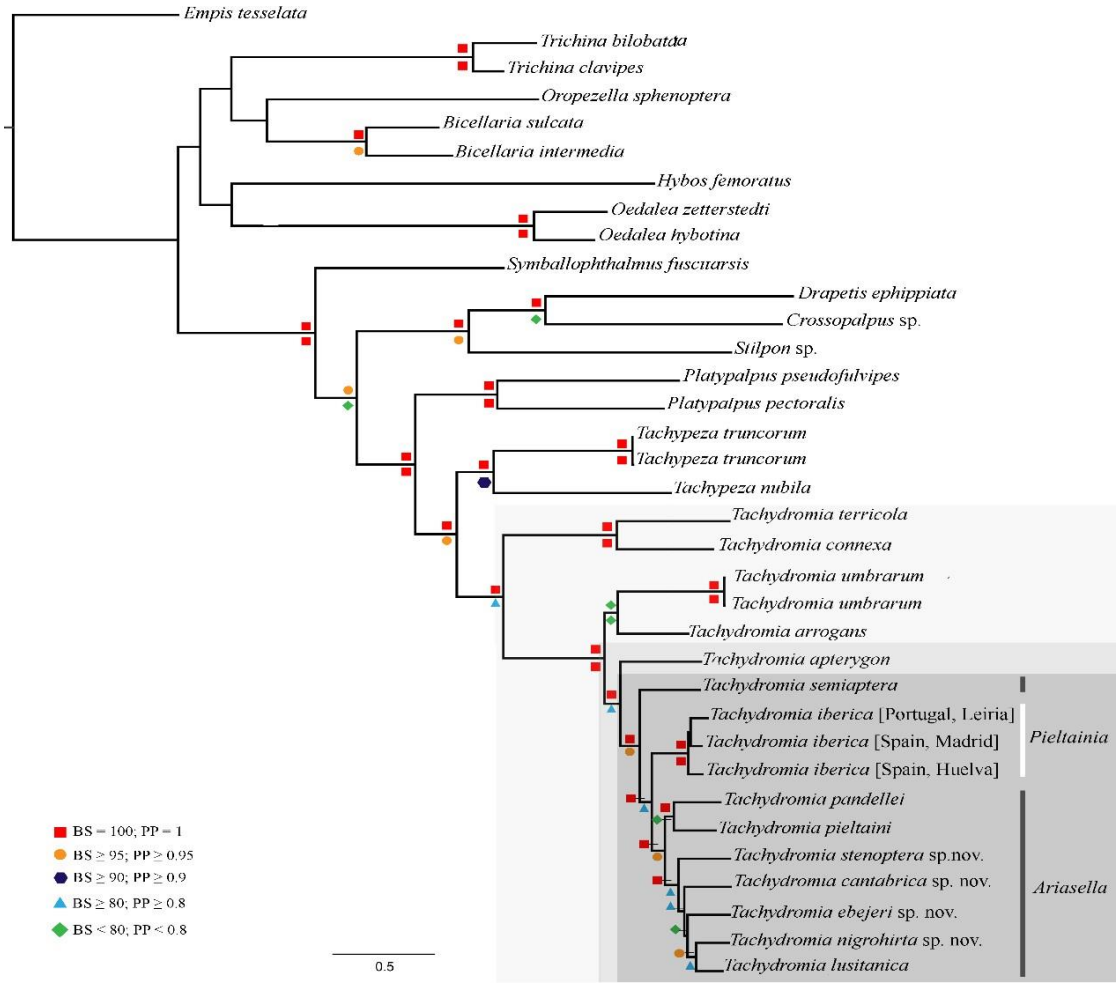

Fig. 1. Maximum-likelihood tree (ln L =-29397.646621) based on the combined dataset (COI, non-trimmed 28S,12S, AATS and PGD) using Garli v.2.01.1067 and the structural alignment for 28S. Bootstrap support values (below) and Bayesian posterior probabilities (above) are depicted at the nodes (only >50 or >0.5, respectively). BS = Bootstrap support values; PP = Bayesian posterior probabilities. A grey-scale is used to highlight the ingroup, where the darkest shade of grey highlights the Iberian flightless ant-like *Tachydromia* species, followed by a lighter shade which includes *T. apterygon*, hence representing all the flightless species occurring in southern Europe and, finally, the lighter shade covers all *Tachydromia* analysed, including the macropterous species assigned to different species-groups *sensu* Chvála (1970). The white bar indicates the species originally assigned to genus *Pieltainia*, while the grey bars indicate the taxa originally assigned to genus different species-groups *sensu* Chvála (1970).

remaining *Tachydromia*, including the Iberian species. Within the latter, (*T. arrogans* + *T. umbrarum*) - here the respective representatives of the *arrogans* and *annulimana* species-group - was resolved as a sister clade to the flightless species with very high support values (BS = 100; PP = 1). *T. apterygon* was placed in the cluster containing all flightless species, which is supported by moderate values of bootstrap and high values of posterior probability (BS = 88; PP = 1), indicating that it is more phylogenetically related to the *arrogans* than to the *terricola* species-group. The Iberian flightless ant-like species ("*Ariasella*" + "*Pieltainia*"), is a well-supported group with internal nodes generally with high support and with short length branches. *Tachydromia semiaptera*, originally the type species of genus *Ariasella*, was recovered as the first branch, with very high support (BS= 97; PP = 1), being the least related to the remaining Iberian species. Moreover, *Tachydromia iberica*, originally the type species of the monotypic genus *Pieltainia*, appears as being closer related to remaining Iberian species, than *Tachydromia semiaptera* is. *T. iberica* is represented by taxa obtained from its three main occurrence localities. While there is a significant geographic distance and small differences concerning the male terminalia morphology (see Figs. 6, 7, 8), these populations are phylogenetically very related, as can be denoted by the very short branch length. *T. pandellei* and *T. pieltaini* were recovered as sister species with a very high Bayesian posterior probability (PP = 1) but only moderate bootstrap support values (BS = 75). The previously undescribed species were placed in the same grouping (PP = 1; BS= 84), being closely related. *T. nigrohirta* sp. nov. and *T. lusitanica* were recovered as sister species (BS = 80; PP = 0.98). The most related species to (*T. nigrohirta* sp. nov. + *T. lusitanica* are *T. cantabrica* sp. nov. and *T. ebejeri* sp. nov., however, the evolutive relationships between these taxa remains unclear due to lack of statistical support. The phylogenetic position of *T. stenoptera* sp. nov. was recovered, with high support (BS = 84; PP = 1) as a sister taxon to [*T. cantabrica* sp. nov. + *T. ebejeri* sp. nov. + (*T. nigrohirta* sp. nov. + *T. lusitanica*)].

The topology of the majority-rule consensus tree resulting from Bayesian inference agrees with the most likely tree regarding all Tachydromiinae taxa, while the other Hybotidae subfamilies had no support in both approaches. Additionally, and regarding the datasets with trimmed and non-trimmed 28S, the analysis (by ML and BI) of both produced trees (Fig. 1, Fig. 2 of supplementary material) with the same topology for the Tachydromiinae phylogenetic placement. The BI analysis of the trimmed dataset produced a tree with slight differences in the PP support values, where the exterior nodes received less support and the interior nodes more support in comparison with the non-trimmed dataset. Considering the support values resulting from the ML approach, there is only a single notable case where the support value of the clade grouping all *Tachydromia* species is much higher in the non-trimmed version (BS= 87 in the non-trimmed; BS=67 in the trimmed).

4. Notes on habitat, distribution and phenology

The fieldwork carried out in the previous years yielded a significant amount of new data regarding both the geographic distribution of most species (Fig. 2) and the (micro) habitat where they occur. Currently, the following localities are known for each species (number of new localities between brackets): *T. cantabrica* sp. nov. is known from 3 localities, *T. ebejeri* sp. nov. from 23, *T. lusitanica* from 22 (19), *T. nigrohirta* sp. nov. from 3, *T. pandellei* from 7 (6), *T. pieltaini* from 3 (2), *T. semiaptera* from 15 (12) and *T. stenoptera* sp. nov. from 7. These species occur in temperate and supra-mediterranean regions, mainly in mountains, where they often inhabit forests dominated by deciduous or marcescent trees, such as *Quercus robur*, *Q. pyrenaica* and *Fagus sylvatica*. They are only to be found among the leaf litter and very low herbaceous vegetation. Furthermore, most Iberian ant-like *Tachydromia* tend to become active in early spring (mostly from February until May), however, species as *T. pandellei* and *T. pieltaini* also occur at higher altitude and, hence, become active much later in the year (likely May onwards). These species occur in the *Fagus sylvatica* forests but seem to be only found on the edge of the forest.

The Iberian ant-like *Tachydromia* are often found among other flightless insects, including ants, but no direct predation carried out by those was ever observed. These flies seem to only prey on Sciaridae (fungus gnats) and Chironomidae (non-biting midges). They are restricted to the Iberian Peninsula and the Pyrenees.

5. Morphological analysis

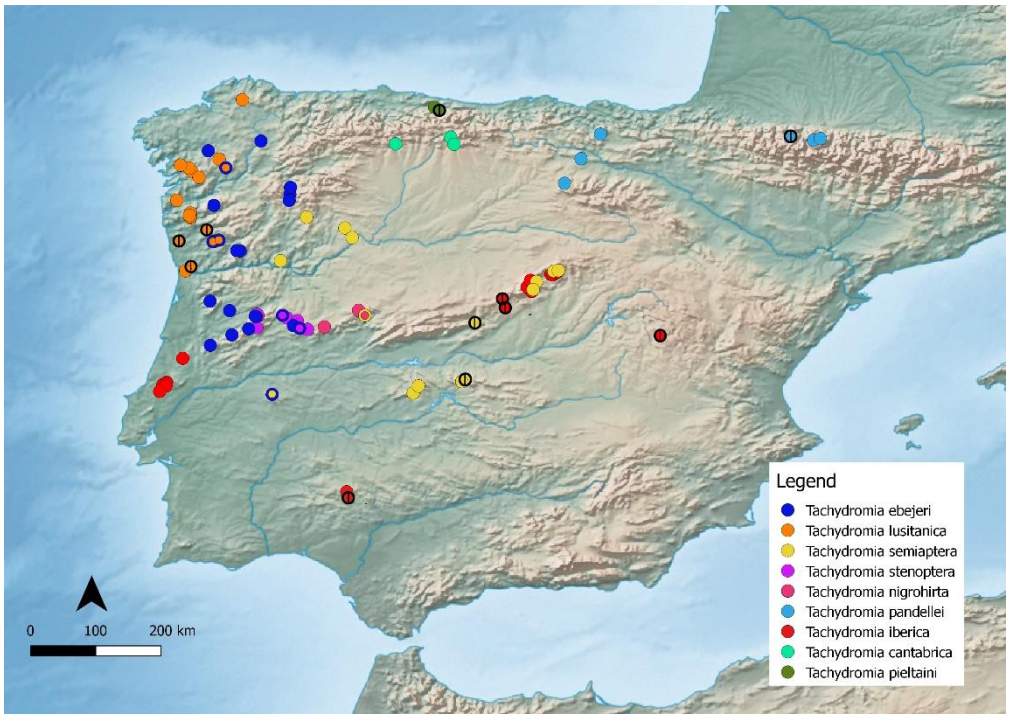

Fig. 2. Current known distribution of the Iberian ant-like *Tachydromia*, originally placed in genera *Ariasella* and *Pieltainia*. Each dot represents a presence point, with each colour corresponding to a different species. When two species co-occur in the same area, their presence is represented by a smaller dot on top of a dot of regular dimension, each of those with the colour corresponding to the co-occurring species. The dots surrounded by a black circle with a vertical line represent localities previously known from the bibliography.

5.1. Identification key to the flightless ant-like *Tachydromia* from Iberia and Italy

5.1.1. Key to males

1a Wings completely absent..... ***Tachydromia iberica* (Arias, 1919)**

b Wings either present as vestiges (micropterous) or stalk-like (stenopterous) **2**

2a Micropterous (Figs 20A, 22) **3**

b Stenopterous (Figs 20C, E, G, 21A–B, D, F) **4**

3a Palpi and antennae black; pospedicel lanceolate, *ca.* 1.5 times longer than pedicel; wing is a single lobe, usually longer than wide, brownish, covered with microtrichia, at most with a single distinct seta (Fig. 20A).....***Tachydromia ebejeri* sp. nov.**

b Palpi yellow; scape and pedicel yellow, postpedicel brown; pospedicel sub-conical, similar lengthas of pedicel; wing roundish, bilobed, brownish, covered in microtrichia and bearing distinct setae (Fig. 22)…….***Tachydromia apterygon* Plant & Deeming, 2006**

4a Antennae overall yellow and/ or light brown, postpedicel roundish, approximately as long as pedicel **5**

b Antennae black; postpedicel sub-conical or lanceolate, longer than pedicel..... **7**

5a Fore tibia black, stout and inflated; mid tibia bearing 2 projections and a row of spinose setae on its posteroventral basal surface (Figs. 19E–F).....***Tachydromia semiaptera* (Gil, 1923)**

b Fore tibia yellow, as wide as mid and hind tibiae; mid tibia bearing 1 single projection (spur) (Figs. 19B–C).....**6**

6a Wing lobe with a complex black and white pattern (Fig. 21B), antennae and legs overall yellow, scutum only slightly convex.....***Tachydromia pieltaini* (Gil, 1936)**

b Wing lobe white at basal half and black distally (Fig. 21A), antenna and legs yellow with brown segments, mesopleuron convex and somewhat humped.....***Tachydromia pandellei* (Séguy, 1941)**

7a Wing with only slightly lobed distal apex (Fig. 21F); tergites bearing few and scattered setae; legs black, except for the pale brown ventral and dorsal posterior surface of coxae, trochanter, basal 0.1 ventral surface of femora and knees; right epandrial lamella of Terminalia convex, globose (Fig. 14A) ..... ***Tachydromia stenoptera* sp. nov.**

b Wing with distinctively lobed distal apex (Figs. 20C, E, G); tergites totally covered by setae; legs with black and yellow/pale brown pattern **8**

8a Distinct prescutellar setae absent, scutellum with 2 setae present medially; front tibia bearing a sparse ciliation of hair-like setae (shorter than the maximum diameter of the tibia) with slightly curved tips; left surstylus of Terminalia with long setae on apical margin (Fig. 4C) ..... ***Tachydromia cantabrica* sp. nov.**

b Distinct prescutellar and scutellar setae present; front tibia bearing a dense ciliation of long hair-like setae with curved tips; left surstylus of Terminalia with setulae on apical margin (Figs. 9C, 10C) **9**

9a Wing with bilobed distal apex, a lobe produced into a long digitation on distal margin (Fig. 20E); Legs black with the following segments yellowish-brown: coxa, trochanter, knee, anterior and ventral

surface of fore and mid femur, base of fore and mid tibia, basal ⅘ surface of tarsomere 1, basal surface ⅔ of mid and hind tarsomere 2….***Tachydromia lusitanica*** **(Grootaert, Shamshev & Andrade, 2009)**

b Wing with lobed distal apex, without a distinct digitation on distal margin (Fig. 20G); legs black, except for trochanters, knees and tarsomere 1 and 2 which are yellowish to brown…..……...……...***Tachydromia nigrohirta*** **sp. nov.**

5.1.2. Key to females

1a Wings completely absent.....***Tachydromia iberica*** **(Arias, 1919)**

b Wings either present as vestiges (micropterous) or stalk-like (stenopterous)..... **2**

2a Antennae overall yellow and/or light brown, postpedicel roundish, approximately as long as pedicel **3**

b Antennae black; postpedicel sub-conical or lanceolate, longer than pedicel..... **6**

3a Stenopterous (Fig. 21C) **4**

b Micropterous (Fig. 21E)..... **5**

4a Antennae and legs overall yellow; 4 prescutellar setae present, scutellum bearing 2 setae; abdomen shining black with grey microtrichia covering only the last apical tergite and almost all sternites .....***Tachydromia pieltaini*** **(Gil, 1936)**

b Antenna and legs yellow with brown segments; 4 prescutellar setae present, scutellum lacking distinct setae; abdomen black, uniformly covered with grey microtrichia.....…..***Tachydromia pandellei*** **(Séguy, 1941)**

5a Palpi yellow in ground colour; wing bilobed, roundish, apical margin of posterior lobe bearing 1 long seta, anterior lobe bearing 3 shorter setae…….....…. .....***Tachydromia apterygon*** **Plant & Deeming, 2006**

b Palpi black in-ground colour; wing is a single lobe, roundish, bearing 1 long seta and 1 short seta on the apical margin(Fig. 21E)..... ***Tachydromia semiaptera*** **(Gil,1936)**

6a 4 prescutellar setae and 4 scutellar setae; wing is a single lobe, usually longer than wide, at most with a single distinct seta present (Fig. 20B); coxae, femora and most of tibia dark brown to black; trochanters, knee, anteroventral surface of tibia, distal ¼ of tarsomere 1, distal half of tarsomere 2 and following tarsomeres, yellowish.....***Tachydromia ebejeri*** **sp. nov.**

b Different number of prescutellar and scutellar setae or setae; wing bilobate, roundish, with distinct setae (Figs. 20D, F, H, 21G).....**7**

7a Tergites with few and scattered setae, mostly present on the posteromarginal surface and near the spiracles; sternites with evenly distributed setae; legs almost completely black, except for very small portions which are brown, specifically: ventral and dorsal posterior surface of coxae, trochanter, basal 0.1 ventral surface of femora and knees..... ***Tachydromia stenoptera*** **sp. nov.**

b Tergites and sternites with evenly distributed setae; legs with a different colour pattern of black and yellow to brown **8**

8a Humeral callus bearing *ca*. 4 setae laterally; wings bilobate, bearing 1 seta on the apical margin of each lobe (Fig. 20D); legs black with the following segments pale brown/yellowish: apical portion of coxae, trochanters, basal portion of mid and hind femora, distal apical surface of front and mid femora, knees, basal ⅘ surface of tarsomere 1 and basal ⅔ surface of tarsomere 2…… .....***Tachydromia cantabrica*** **sp. nov.**

b Humeral callus bearing more than 4 setae laterally; wings bilobate, bearing more than 2 setae in both lobes combined (Figs. 20F, H); legs with a different colour pattern… **9**

9a Legs black, except for trochanters, knees and tarsomere 1 and 2 which are yellowish to brown …..……..……..……***Tachydromia nigrohirta*** **sp. nov.**

b Legs black with the following segments yellowish-brown: coxa, trochanter, knee, anterior and ventral surface of fore and mid femur, base of fore and mid tibia, basal ⅘ surface of tarsomere 1, basal surface ⅔ of mid and hind tarsomere 2 .....***Tachydromia lusitanica*** **(Grootaert, Shamshev & Andrade, 2008)**

5.2. Species description

General description of the Iberian ant-like *Tachydromia*

The Iberian ant-like *Tachydromia* can be jointly characterized by being flightless (apterous, micropterous or stenopterous), lacking halteres, having one single notopleural seta, a silvery patch on prothorax; palpi dark in ground colour and the stylus at least 1.5 times as long as antenna. Considering their morphology, it can be said they are generally more related to the *arrogans* species-group than to any other, however, various species differ in having a shorter stylus 1.5 times as long as the antenna and/or for having male genitalia large and globular. They are quick and agile runners, which often move their abdomens up and downwards, resembling ants or certain flightless parasitic wasps.

5.2.1. .*Tachydromia apterygon* Plant & Deeming, 2006

Figs 3, 16I–J, 19J, 22, 23

Holotype
ITALY • 1 ♂; Lazio (10 km south of Forca d'Acero, on road to Sora); 1 Jul. 2005; J. C. Deeming leg.; NMGW.

Other material examined
ITALY • 5 ♂♂, 10 ♀♀; Frosinone, San Donato Val di Comino; 41°43'58.7" N, 13°49'12.2" E; 26 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 2 ♂♂; L'Aquila, Gioia dei Marsi (Parco Nazionale D'Abruzzo Lazio e Molise); 41°51'34.0" N, 13°46'52.1" E; 26 Jun. 2017; A.R. Gonçalves & R. Andrade

leg. • 19 ♂♂, 13 ♀♀; L'Aquila, Pacentro; 42°02'56.7" N, 14°02'51.7" E; 27 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 18 ♂♂, 14 ♀♀; Pescara, Sant'Eufemia a Maiella; 42°06'01.2" N, 14°02'10.5" E; 27 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 13 ♂♂, 13 ♀♀; L'Aquila, Ofena; 42°23'06.1" N, 13°45'37.8" E; 28 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 2 ♂♂; Pescara, Farindola; 42°23'45.7" N, 13°47'06.8" E; 28 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 2 ♂♂, 3 ♀♀; Lazio, Leonessa; 42°30'59.3" N, 12°59'10.9" E; 29 Jun. 2017; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 3 ♀♀; Lazio, Posta; 42°31'51.2" N, 13°03'37.4" E; 29 Jun. 2017; A.R. Gonçalves & R. Andrade leg.

Diagnosis

Small (length= 2mm), slender, overall dark, species with minute bilobed squamiform wings (Fig. 22) in both sexes and halteres absent. Frons, vertex and occiput largely covered by grey microtrichia; one pair of lateroclinate ocellars; one pair of proclinate verticals; palpi pale yellow, antennae with scape and pedicel yellow, postpedicel brown; proboscis shining black; pospedicel sub-conical, similar length as of pedicel. Thorax black with portion of prothoracic episternum densely covered with grey setulae. Legs with a colour pattern of yellowish and dark brown to black; fore tibia with ciliation of short coarse setae. Male mid tibia has, at its anteroventral apex, a spur, produced into a short apical elongation, about as long as diameter of tibia at apex (Fig. 19J); the spur possesses several distinct setae and, on the distal half margin a few short, stout, curved, spinose setae. Abdomen black covered with grey setulae and short setae, longer at hind margin of last sternite.

Description

Female (previously undescribed)**.** Similar to male. Micropterous, with wing round, brownish, bilobate, apical margin of posterior lobe bearing 1 long seta, anterior lobe bearing 3 shorter setae - both covered with grey microtrichia. Differs from the male in the following: legs mainly covered with microtrichia and regular rows of setae, no distinct setae; spur on mid tibia absent; cerci pale brown with grey microtrichia and long setae; a few longer setae on apical sternites.

Distribution and habitat

Currently, this species is only known from the central Apennine Mountains (Fig. 23). It occurs on the edge of *Fagus sylvatica* forests (Fig. 24), on the leaf litter and short herbaceous vegetation, where it can be abundant. It has also been found in mixed deciduous woodland at lower altitudes, inside the forest, on the leaf litter.

Remarks

Despite the sampling effort, the species was not found at the type locality, which is a mixed deciduous woodland, at the end of July. However, it was still very abundant at higher altitudes. The type specimen was collected at the beginning of July, so by the end of the month, it may already be too hot and dry for this species to survive at lower altitudes.

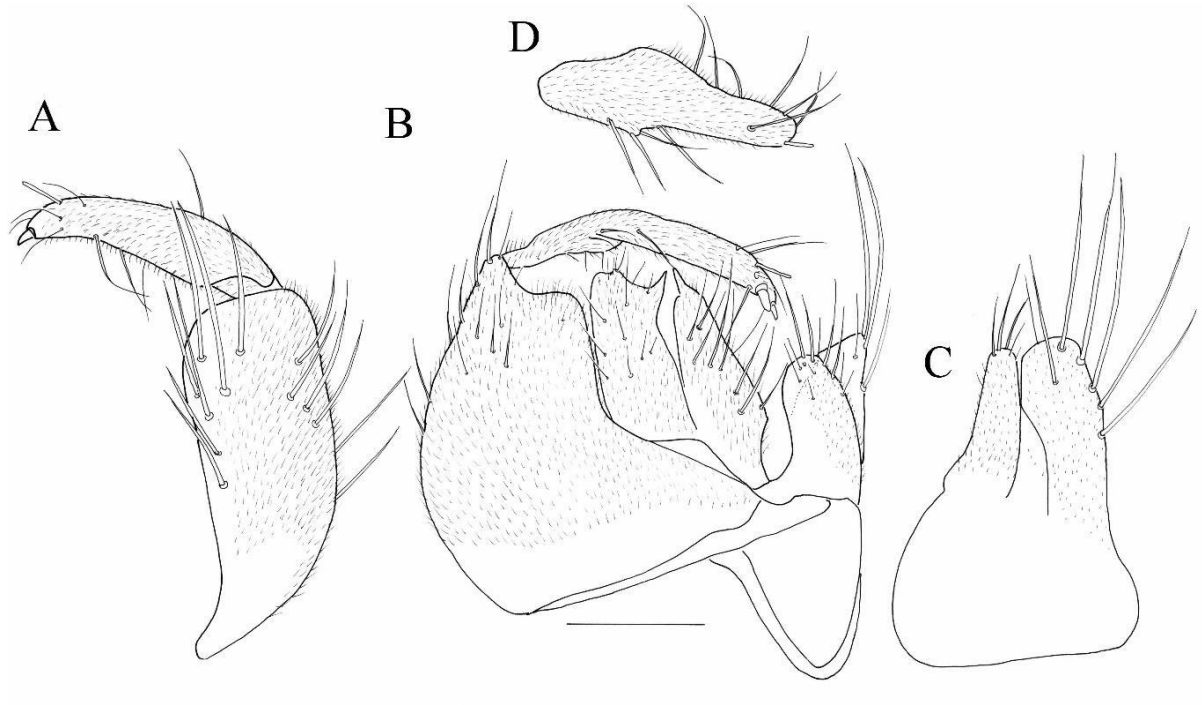

Fig. 3. Terminalia of *Tachydromia apterygon* Plant & Deeming, 2006 from ITALY, Lazio, Posta. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

5.2.2. *Tachydromia cantabrica* sp. nov. Gonçalves, Grootaert & Andrade

Figs 4, 15I–J, 18A–B, 19H, 20C–D

Etymology. This species is named after the Spanish Cantabrian mountain range, where it was found.

Material examined

Holotype
SPAIN • 1 ♂; Palencia, Río Carrión; 42º50'34.9'' N, 4º51'16.9'' W; 14 May 2014; A.R. Gonçalves & R. Andrade leg.

Paratypes
SPAIN • 4 ♂♂, 2 ♀♀; Palencia, Río Carrión; 42º50'34.9'' N, 4º51'16.9'' W; 14 May 2014; A.R. Gonçalves & R. Andrade leg.

Other material
SPAIN • 2 ♂♂; León, La Pola de Gordón; 42°51'00.5" N, 5°39'46.0" W; 13 May 2014; A.R. Gonçalves & R. Andrade leg. • 1 ♀; León, Boca de Huérgano; 42º56'27.3'' N, 4º54'10.0'' W; 14 May 2014; A.R. Gonçalves & R. Andrade leg.

Diagnosis

Small, slender, overall dark, species. Wing-dimorphic: male is stenopterous; wing with lobed distal apex, no veins distinguishable, dark brown for the most part with lobe black and white; female is

micropterous, wing bilobate, bearing 1 seta on each lobe. Halteres absent. Occiput largely covered by grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel sub-conical, *ca*. 1.5 times longer than pedicel. Thorax black with part of the prothoracic episternum densely covered with grey setulae, appearing sub-triangular in lateral view. Legs with a colour pattern of yellowish and dark brown to black; male fore tibia with ciliation of long hair-like setae. Abdomen black, tergites and sternites with evenly distributed setae and covered with grey microtrichia.

Description

Male. Body length: 2.1 - 2.3 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=3). Wing length: 0.8 - 0.9 mm (dry-mounted specimens, n=3). **Head.** Globular, black. Face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle shining anteriorly and finely pollinose posteriorly. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for a glabrous triangular patch posterior to the occiput and for the lower half. One pair lateroclinate ocellars, same length of postpedicel, no posterior ocellars. One pair proclinate verticals, *ca*. ¼ shorter than the length of postpedicel, and numerous setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Black, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel sub-conical, *ca*. 1.5 times as long as pedicel, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being almost twice as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae and black setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a distinct seta on the ventral apex. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with long setae black, short ones pale. Humeral callus and prothoracic episternum, mesopleuron and sternopleuron, almost undifferentiated, scutellum short; notopleuron, pteropleuron, hypopleuron and postscutellum well developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum partially, but densely, covered, appearing sub-triangular in lateral view; hypopleuron, scutellum and upper part of the metathorax thinly covered. Humeral callus bearing *ca*. 4 setae laterally, no dorsocentrals, 8 acrostichal setae, biseriate, of equal length; notopleuron with 1 strong, prominent, seta plus 2 small setae; 4 small, incurvated, prescutellar setae; scutellum bearing 2 strong, long setae medially; 1 pre-alar seta **Wing.** Stenopterous. Lobed distal apex, sub-oval, with a minute digitation. Stalk-like portion dark brown, distal ⅔ of lobe black with basal ⅓ and digitation translucid (appearing white under most light conditions) (Fig. 20C). No veins distinguishable. Uniformly covered in microtrichia. Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Black with the following pale brown/yellowish: basal portion of coxae, trochanters, basal portion of mid and hind femora, distal apical surface of front and mid femora, knees, basal ⅘ surface of tarsomere 1 and basal ⅔ surface of tarsomere 2. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several short, downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae. Front tibia bearing a sparse ciliation of hair-like setae (shorter than the maximum diameter of the tibia) with slightly curved tips, and a row of long, erect, setae present at its distal ⅓ posterodorsal surface.

Tarsomere 1 of fore tarsus with strong and long (twice the tarsomere 1 width) setae; at the posterior distal surface with a row of distinct, short, black setae. Mid tibia at its anteroventral apex has a spur, with a barely noticeable apical elongation, about as long as diameter of tibia at apex (Fig. 19H); the spur possesses several distinct setae and, on the distal half margin, 5 short, stout, curved, spinose setae. **Abdomen.** Black, uniformly covered with grey microtrichia. Tergites and sternites with evenly distributed setae; apical sternites with long, distinct, setae. **Terminalia** (Fig. 4). Subglobular, mostly covered with grey microtrichia, dark brown. Right surstylus short with anterior margin produced into short slender projection, mostly glabrous, without microtrichia, bearing stronger setae mostly on the dorsal margin, with several (*ca.* 8) distinct denticles, most of which aggregated on distal apex. Right epandrial lamella with irregularly placed long setae and with a patch of similar setae on lateral surface. Cerci of similar length, both with long setae of unequal length, enclosed in epandrial lamellae. Left epandrial lamella slightly shorter than left cercus, bilobed, with a few long setulae on apical margin. Left surstylus sub- rectangular with rounded apical margin, 3 times as long as wide, distinctively longer than cerci, densely covered with grey microtrichia and with equally long setae on apical margin.

Female. Similar to male, except for the following features: micropterous, with wings round, brownish, bilobate, bearing 1 seta on the apical margin of each lobe, covered by grey microtrichia (Fig. 20D); legs covered with regular rows of setae; spur on mid tibia absent; cerci pale brown with grey microtrichia and setae; no longer setae on apical sternites.

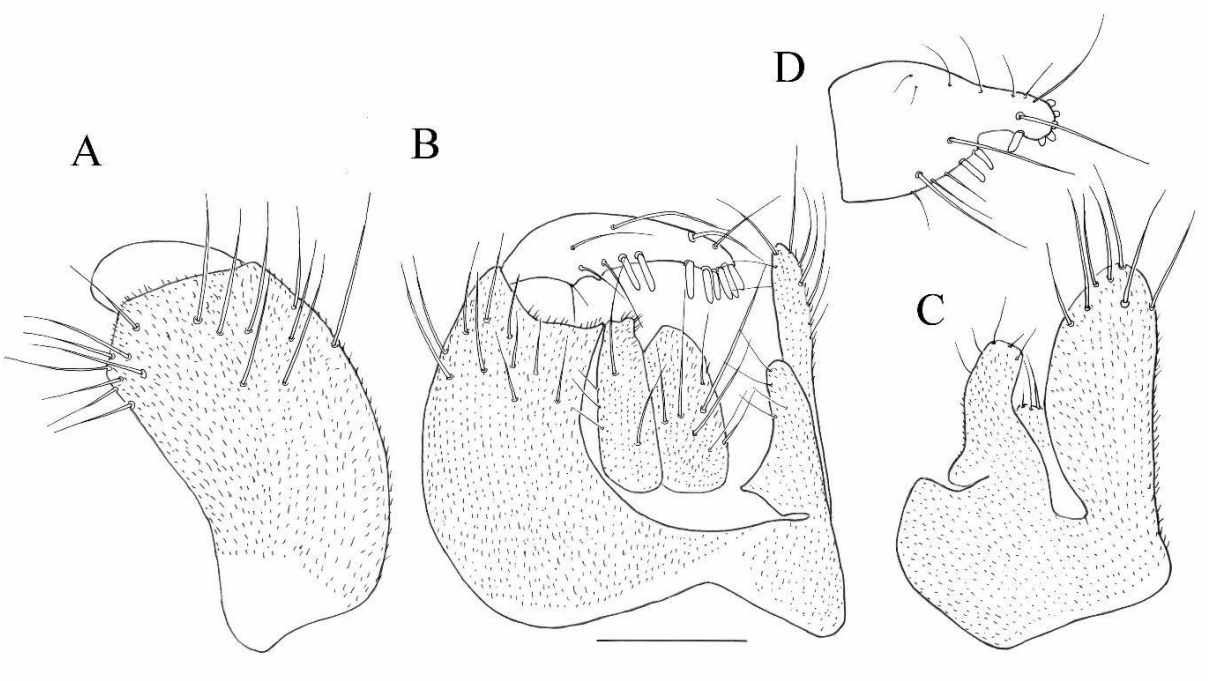

Fig. 4. Terminalia of *Tachydromia cantabrica* sp. nov. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

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5.2.3. *Tachydromia ebejeri* sp. nov. Gonçalves, Grootaert & Andrade

Figs 5, 15E–F, 17E–F, 19D, 20A–B

Etymology. Species named after dipterologist Martin Ebejer for his contribution to the advancement of the knowledge on Portuguese Diptera.

Material examined

Holotype
PORTUGAL • 1 ♂, Marvão, Santa Maria de Marvão; 39º23'49.2'' N, 7º21'51.4'' W; 14 Mar. 2015; A.R. Gonçalves & R. Andrade leg.; RBINS.

Paratypes
PORTUGAL • 5 ♂♂, 5 ♀♀ Marvão, Santa Maria de Marvão; 39º23'49.2'' N, 7º21'51.4'' W; 14 Mar. 2015; A.R. Gonçalves & R. Andrade leg.; RBINS.

Other material
PORTUGAL • 5 ♂♂, 1 ♀; Gouveia, Aldeias; 40º28'05.4'' N, 7º35'15.7'' W; 25 Apr. 2013; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 4 ♀♀; Arganil, Benfeita (Mata da Margaraça); 40º12'59.6' 'N, 7º55'12.5'' W; 22 Feb. 2014; A.R. Gonçalves leg. • 10 ♂♂, 8 ♀♀; Marvão, Santa Maria de Marvão; 39º23'49.2'' N, 7º21'51.4'' W; 14 Mar. 2015; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 10 ♀♀; Guarda, Gouveia, São Cosmado; 40°28'4.49" N, 7°35'25.18" W; 20 Apr. 2015; A. R. Gonçalves leg. • 1 ♀; Guarda, Seia, Alvoco da Serra; 40°18'03.4" N, 7°41'22.6" W; Apr. 2015; A. R. Gonçalves leg. • 1 ♂, 2 ♀♀; Guarda, Gouveia, Aldeias (Eiras); 40°28'26.5" N 7°35'49.6" W; 20 Apr. 2015; A. R. Gonçalves leg. • 3 ♂♂, 4 ♀♀; Vila Real, Lamas de Ôlo; 41º22'27.6'' N, 7º48'23.7'' W; 30 Apr. 2016; R. Andrade leg. • 4 ♂♂, 4 ♀♀; Mondim de Basto, Ermelo; 41º22'44.0'' N, 7º51'14.4'' W; 30 Apr. 2016; R. Andrade leg. • 1 ♂, 3 ♀♀; Castro Laboreiro; 42º00'09.3'' N, 8º10'09.6'' W; 29 May 2016; R. Andrade leg. • 4 ♀♀;; Viseu, Póvoa Dão; 40°33'03.9" N 7°57'05.6" W; 18 Mar. 2017; J. Almeida leg. • 1 ♂, 1 ♀; Vouzela, Campia; 40°40'53.0" N, 8°13'18.5" W; 21 Mar. 2017; J. Almeida leg. • 3 ♂♂, 1 ♀; Guarda, Panóias de Cima; 40°29'10.2" N, 7°13'18.4" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 3 ♂, 2 ♀♀; Sabugal, Sabugal; 40°20'39.9" N, 7°04'18.6" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 1 ♂, 2 ♀♀; Sabugal, Quadrazais; 40°18'30.6" N, 6°59'08.7" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 1 ♂, 5 ♀♀; Guarda, Adão; 40°27'03.0" N, 7°09'41.8" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 2 ♀♀; Fafe, Felgueiras; 41°31'24.0" N, 8°06'01.2" W; 9 Apr. 2017; R. Andrade & J. Almeida leg. • 1 ♂; Fafe, Travassós; 41°30'06.4" N, 8°10'55.6" W; 9 Apr. 2017; R. Andrade & J. Almeida leg. • 3 ♂♂, 7 ♀♀; Coimbra, Lousã, Catarredor; 40°04'20.9" N, 8°13'04.2" W; 19 Apr. 2017; A.R. Gonçalves & D. Rodrigues leg. • SPAIN • 10 ♂♂, 21 ♀♀; Ourense, Viana do Bolo; 42º09'06.2'' N, 7º07'12.3'' W; 11 May 2014; A.R. Gonçalves & R. Andrade leg. • 3 ♀♀; Ourense, O Bolo; 42º15'03.2'' N, 7º06'46.9'' W; 11 May 2014; A.R. Gonçalves & R. Andrade leg. • 8 ♂♂, 12 ♀♀; Ourense, A Gudiña; 42º04'14.6'' N, 7º07'47.5'' W; 11 May 2014; A.R. Gonçalves & R. Andrade leg. • 1 ♂; Mosteiro de Carboeiro; 42°45'18.8" N, 8°14'46.6" W; 6 May 2018; A.R. Gonçalves leg. • 3 ♂♂, 2 ♀♀; Galiza, Ourense, Piñor; 42°31'17.3" N, 8°00'14.3" W; 7 May 2018; A.R. Gonçalves leg.

Diagnosis

Small, slender, overall dark, species with minute squamiform wings in both sexes and halteres absent. Occiput largely covered by grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel lanceolate, *ca.* 1.5 times longer than pedicel. Thorax black with prothoracic episternum densely covered with grey setulae, appearing triangular in lateral view. Legs with a colour pattern of yellowish and dark brown to black; fore tibia with ciliation of long hair-like setae. Abdomen black, covered with grey setulae and with distinct, strong, setae on posteromarginal 1st sternite.

Description

Male. Body length: 1.9 - 2.4 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 0.05 - 0.16 mm (dry-mounted specimens, n=5). **Head.** Globular, black. Face and

frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle shining anteriorly and finely pollinose posteriorly. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for a glabrous triangular patch on upper half and for the lower half, which is shining black with a narrow middle stripe of grey setulae. One pair lateroclinate ocellars, ¾ the length of postpedicel, no posterior ocellars. One pair proclinate verticals, *ca*. half the length of postpedicel, and about 60 scattered setae on the occiput, in the upper half twice as long as in the lower half; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Black, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide with apical circlet of black setae, ventrals longer; postpedicel lanceolate, *ca*. 2 times as long as pedicel, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being twice as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery white setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a distinct seta on the ventral apex. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with long setae black, short ones pale. Humeral callus and prothoracic episternum, mesopleuron and sternopleuron, almost undifferentiated, scutellum short; notopleuron, pteropleuron, hypopleuron and postscutellum well developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum densely covered, appearing sub-triangular in lateral view; hypopleuron, scutellum and upper part of the metathorax thinly covered. Humeral callus bearing *ca*. 6 setae laterally, no dorsocentrals, 7 acrostichal setae, biseriate, of equal length; notopleuron with 1 strong, prominent, seta plus 4 small setae; 4 small, incurvated, prescutellar setae; scutellum bearing 4 setae medially, central pair long and thick, lateral thick but shorter. **Wing.** Micropterous, covered by grey microscopic setulae, no setae present (Fig. 20A). Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Coxae, femora and most of tibia dark brown to black; trochanters, knee, anteroventral surface of tibia, distal ¼ of tarsomere 1, distal half of tarsomere 2 and following tarsomeres, yellowish. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several short, downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae. Front tibia bearing a sparse ciliation of hair-like setae (slightly longer than the maximum diameter of the tibia) with curved tips, and a row of long, erect, setae present at its distal ⅓ posterodorsal surface. Tarsomere 1 of fore tarsus with long hairs and a patch of distinct, strong, black setae on the posterior distal surface. Mid tibia at its anteroventral apex has a spur, with a barely noticeable apical elongation, about as long as diameter of tibia at apex (Fig. 19D); the spur possesses several distinct setae and, on the distal half margin, 5 short, stout, curved, spinose setae. **Abdomen.** Black, uniformly covered with grey setulae. Tergites and sternites with evenly distributed setae; first tergite with differentiated, strong, posteromarginal setae and apical sternites with long, distinct, setae. **Terminalia** (Fig. 5)**.** Subglobular, mostly covered by grey microtrichia, dark brown. Right epandrial lamella with irregular rows of long setae. Right surstylus short with anterior margin produced into short slender projection, covered with long setulae, without microtrichia, bearing stronger setae mostly on the dorsal margin, with several (*ca.* 10) distinct denticles, ⅔ of which aggregated on distal apex. Cerci of similar length, right with broad tip, left narrowing distally, both with long setae of unequal length on the apical half, enclosed in epandrial lamellae. Left epandrial lamella ⅔ the length of the left cercus, bilobed, with a few setulae on apical margin. Left surstylus sub-rectangular, twice as long as wide, longer than cerci, densely covered with microtrichia and with short setulae of equal length on apical margin.

Female. Similar to male, including being micropterous (Fig. 20B), except for the following features: no distinctive hairs or setae on fore femora; spur on mid tibia absent; cerci pale brown with grey microtrichia and setae; no longer setae on apical sternites.

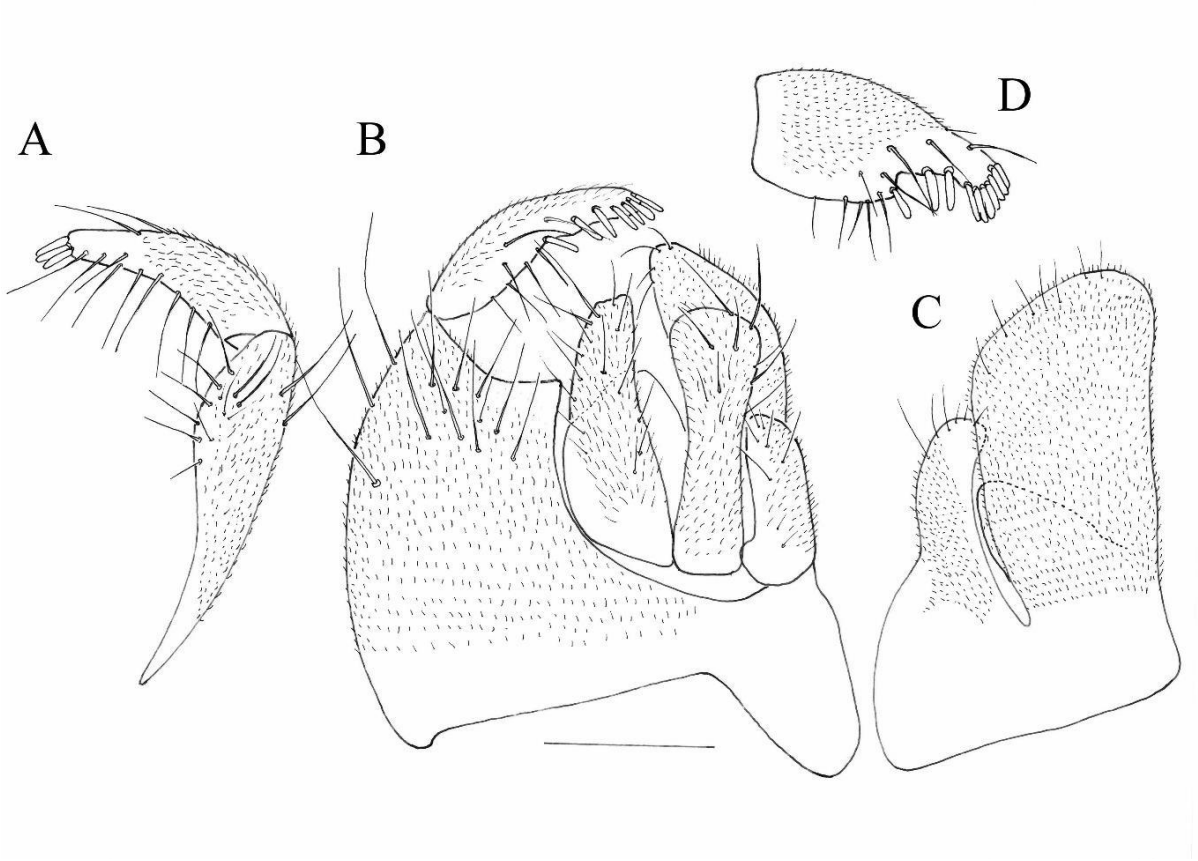

Fig.5. Terminalia of the holotype of *Tachydromia ebejeri* sp. nov. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

5.2.4. *Tachydromia iberica* (Arias, 1919)

Figs 6–8 16A–B, 18C–D

Pieltainia iberica Arias, 1919: 479-480

Tachydromia iberica comb. nov. Shamshev & Grootaert, 2018

Material not examined

Type material (probably lost)
SPAIN • Huelva, Cala; 22 Feb. 1915; C. Bolívar leg.

Paratype material (probably lost)
SPAIN • Segovia, San Rafael; (spring) 1917; C. Bolívar leg. (several specimens)

Other records
SPAIN • Madrid, El Escorial; C. Bolívar leg. • Cuenca, Ciudad Encantada; J. G. Collado leg.

Material examined
SPAIN • 17 ♂♂, 12 ♀♀; Badajoz, Tentudía; 38º03’16.17’’ N, 6º20’14.7’’ W; 3 Apr. 2015; A.R.

Gonçalves & R. Andrade leg. • 8 ♂♂, 13 ♀♀; Segovia, El Espinar; 40º42'35.3'' N, 4º14'08.3'' W; 1 Apr. 2015; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 4 ♀♀; Madrid, Montejo de la Sierra, Prádena del Rincón; 41°03'03.6" N, 3°31'10.8" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 3 ♂♂; Madrid, Montejo de la Sierra, Prádena del Rincón; 41°02'40.8" N, 3°29'28.7" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 3 ♂♂, 9 ♀♀; Madrid, Arroyo de la Fuentecilla (La Hiruela); 41°04'18.0" N, 3°27'28.2" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 2 ♂♂, 1 ♀; Madrid, La Hiruela; 41°04'59.3" N, 3°25'45.3" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 2 ♂♂, 1 ♀; Guadalajara, El Cardoso de la Sierra; 41°05'46.2" N, 3°28'56.1" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 6 ♂♂, 9 ♀♀; Madrid, Pinilla de Buitrago; 40°57'02.0" N, 3°43'11.6" W; 24 Apr. 2017; A. R. Gonçalves & P. Álvarez leg • 3 ♂♂, 2 ♀♀; Madrid, Miraflores de la Sierra; 40°50'24.4" N, 3°48'24.4" W; 24 Apr. 2017; P. Álvarez leg. • 4 ♂♂, 5 ♀♀; Madrid, Rascafría, Calle del Aguilón; 40°52'35.6" N, 3°50'44.0" W; 24 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 5 ♂♂, 2 ♀♀; Madrid, Lozoya; 40°58'07.9" N, 3°48'01.6" W; 24 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 3 ♂♂, 5 ♀♀; Madrid, Miraflores de la Sierra (Fuente del Cura); 40°48'51.1" N, 3°47'02.2" W; 7 May 2017; P. Álvarez leg. • 5 ♂♂, 3 ♀♀; Madrid, Miraflores de la Sierra (Fuente del Cura); 40°49'19.2" N, 3°47'21.5" W; 7 May 2017; P. Álvarez leg. • PORTUGAL • 1 ♂, 2 ♀♀; Porto de Mós, Arrimal; 39º31'54.6'' N, 8º52'20.7'' W; 9 Mar. 2014; A.R. Gonçalves leg. • 1 ♂; Leiria, Pombal, Cumieira; 39°53'33.5" N, 8°35'53.6" W; 18 Feb. 2017; S. Henriques leg.; LNHM • A. R. Gonçalves & F. Barros; Porto de Mós, Arrimal; 39º31'54.6'' N, 8º52'20.7'' W; 11 Mar. 2017; A. R. Gonçalves & F. Barros • 4 ♂♂, 5 ♀♀; Leiria, Pombal, Cumieira; 39°53'33.5" N, 8°35'53.6" W; 18 Mar. 2017; A. R. Gonçalves & J. Rosete leg. • 3 ♂♂, 7 ♀♀; Porto de Mós, Arrimal; 39º31'54.6'' N, 8º52'20.7'' W; 8 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 15 ♂♂, 13 ♀♀; Porto de Mós, Arrimal; 39º31'54.6'' N, 8º52'20.7'' W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 4 ♂♂, 6 ♀♀; Porto de Mós; 39°31'56.53" N, 8°52'17.86" W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 1 ♂; Porto de Mós; 39°33'52.41" N, 8°49'13.07" W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 2 ♂♂, 3 ♀♀; Porto de Mós; 39°32'20.18" N, 8°49'22.41" W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 2 ♂♂, 2 ♀♀; Porto de Mós; 39°30'37.37" N, 8°50'3.13" W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg. • 1 ♂, 2 ♀♀; Porto de Mós; 39°26'16.57" N, 8°55'6.93" W; 16 Apr. 2018; A.R. Gonçalves, S. Martins & F. Barros leg.

Diagnosis

Small, slender, dark and glabrous, species. Wings and halteres absent. Occiput largely covered by grey microtrichia, no long lateroclinate ocellars present; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel subconical, 2 times longer than pedicel; stylus 2 times longer than scape, pedicel and postpedicel combined. Thorax black with prothoracic episternum densely covered with grey setulae, appearing sub-triangular in lateral view. Legs with dark brown to black and yellow colour pattern. Abdomen black, tergites and sternites mostly covered by grey microtrichia and by sparse, very short, setae; apical sternites with long, distinct, setae.

Description

Male. Body length: 1.9 - 2.5 mm (measured from frons to end of terminalia, dry-mounted specimens, n=5). **Head.** Globular, black. Ocellar tubercle, face and frons largely glabrous, shining black; clypeus and occiput largely covered with grey microtrichia. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. One pair lateroclinate ocellars, *ca.* ½ smaller than the length of postpedicel, no anterior ocellars. Several equally long setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Black, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel subconical, 2 times as long as pedicel, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being 2 times longer than

scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery white setae and black setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a few distinct setae. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with long setae black, short ones pale. Humeral callus and prothoracic episternum, mesopleuron and sternopleuron, almost undifferentiated, scutellum almost undistinguishable and postscutellum poorly developed; metanotum, notopleuron, pteropleuron and hypopleuron well developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum densely covered, appearing sub-triangular in lateral view; hypopleuron thinly covered. Thorax black, glabrous expect for posterior portion of mesonotum, scutellum and metanotum, which are covered by grey microtrichia. Humeral callus bearing *ca*. 8 setae laterally, no dorsocentrals, *ca.* 8 acrostichal setae, irregular biseriate, of equal length, and 6 similar other setae placed irregularly; notopleuron with 2 setae; 2 setae near mesothoracic spiracle; 2 long, incurvated, prescutellar setae. **Wing** and halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Dark brown to black with the following yellow: trochanters, apical half of fore and mid femora, dorsal surface and basal ⅓ of fore and mid tibia, most of tarsomere 1 and 2, except for their black apical margin. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several short, downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae. **Abdomen.** Black, uniformly covered with grey microtrichia. Tergites and sternites with very short, sparse, setae, except for apical sternites with longer, distinct, setae. **Terminalia** (Figs 6–8). Subglobular, mostly covered by minute pale setulae, dark brown. Right epandrial lamella with long, irregularly placed, setae on apical ⅓. Right surstylus long, mostly glabrous, bearing stronger setae mostly along the dorsal margin, with several (*ca.* 8) distinct denticles, most of which aggregated on distal apex. Cerci of similar length, both with long setae of unequal length mostly placed on apical ⅓, enclosed in epandrial lamellae. Left epandrial lamella ⅓ shorter than left cercus, deeply bilobed, with a few long setulae on the apex. Left surstylus sub-rectangular with round apex margin, almost twice as long as wide, ⅓ shorter than cerci, densely covered with minute pale setulae and with unequally long setulae on the apex.

Female. Except for the terminalia, there are no distinguishing features between the sexes. Cerci pale brown with grey microtrichia and setae.

Variability. The main sources of variability are related to the leg colouration, number of prescutellar setae and shape of the right surstylus. In all populations, it is possible to find specimens with dark tarsi, instead of yellow. Regarding the right surstylus, in the southern population, this structure is usually long, narrowing distally (Fig. 6); in the Spanish central system, the specimens usually present a very short, sub-triangular, right surstylus (Fig. 7); the Portuguese specimens, often present a very long and narrow surstylus (Fig. 8). However, it is possible to find variability concerning the shapes of right surstylus within the same population. Concerning the number of prescutellar setae, the usual number is 2 setae, but in all populations analysed it is possible to observe specimens with several prescutellar setae (*ca.* 6).

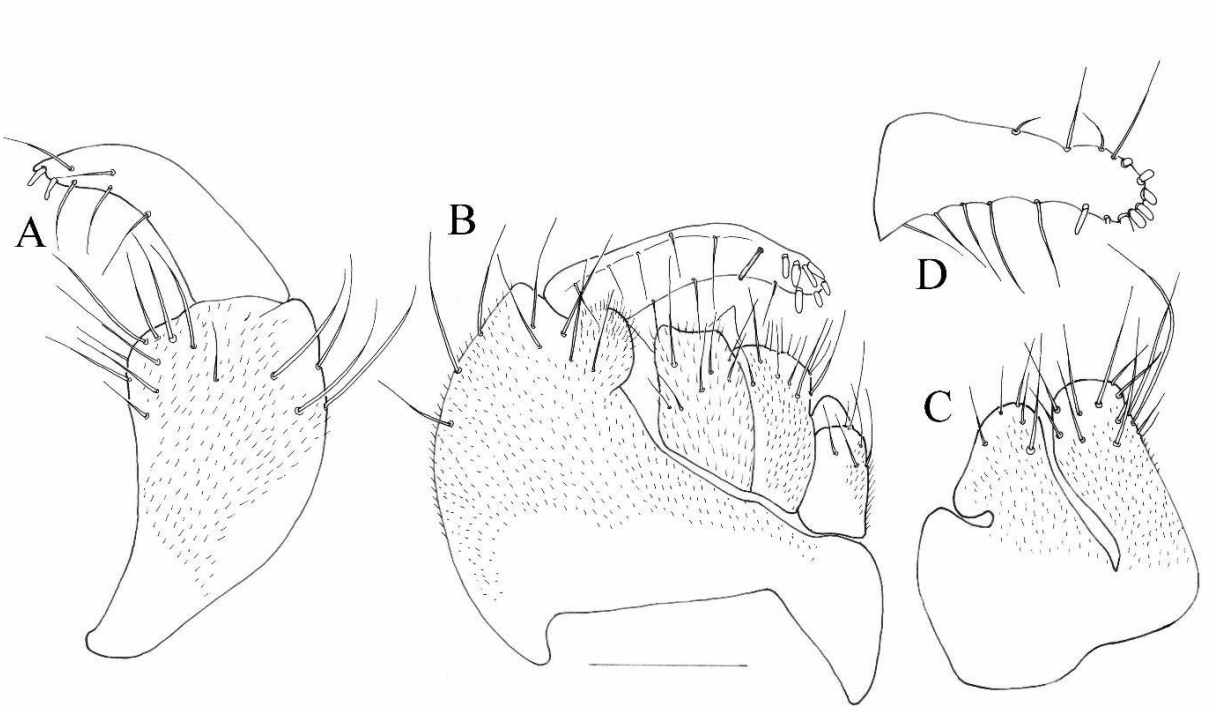

Fig. 6. Terminalia of *Tachydromia iberica* (Arias, 1919) from SPAIN, Madrid. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

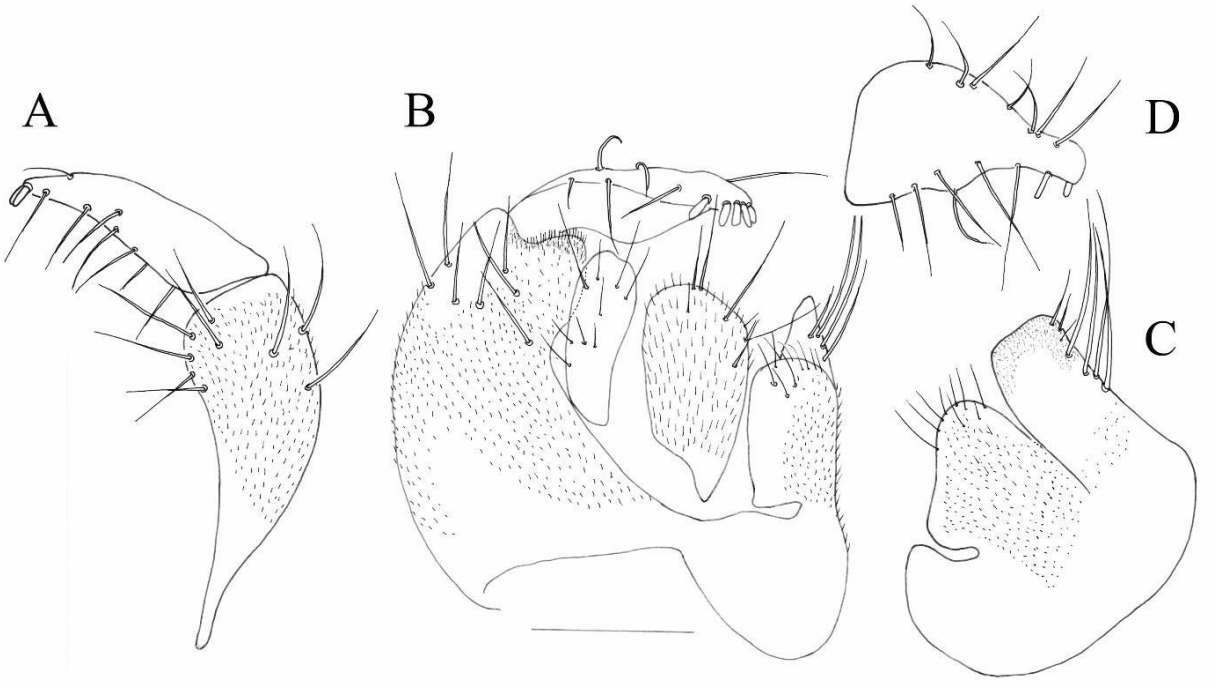

Fig. 7. Terminalia of *Tachydromia iberica* (Arias, 1919) from SPAIN, Segovia, El Espinar (Central System). A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

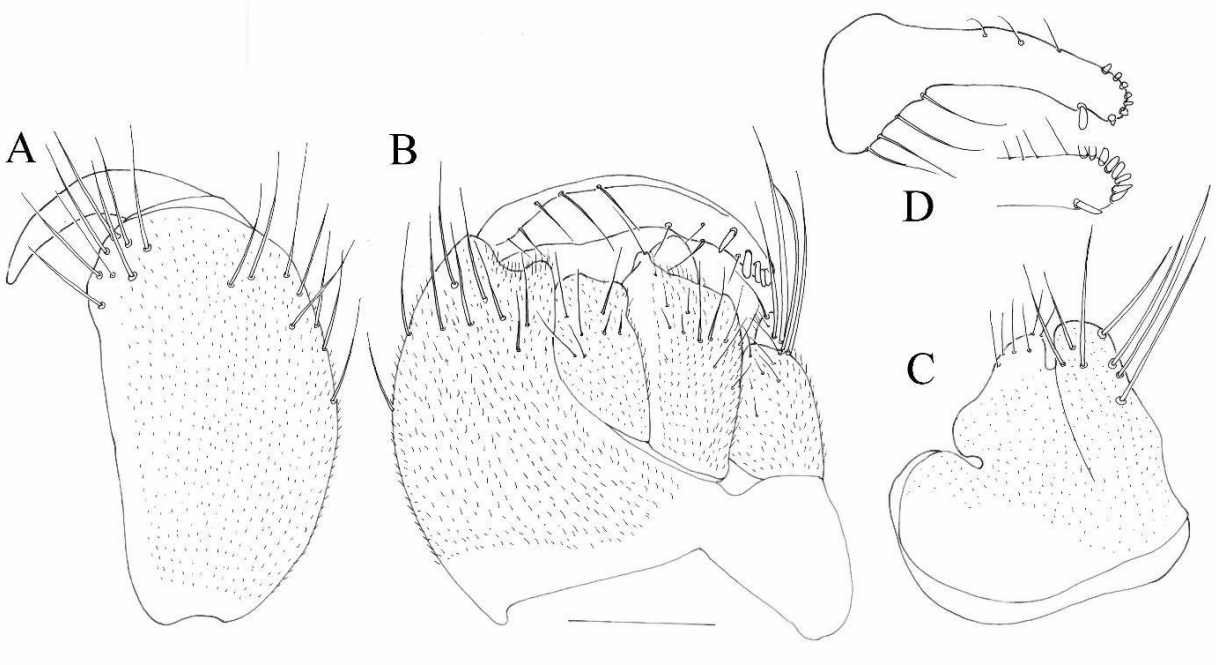

Fig. 8. Terminalia of *Tachydromia iberica* (Arias, 1919) from PORTUGAL, Leiria, Porto de Mós. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm

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5.2.5. *Tachydromia lusitanica* (Grootaert, Shamshev & Andrade, 2009)

Figs 9, 15A–B, 17A–B, 19A, 20E–F

Ariasella lusitanica Grootaert, Shamshev & Andrade, 2009: 45, 47

Tachydromia lusitanica comb. nov. Shamshev & Grootaert, 2018

Holotype
PORTUGAL • 1 ♂; Barcelos, Gilmonde; 41º30’42.54’’ N, 8º38’57.33’’ W; 8 Mar. 2008; R. Andrade leg.; RBINS

Paratypes
PORTUGAL • 2 ♂♂; Barcelos, Gilmonde; 41º30’42.54’’ N, 8º38’57.33’’ W; 8 Mar. 2008; R. Andrade leg.; RBINS • 1 ♂; 3 ♀♀; same collection data as for preceding; 9 Mar. 2008; R. Andrade leg.; RBINS • 1 ♂; same collection data as for preceding; 17 Feb. 2009; R. Andrade leg.; RBINS • 1 ♂; same collection data as for preceding; 18 Feb. 2009; R. Andrade leg.; RBINS • 3 ♂♂, 3 ♀♀; same collection data as for preceding; 23 Feb. 2009; R. Andrade leg.; RBINS

Other material examined
PORTUGAL • 1 ♂; Barcelos, Gilmonde; 41º30’42.54’’ N, 8º38’57.33’’ W; 28 Jan. 2013; R. Andrade leg. • 9 ♂♂, 6 ♀♀; Vila Nova de Gaia, Avintes; 41°06’00.0” N, 8°33’35.3” W; 23 Feb. 2013; R. Andrade leg. • 1 ♂; Barcelos, Gilmonde; 41º30’42.54’’ N, 8º38’57.33’’ W; 23 Feb. 2015; R. Andrade leg. • 1 ♂; same collection data as for preceding; 24 Feb. 2015 • 1 ♂, 1 ♀; same collection data as for preceding; 25 Feb. 2015 • 4 ♂♂, 6 ♀♀; same collection data as for preceding; 15 Apr. 2015 • 10 ♂♂, 8 ♀♀; same collection data as for preceding; 16 Apr. 2015 • 7 ♂♂, 3 ♀♀; Valongo, Valongo; 41º09’33.44’’ N, 8º29’05.6’’ W; 21 Feb. 2016; R. Andrade leg. • 2 ♂♂, 1 ♀; Arcos de Valdevez, Miranda; 41°49’49.1” N, 8°29’37.5” W; 8 Apr. 2017; R. Andrade & J. Almeida leg. • 10 ♂♂, 9 ♀♀; Arcos de Valdevez,

Miranda; 41°50’49.6” N, 8°29’27.9” W; 8 Apr. 2017; R. Andrade & J. Almeida leg. • 5 ♂♂, 9 ♀♀; Paredes de Coura, Vascões; 41°53’50.4” N, 8°29’33.2” W; 8 Apr. 2017; R. Andrade & J. Almeida leg. • 2 ♂♂, 3 ♀♀; Arcos de Valdevez, Rio Frio; 41°51’51.7” N, 8°30’21.4” W; 8 Apr. 2017; R. Andrade & J. Almeida leg. • 1 ♂, 3 ♀♀; Fafe, Travassós; 41°30’13.4” N, 8°09’52.7” W; 9 Apr. 2017; R. Andrade & J. Almeida leg. • 5 ♂♂, 9 ♀♀; Fafe, Travassós; 41°30’06.4” N, 8°10’55.6” W; 9 Apr. 2017; R. Andrade & J. Almeida leg. • 3 ♂♂, 6 ♀♀; Fafe, Felgueiras; 41°31’24.0” N, 8°06’01.2” W; 9 Apr. 2017; R. Andrade & J. Almeida leg. • x; Amares, Santa Maria do Bouro; 41°39’52.5” N, 8°16’06.5” W; x; R. Andrade leg. • 2 ♀♀; Maia, Gemunde; 41°16’42.6”N, 8°38’09.4”W; 10 March 2019; C. Silva leg. • SPAIN • 1 ♂, 1 ♀; Galiza, Parque Natural Monte Aloia; 42°04’22.0” N, 8°40’45.8” W; 4 May 2018; A.R. Gonçalves leg. • 2 ♂♂; Galiza, Pontevedra, Tui; 42°27’59.9” N, 8°28’09.7” W; 4 May 2018; A.R. Gonçalves leg. • 4 ♂♂, 3 ♀♀; Galiza, Pontevedra, Carballeira do Río Xesta; 42°23’18.3” N, 8°22’36.3” W; 4 May 2018; A.R. Gonçalves leg. • 1 ♂, 3 ♀♀; Galiza, Pontevedra, Carballeira de San Xusto; 42°31’02.9” N, 8°30’42.6” W; 4 May 2018; A.R. Gonçalves leg. • 2 ♂♂; Galiza, Cascada del Río Barosa; 42°33’24.4” N, 8°37’45.0” W; 4 May 2018; A.R. Gonçalves leg. • 2 ♂♂, 8 ♀♀; Galiza, Lugo, Muras; 43°27’27.3” N, 7°46’31.5” W; 6 May 2018; A.R. Gonçalves leg. • 9 ♂♂, 5 ♀♀; Galiza, Bosque de Fervenza; 42°53’22.7” N, 7°31’09.5” W; 7 May 2018; A.R. Gonçalves leg. • 5 ♂♂, 8 ♀♀; Galiza, Fraga de Catasós; 42°38’18.3” N, 8°05’45.3” W; 7 May 2018; A.R. Gonçalves leg. • 3 ♂♂, 1 ♀; Galiza, Ourense, Piñor; 42°31’17.3” N, 8°00’14.3” W; 7 May 2018; A.R. Gonçalves leg.

Diagnosis

Small [2.25 - 2.40 mm (n=3)], slender, dark and hirsute, species. Wing-dimorphic: male is stenopterous; bilobed distal apex, with a very long digitation on distal margin (Fig. 20E). Stalk-like portion dark brown on basal half and pale on distal half; lobed apex mostly black, digitation hyaline with black distal ⅓ margin. Female is micropterous, wing bilobate, squamiform, apical margin of each lobe bearing setae - the posterior lobe has 2 long setae and the anterior has 2 short setae (Fig. 20F). Wing veins and halteres absent in both sexes. Occiput largely covered by grey microtrichia, except for lower half; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel lanceolate, 2.5 times longer than pedicel; stylus 2 times longer than scape, pedicel and postpedicel combined. Thorax black with prothoracic episternum densely covered with grey microtrichia, appearing sub-triangular in lateral view. Humeral callus bearing 7 setae laterally, *ca.* 9 acrostichal setae, irregular biseriate, of equal length; notopleuron with 1 strong, prominent, seta plus 5 small setae; 2 strong prescutellar setae present laterally plus several setulae, scutellum bearing 2 similar setae medially. Legs mainly dark but with a pattern of yellow and brown to black; male fore tibia with dense ciliation of long hair-like setae; male mid tibia has, at its anteroventral apex, a spur, produced into a barely noticeable apical elongation, about as long as diameter of tibia at apex (Fig. 19A); the spur possesses several distinct setae and, on the distal half margin, 4 short, stout, curved, spinose setae. Abdomen black, tergites and sternites overall uniformly covered by grey microtrichia and long setae; apical sternites with longer setae. Terminalia (Fig. 9) Subglobular, mostly covered with grey microtrichia, dark brown. Right surstylus produced into a short projection, bearing long setae at the lateral margins and *ca.* 11 denticles. Right cerci slightly longer than left, both enclosed in epandrial lamellae. Left epandrial lamella bilobed.

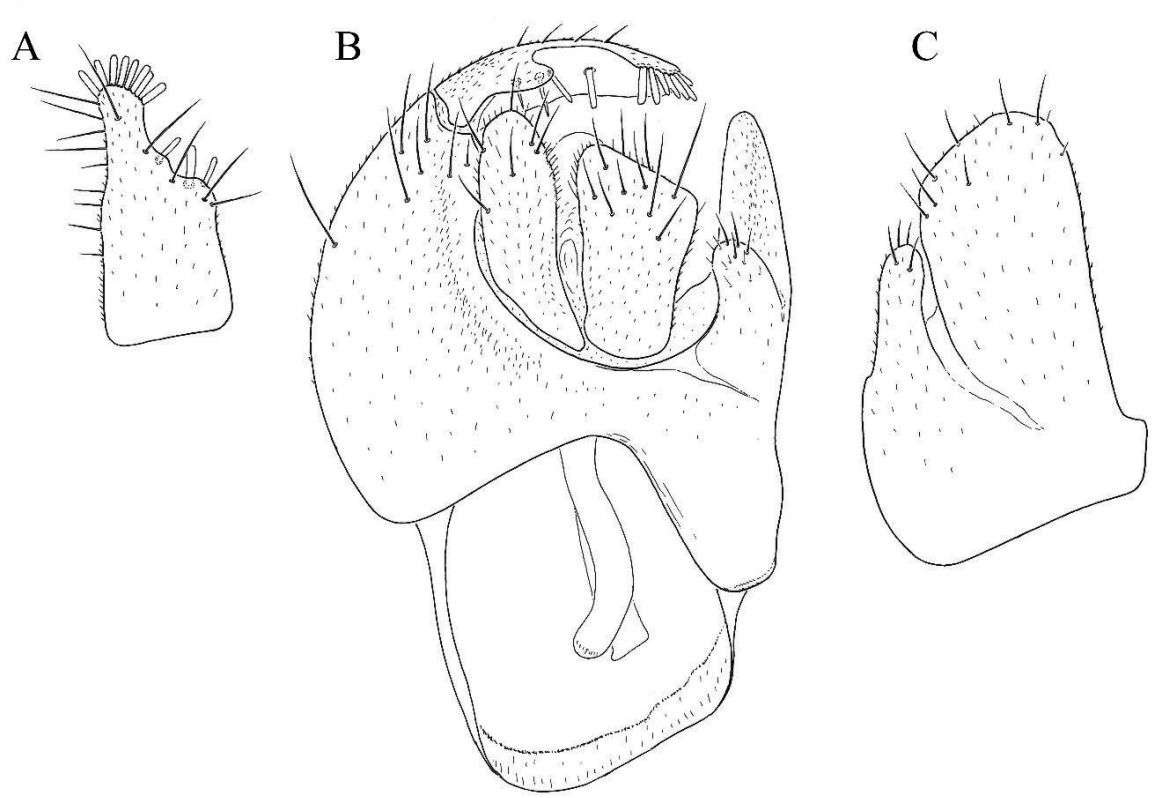

Fig. 9. Terminalia of the holotype of *Tachydromia lusitanica* (Grootaert, Shamshev & Andrade, 2009) from PORTUGAL, Braga, Gilmonde. A, right surstylus, B, epandrium with cerci, C, left epandrial lamella and left surstylus. Figure adapted from Grootaert et al., 2009.

5.2.6. *Tachydromia nigrohirta* sp. nov. Gonçalves, Grootaert & Andrade.

Figs 10, 15C–D, 17C–D, 19I, 20G–H

Etymology. The name of this species is composed of two Latin words, *niger* (nigro) meaning black and *hīrtus* (hirta) meaning hairy. The name was selected due to being characteristically hirsute and dark.

Material examined

Holotype
SPAIN • 1 ♂; Salamanca, El Maíllo; 40º33'24.8'' N, 6º10'23.9'' W; 30 Mar. 2015; A.R. Gonçalves & R. Andrade leg.
Paratypes
SPAIN • 2 ♂♂, 2 ♀♀; Salamanca, El Maíllo; 40º33'24.8'' N, 6º10'23.9'' W; 30 Mar. 2015; A.R. Gonçalves & R. Andrade leg.; 3 ♂♂, 2 ♀♀; Salamanca, El Maíllo; 40º33'24.8'' N, 6º10'23.9'' W; 25 Apr. 2017; A.R. Gonçalves leg.

Other material
SPAIN • 3 ♂♂, 2 ♀♀; Salamanca, El Maíllo; 40º33'24.8'' N, 6º10'23.9'' W; 30 Mar. 2015; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 5 ♀♀; Salamanca, La Alberca; 40º29'04.0'' N, 6º05'10.2'' W; 31

Mar. 2015; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 5 ♀♀; Salamanca, La Alberca; 40º29'04.0'' N, 6º05'10.2'' W; 25 Apr. 2017; A.R. Gonçalves leg. • 4 ♂♂, 2 ♀♀; Salamanca, El Maíllo; 40º33'24.8'' N, 6º10'23.9'' W; 25 Apr. 2017; A.R. Gonçalves leg. • 2 ♂♂, 1 ♀; Salamanca, Villasrubías; 40°19'42.3" N, 6°38'42.1" W; 25 Apr. 2017; A.R. Gonçalves leg.

Diagnosis

Small, slender, very dark and hirsute, species. Wing-dimorphic: male is stenopterous; lobed distal apex, sub oval, with a minute digitation on apical margin. Stalk-like portion dark brown on basal half and pale on distal half; distal ⅔ of lobe black with basal ⅓ and digitation translucid; female is micropterous, wing bilobate, apical margin of each lobe bearing setae - the posterior lobe has 1 long seta and the anterior has 3 short setae. Halteres absent. Occiput largely covered by grey microtrichia, except for lower half; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel lanceolate, 2 times longer than pedicel; stylus 1.5 longer than scape, pedicel and postpedicel combined. Thorax black with prothoracic episternum densely covered with grey setulae, appearing sub-triangular in lateral view. Legs mostly black except for yellowish to pale brown trochanters, knees and tarsomere 1 and 2; male fore tibia with dense ciliation of long hair-like setae. Abdomen black, tergites uniformly covered by grey microtrichia and long setae.

Description

Male. Body length: 2.4 - 2.6 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 0.7 - 0.8 mm (dry-mounted specimens, n=5). **Head.** Globular, black. Ocellar tubercle, face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; occiput largely covered with grey microtrichia, except for the glabrous lower half. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. One pair lateroclinate ocellars, *ca.* ⅓ smaller than the length of postpedicel, no anterior ocellars. One pair proclinate verticals, approximately half the length of postpedicel, and *ca.* 40 long setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Black, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel lanceolate, 2 times as long as pedicel, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being 1.5 times longer than scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae and black setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a distinct seta on the ventral apex. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with long setae black, short ones pale. Humeral callus and prothoracic episternum, mesopleuron and sternopleuron, almost undifferentiated, scutellum very short and poorly differentiated; notopleuron, pteropleuron, hypopleuron and postscutellum well developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum densely covered, appearing sub-triangular in lateral view; hypopleuron, scutellum and upper part of the metathorax thinly covered. Humeral callus bearing *ca*. 7 setae laterally, no dorsocentrals, 8 acrostichal setae, irregular biseriate, of equal length; 1 small pre-alar seta; notopleuron with 1 strong, prominent, seta plus 2 small setae; 4 long, incurvated, prescutellar setae; scutellum bearing 2 strong, long setae medially. **Wing.** Stenopterous. Lobed distal apex, sub-oval, with a minute digitation. Stalk-like portion dark brown on basal half and pale on distal half; distal ⅔ of lobe black with basal ⅓ and digitation translucid (appearing white under most light

conditions) (Fig. 20G). No veins distinguishable. Uniformly covered in microtrichia. Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Black, except for trochanters, knees and tarsomere 1 and 2 which are yellowish to brown. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several short, downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae. Front tibia bearing a dense ciliation of long hairs (distinctively larger than the maximum diameter of the tibia) with curved tips, and a row of long, erect, setae present at its distal ⅓ posterodorsal surface. Tarsomere 1 of fore tarsus with long hairs and a row of distinct black setae on the posterior distal surface. Mid tibia at its anteroventral apex has a spur, with a distinct apical elongation, about as long as diameter of tibia at apex (Fig. 19I); the spur possesses a few distinct setae and, on the basal half margin, 4 short, stout, curved, spinose setae. **Abdomen.** Black, uniformly covered with grey microtrichia. Tergites and sternites with evenly distributed long setae, except for apical sternites with longer, distinct, setae. **Terminalia** (Fig. 10). Subglobular, mostly covered by minute pale setulae, dark brown. Right epandrial lamella with irregularly placed setae and with a patch of similar setae on lateral surface. Right surstylus short with anterior margin produced into short slender projection, mostly glabrous, with a small patch of microtrichia, bearing stronger setae mostly on the apical dorsal margin, with several (*ca.* 8) distinct denticles, most of which aggregated on distal apex. Cerci of similar length, both with long setae of unequal length on apical half, enclosed in epandrial lamellae. Left epandrial lamella ⅓ shorter than left cercus, deeply bilobed, with a few long setulae on the distal apex. Left surstylus sub-rectangular with round apex margin, twice as long as wide, distinctively longer than cerci, densely covered with minute pale setulae and with equally long setulae on the apex.

Female. Similar to male, except for the following features: micropterous, with wing round, brownish, bilobate, apical margin of each lobe bearing setae - the posterior lobe has 1 long seta and the anterior has 3 short setae - both covered with grey microtrichia (Fig. 20H); legs covered with regular rows of setae; spur on mid tibia absent; cerci pale brown with grey microtrichia and setae; no longer setae on apical sternites.

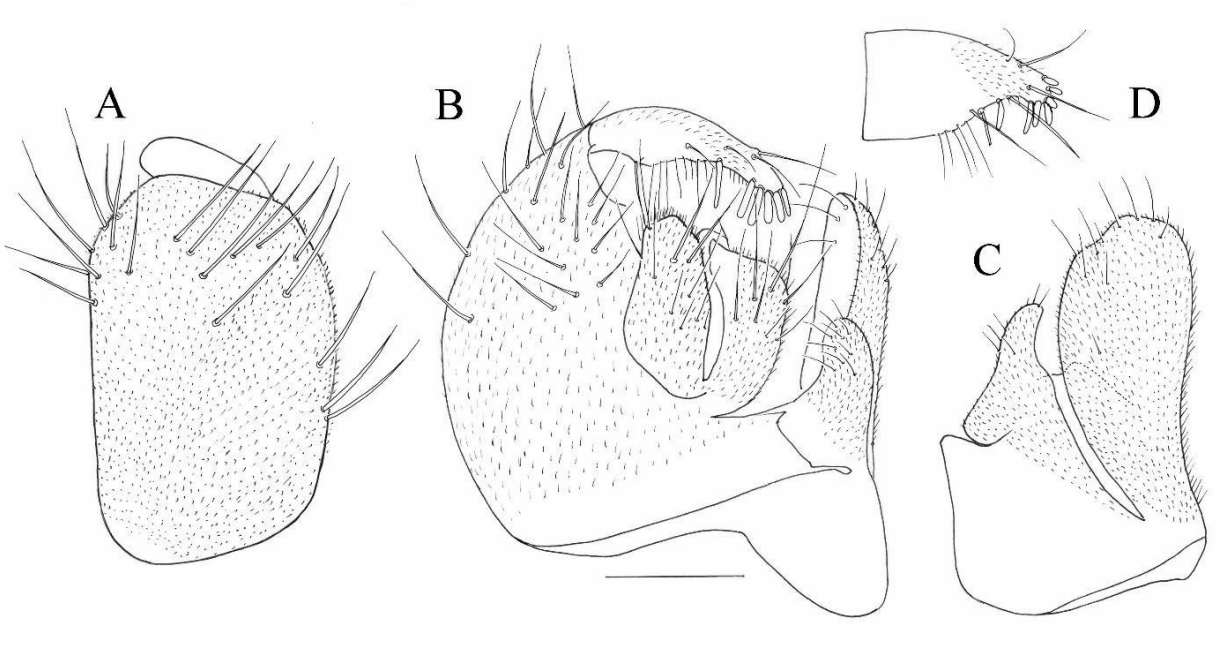

Fig. 10. Terminalia of *Tachydromia nigrohirta* sp. nov. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm

5.2.7. *Tachydromia pandellei* (Séguy, 1941)

Figs 11, 16G–H, 18I–J, 19B, 21A,C

Ariasella pandellei Séguy, 1941: 4-5

Tachydromia pandellei comb. nov. Shamshev & Grootaert, 2018

Material examined

Lectotype
FRANCE • 1 ♂ ; Hautes-Pyrénées, Arrens-Marsous ; 28 Jun. 1879 ; L. Pandellé leg., MHNP-1256.

Paralectotype
FRANCE • 2 ♂♂, 2 ♀♀ ; Hautes-Pyrénées, Arrens-Marsous ; 28 Jun. 1879 ; L. Pandellé leg., MHNP-1256.

Other material examined
FRANCE • 2 ♂♂, 1 ♀ ; Hautes-Pyrénées, Arrens-Marsous ; 42°57'20.3 N, 0°12'52.1 W ; 18 Jun. 2017 ; A.R. Gonçalves & E. Marabuto leg. • 2 ♂♂, 1 ♀ ; Hautes-Pyrénées, Arrens-Marsous, Arrens, Route d'Aste ; 42°56'14.9 N, 0°13'45.9 W ; 18 Jun. 2017 ; A.R. Gonçalves & E. Marabuto leg. • 3 ♂♂, 6 ♀♀ ; Hautes-Pyrénées, Barèges, Touët et Labach ; 42°54'08.1 N, 0°06'04.0 E ; 19 Jun. 2017 ; A.R. Gonçalves & E. Marabuto leg. • 1 ♀; Hautes-Pyrénées, Campan; 42º 55' 31'' N, 0º11'49'' E; 20 Jun. 2017; E. Marabuto leg. • SPAIN • 5 ♀♀; Burgos, Villasur de Herreros; 42º18'26.4'' N, 3º19'59.1'' W; 15 May 2014; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 9 ♀♀; Burgos, Pancorbo; 42º38'42.4'' N, 3º06'17.1'' W; 11 May 2015; A.R. Gonçalves & R. Andrade leg. • 2 ♂♂, 6 ♀♀; Basque Country, Araba/Álava (Gorbea Natural Park); 42°59'02.6" N, 2°49'59.4" W; 17 Jun. 2017; A.R. Gonçalves leg.

Diagnosis
Small, shining black species. Wing-dimorphic: male is stenopterous; wing with cordiform apex; stalk-like portion rigid, yellow to light brown; lobed apex with basal half hyaline and distal half black; female is stenopterous, stalk-like portion yellowish with minute lobed apex pale brown. Venation absent. Halteres absent in both sexes. Occiput largely covered with grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; antennae yellow with brownish postpedicel; palpi yellow; proboscis black; postpedicel roundish, approximately as long as pedicel. Thorax black, very shining, with prothoracic episternum densely covered with grey setulae, appearing sub-triangular in lateral view; mesopleuron somewhat humped. Legs overall yellow with brownish hind femora and tibia, tarsomeres yellow and black. Male fore tibia with sparse ciliation of long hair-like setae. Abdomen black, tergites and sternites overall covered with grey microtrichia and setae.

Description

Male. Body length: 2.4 - 2.8 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 0.9 - 1.1 mm (dry-mounted specimens, n=5). **Head.** Globular, black. Face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle glabrous. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for a glabrous patch posterior to the occiput. One pair lateroclinate ocellars, 2 times the length of postpedicel, no posterior ocellars. One pair proclinate verticals, 1.5 times the length of postpedicel, and numerous very long setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Scape and pedicel yellow, postpedicel brown; covered by

grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel oval, twice as long as wide and slightly longer than pedicel, with numerous pale setulae that are longer ventrally and apically; stylus black, bare and arising dorsoapically, being slightly more than 3 times as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Brown in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally. **Proboscis.** Black, mostly glabrous, thin, long setae present ventrally, larger than palpus. **Thorax.** Elongated, overall black and largely shining, with black setae. Thoracic pleuron with poorly differentiated sclerites, however, it's possible to distinguish a separation between humeral callus, notopleuron, prothoracic episternum, mesopleuron, pteropleuron, sternopleuron and hypopleuron. Humeral callus very small, mesopleuron somewhat humped, scutellum well defined but very short, postscutellum longer and metanotum well developed. Pleura black, overall glabrous with small defined patches of grey microtrichia: prosternum and prothoracic episternum densely covered, appearing sub-triangular in lateral view; hypopleuron, scutellum, postscutellum and metanotum thinly covered; pteuropleuron with 3 setae on apical margin, near the base of the wing. Humeral callus bearing 12 setae laterally, no dorsocentrals, *ca*. 8 acrostichal setae, biseriate, of equal length; notopleuron with 1 strong, prominent, very long seta, plus 3 smaller ones; prescutellum bearing 4 setae, inner setae longer. **Wing.** Stenopterous. Lobed apex cordiform. Stalk-like portion rigid, yellow except for brown basal portion; lobed apex with basal half hyaline (appearing silver under certain light conditions), distal half black. Venation absent. Uniformly covered in microtrichia (Fig. 21A). Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Overall yellow, except for the following segments, which are brown to black: posterodorsal surface of coxae, apical ¼ dorsal surface, posterior and anterior surface of hind femora, most of hind tibia (except for yellow basal and apical portion); tarsomere 5 of fore and mid-leg, distal apex of tarsomere 1 and 2, distal ⅓ of tarsomere 3, distal ¾ of tarsomere 4 and tarsomere 5. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora. Coxae with several short, downward directed, dorsal anteroapical setae. Femora of all legs with a circlet of a few distinct apical setae; mid femora at its posteroventral basal surface bears several black denticles and black setae. Fore tibia bearing a sparse ciliation of hair-like setae (slightly shorter than maximum diameter of the tibia) with curved tips, and a row of long, erect, setae present at its distal ⅓ anterodorsal surface; mid tibia at its anteroventral apex has 1 spur produced into a distinct and long apical elongation (Fig. 19B); both are longer than the diameter of tibia at apex. Spur is mostly glabrous ventrally but dorsally is covered by microtrichia and a few setae; additionally possesses a few distinct setae and, on the basal half margin, 3 short, stout, curved, setae. Elongation is mostly glabrous but with a few setae arising marginally and apically. Tarsomere 1 of foreleg has long pale setae and a patch of black setae at the apical margin of its posteroventral surface. **Abdomen.** Black, uniformly covered with grey microtrichia. Tergites and sternites with evenly distributed setae, except for last sternite with long setae. **Terminalia** (Fig. 11). Subglobular, mostly covered with grey microtrichia, dark brown. Right surstylus bifurcated into a short projection and very long and slender projection; mostly glabrous but bearing long, curved, setae at the lateral margin and 2 denticles at the distal margin of longer projection; short projection with similar setae at its distal margin. Right epandrial lamella with long, erect setae mostly on apical half. Right cercus with setae on apical half, left cercus with setae on apical half. Right cerci slightly longer than left, both enclosed in epandrial lamellae. Left epandrial lamella as long as left cercus, bilobed, with setae and setae of unequal length on apical half. Left surstylus sub-cylindrical, narrowing proximately, with rounded apex, 4 times as long as wide, as long as right cercus, with setae on lateral margins and apical ⅓ surface.

Female. Similar to male, except for the following features: stenopterous [length of wing (n=2): 0.7 - 0.8 mm], with a very minute lobed apex, stalk-like portion yellowish with the lobe light brown, covered by

grey microtrichia (Fig. 21C); legs covered with regular rows of setae, with distinct circle of setae on front femora and anteroapical setae on coxae, but no other distinctive hairs or setae; spurs on mid tibia absent; cerci pale brown with grey microtrichia and setae.

Variability. There is variability in the shape of the wing lobe in males between the populations sampled in the French Pyrenees and those from Spain. In the first, the lobed apex is distinctively large and cordiform, while in the latter the lobed apex is comparatively smaller and oval.

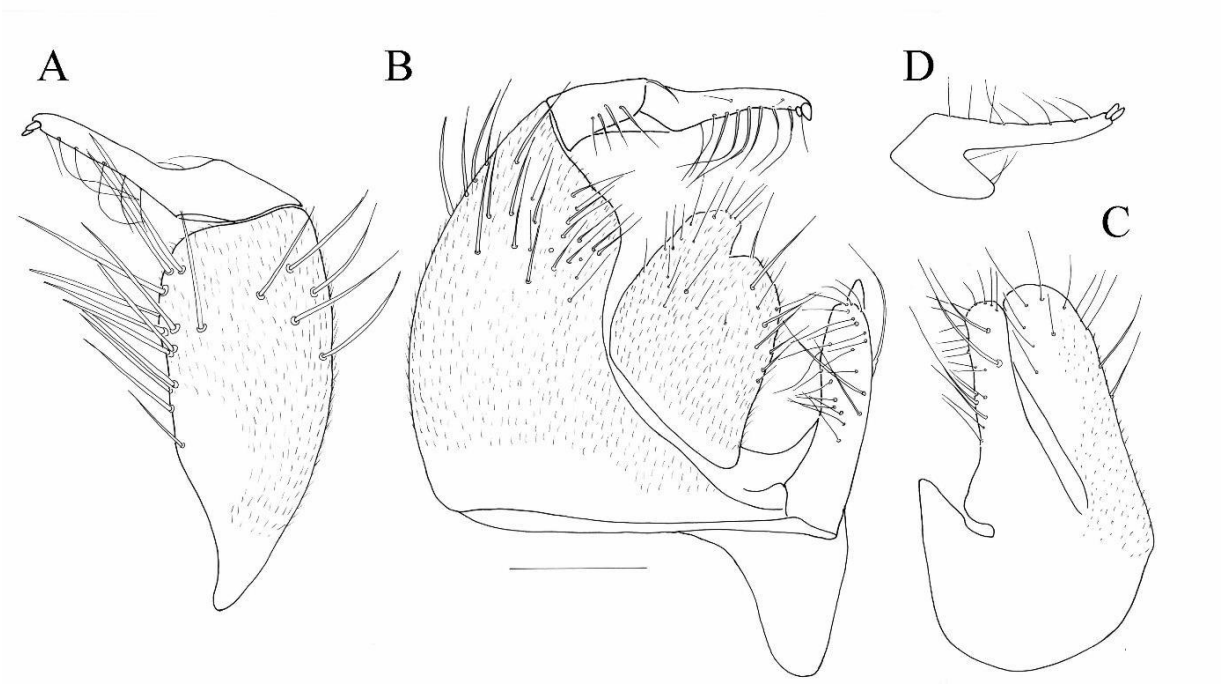

Fig. 11. Terminalia of the topotype of *Tachydromia pandellei* (Séguy, 1941) from FRANCE, Hautes-Pyrénées, Arrens-Marsous. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm

5.2.8. *Tachydromia pieltaini* (Gil, 1936)

Figs 12, 16E–F, 18G–H, 19C, 21B–C

Ariasella pieltaini Gil, 1936: 191-193

Tachydromia pieltaini comb. nov. Shamshev & Grootaert, 2018

Material not examined

Holotype
SPAIN • 1 ♀; Asturias, Porra de Enol; C. Bolívar leg.

Material examined
SPAIN • 5 ♂♂, 5 ♀♀; Asturias, Cangas de Onís, Covadonga; 43°18’42.4” N, 5°03’28.0” W; 26. Apr. 2011; R. Andrade leg. • 2 ♂♂, 4 ♀♀; Asturias, Cangas de Onís; 43º21’14.6’’ N, 5º07’25.9’’ W; 9 Apr. 2016; A.R. Gonçalves leg.

Diagnosis

Small, shining black species. Wing-dimorphic: male is stenopterous; wing with oval apex; stalk-like

portion rigid, yellow to light brown; lobed apex with hyaline cells, veins and distal margin black; venation very reduced (costal, longitudinal and cross veins distinguishable); female is stenopterous, stalk-like portion yellowish with minute lobed apex pale brown. Halteres absent in both sexes. Occiput largely covered with grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; proboscis and antennae black; palpi yellow; postpedicel roundish, approximately as long as pedicel. Thorax black, very shining, with the basal half of prothoracic episternum densely covered with grey setulae. Legs overall yellow only with the distal tarsomeres black. Male fore tibia with dense ciliation of long hair-like setae. Abdomen black, tergites and sternites overall covered with setae; grey microtrichia present at the last apical tergite and most sternites, resulting shining appearance of the dorsal surface.

Description

Male (previously undescribed)**.** Body length: 2.6 - 2.9 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 0.9 - 1.1 mm (dry-mounted specimens, n=5). **Head.** Globular, black. Face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle glabrous. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for a glabrous patch posterior to the occiput. One pair lateroclinate ocellars, 2 times the length of postpedicel, no posterior ocellars. One pair proclinate verticals, 1.8 times the length of postpedicel, and numerous very long setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Yellow, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel roundish, as long as wide and, approximately, as long as pedicel, with numerous pale setulae that are longer ventrally and apically; stylus black, bare and arising dorsoapically, being almost 4 times as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 4 times as long as wide. Yellow in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally. **Proboscis.** Black, mostly glabrous, thin, long setae present ventrally, larger than palpus. **Thorax.** Elongated, overall black and largely shining, with black setae. Thoracic pleuron with poorly differentiated sclerites, however, it's possible to distinguish a separation between humeral callus, notopleuron, prothoracic episternum, mesopleuron, pteropleuron, sternopleuron and hypopleuron. Scutellum short, postscutellum longer and metanotum well developed. Pleura black, overall glabrous with small defined patches of grey microtrichia: prosternum and basal half of prothoracic episternum densely covered; hypopleuron, scutellum, postscutellum and metanotum thinly covered. Humeral callus bearing 7 setae laterally, no dorsocentrals, 5 acrostichal setae, irregularly placed, of equal length; notopleuron with 1 strong, prominent, very long seta, plus 3 smaller ones; prescutellum bearing 4 setae and scutellum bearing 2 setae medially. **Wing.** Stenopterous. *Ca*. 8 costal setae at the base of the wing. Lobed apex, oval. Stalk-like portion rigid, yellow to light brown; lobed apex with hyaline cells, veins and distal margin black. Venation very reduced (costal, longitudinal and cross veins distinguishable), forming 4 cells. Uniformly covered in microtrichia (Fig. 21B). Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Overall yellow, except for dorsal strip on fore and mid femora, posterior coxae, tarsomere 5 of fore and mid-leg, for the apical portion of tarsomere 2 and 3, tarsomere 4 and 5 of hind leg, which are brown to black. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora. Coxae with several short, downward directed, dorsal anteroapical setae. Mid trochanter at its anteroventral margin has several black setae. Femora of all legs with a circlet of a few distinct apical setae; mid femora at its posteroventral basal surface bears a small, roundish, projection with several black denticles and *ca.* 3 black setae. Fore tibia bearing a ciliation of hair-like setae (slightly longer than

maximum diameter of the tibia) with curved tips, and a row of long, erect, setae present at its distal ⅓ anterodorsal surface; mid tibia at its anteroventral apex has 1 spur produced into a distinct and long apical elongation (Fig. 19C); both are slightly longer than the diameter of tibia at apex. Spur is covered on its surface with microtrichia and >10 short, blunt, denticles; additionally, has 6 longer, stout, curved, setae at its lateral margin. Elongation is mostly glabrous but with a few setae arising marginally and apically. Tarsomere 1 of foreleg has a row of long pale setae at its anterior surface, decreasing in length distally, and a patch of black setae at the apical margin of its posteroventral surface. **Abdomen.** Black, shining, with grey microtrichia present covering only the last tergite and almost all sternites, except for the sternite 1 and 2. Tergites and sternites overall covered with setae, except for the dorsal surface of all tergites and most of sternite 1 (distal margin with a few setae); last apical sternite and tergite with longer setae. **Terminalia** (Fig. 12). Subglobular, mostly covered with grey microtrichia, dark brown. Right surstylus short with irregular margins, narrowing distally, mostly glabrous but bearing long setae, with several (*ca.* 8) distinct denticles, aggregated on distal margin. Right epandrial lamella with long, erect setae on apical half. Right cerci with setae on apical ⅓; left cerci with setae of unequal length on its apical half and 2 long setae on apex. Right cerci longer than left, both enclosed in epandrial lamellae. Left epandrial lamella
⅓ shorter than left cercus, bilobed, with setae of similar length on apical half of ventral surface and on the apex of dorsal margin. Left surstylus sub-rectangular with rounded apex, 3 times as long as wide, longer than right cercus, with very long setae on lateral margins and apex.

Female. Similar to male, except for the following features: stenopterous [length of wing (n=2): 0.7 - 0.8 mm], with a very minute lobed apex, stalk-like portion yellowish with the lobe light brown, covered by grey microtrichia (Fig. 21C); legs covered with regular rows of setae, with distinct circle of setae on front femora and anteroapical setae on coxae, but no other distinctive hairs or setae; spurs on mid tibia absent; cerci pale brown with grey microtrichia and setae.

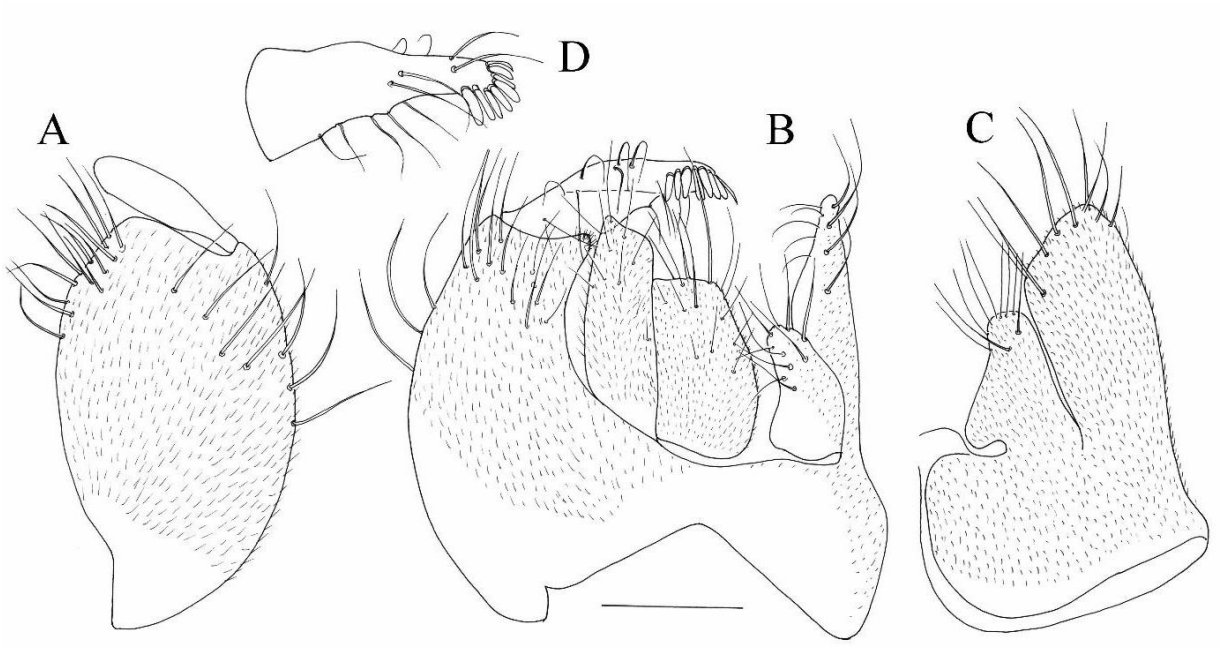

Fig. 12. Terminalia of *Tachydromia pieltaini* (Gil, 1936) from SPAIN, Asturias, Covadonga. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

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5.2.9. *Tachydromia semiaptera* (Gil, 1923)

Figs 13, 16C–D, 18E–F, 19E–F, 21D–E

Ariasella semiaptera Gil, 1923: 150-154

Tachydromia semiaptera comb. nov. Shamshev & Grootaert, 2018

Material not examined

Type material
SPAIN • Ávila, Valle de Iruelas; May 1919; C. Bolívar leg. (*ca*. 20 specimens collected).

Other material
SPAIN • Ciudad Real, Navas de Estena; C. Bolívar leg. • Madrid, El Escorial; C. Bolívar leg.

Material examined
PORTUGAL • 2 ♂♂, 1 ♀; Miranda do Douro; 41°33'25.9" N, 6°15'43.3" W; 3 May 2015; A.R. Gonçalves leg. • 3 ♂♂, 9 ♀♀; Bragança, Castrelos; 41º50'22.1'' N, 6º53'24.2'' W; 10 May 2015; A.R. Gonçalves & R. Andrade leg. • 13 ♂♂, 10 ♀♀; Carrazeda de Ansiães, Fontelonga; 41º14'16.2'' N,7º14'57.6'' W; 1 May 2016; R. Andrade leg. • SPAIN • 10 ♂♂, 11 ♀♀; Zamora, Alcañices; 41º41'26.9'' N, 6º21'45.1'' W; 11 May 2015; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 16 ♀♀; Ávila, El Barraco; 40º23'08.4'' N, 4º34'05.7'' W; 13 Apr. 2016; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 6 ♀♀; Toledo, Los Navalucillos; 39º36'02.0'' N, 4º42'07.0'' W; 15 Apr. 2016; A.R. Gonçalves & R. Andrade leg. • 7 ♂♂, 1 ♀; Toledo, Los Navalucillos; 39º34'25.0'' N, 4º44'57.0'' W; 15 Apr. 2016; A.R. Gonçalves & R. Andrade leg. • 5 ♂♂, 4 ♀♀; Guadalajara, El Cardoso de la Sierra; 41°05'46.2" N, 3°28'56.1" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 4 ♂♂, 3 ♀♀; Guadalajara, El Cardoso de la Sierra (Río Berbellido); 41°06'22.6" N, 3°24'59.7" W; 23 Apr. 2017; A. R. Gonçalves & P. Álvarez leg. • 5 ♂♂, 2 ♀♀; Salamanca, La Alberca; 40º29'04.0'' N, 6º05'10.2'' W; 25 Apr. 2017; A. R. Gonçalves leg. • 9 ♂♂, 6 ♀♀; Madrid, Bustarviejo; 40°50'29.8" N, 3°45'59.1" W; 21 May 2017; P. Álvarez leg. • 17 ♂♂, 23 ♀♀; Cáceres, Alía; 39°30'40.3" N, 5°20'47.7" W; 23 Apr. 2018; P. Álvarez leg. • 3 ♂♂, 5 ♀♀; Cáceres, Berzocana; 39°24'55.6" N, 5°25'10.3" W; 23 Apr. 2018; A. R. Gonçalves & P. Álvarez leg.

Diagnosis

Small, slender, dark, species. Wing-dimorphic: male is stenopterous; oblong apex; stalk-like portion very flexible, light brown with a distinct black spot on the lobed apex; three distinguishable veins (submarginal, marginal and longitudinal); female is micropterous, with wing round, brownish, bearing 1 long seta and 1 short setulae on the apical margin. Halteres absent. Occiput largely covered with grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; postpedicel roundish, approximately as long as pedicel. Thorax black with the prothoracic episternum densely covered with grey setulae, appearing sub-triangular in lateral view. Legs with a colour pattern of yellowish and dark brown to black. Male legs with the following features: fore tibia black, stout and inflated; mid tibia bearing 2 projections and a row of 5 spinose setae on its posteroventral basal surface. Abdomen black, overall covered with microtrichia and sparse setae; last 2 apical sternites with long setae.

Description

Male. Body length: 2.4 - 2.8 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 1.2 - 1.3 mm (dry-mounted specimens, n=5). **Head.** Globular, black. Face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle shining anteriorly and finely pollinose posteriorly. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for the lower half. One pair lateroclinate ocellars, 2.5 times the length of postpedicel, no posterior ocellars. One pair proclinate verticals, twice the length of postpedicel, and numerous long setae on the occiput; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Light brown, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide, with apical circlet of black setae, ventrals longer; postpedicel roundish, as long as wide, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being more than 4 times as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae and black setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a distinct seta on the ventral apex. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with very long black setae. Thoracic pleuron with well-distinguished sclerites, with a clear separation between the mesopleuron and the pteropleuron; humeral calli very large; scutellum and metanotum well developed and postscutellum poorly developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum densely covered, appearing sub-triangular in lateral view; hypopleuron, scutellum and upper part of the metathorax thinly covered. Humeral callus bearing 5 setae laterally, mesonotum bearing 6 setae of equal length, scattered; 5 setae near the alar region; notopleuron with 1 strong, prominent, very long seta; 2 setae near the posterior margin of humeral callus; scutellum bearing 2 strong, long setae medially. **Wing.** Stenopterous. Lobed apex, oblong. Stalk-like portion very flexible, light brown with a distinct black spot on the lobed apex. Venation very reduced (costal, longitudinal and cross veins distinguishable), forming 4 cells. Uniformly covered in microtrichia (Fig. 21D). Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Fore tibia stout and inflated distally. Dark brown to black, while the following portions are yellow: trochanters; anterior apical half and apex of fore and mid tibia; dorsal strip and apical half of fore and mid tibia; tarsomere 1, 2 and 3 - excepting the brown tip - of fore and mid tibia; tarsomere 1 and 2 - excepting the brown tip - of hind tibia. Legs mostly covered with regular rows of numerous black and pale setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several short, downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae; front tibia bearing rows of long, erect, setae; mid tibia bearing a row of 5 strong, spine-like setae on its posteroventral basal surface. Mid tibia at its anteroventral apex has two projections (Figs 19E–F): one is slightly shorter than the diameter of tibia at apex; possesses microtrichia, several distinct setae and, on the distal half margin, 2 short, stout, curved, spinose setae; the other is as long as the diameter of tibia at apex and has a few long setae. **Abdomen.** Black, uniformly covered with grey setulae. Tergites and sternites with short, evenly distributed setae, except for the last two apical sternites with long, distinct, setae. **Terminalia** (Fig. 13). Subglobular, mostly covered with grey microtrichia, dark brown. Right surstylus short, narrowing distally, with sparse microtrichia, bearing long, irregularly curved setae, with several (*ca.* 6) distinct denticles, aggregated on distal margin. Right epandrial lamella with long, erect setae on apical half. Right cerci with setae on its apical half and a distinct seta on the apex; left cerci with setae of unequal length on its apex. Right cerci longer than left, both enclosed in epandrial lamellae. Left epandrial lamella shorter than left cercus, deeply bilobed, with a few long setulae on apex. Left surstylus sub-rectangular with rounded apex, 1.5 times as long as wide, approximately as long as right cercus, with very long setae, lateroclinate, facing

inwards.

Female. Similar to male, except for the following features: micropterous, with wing round, brownish, bearing 1 long seta and 1 short seta on the apical margin, covered by grey microtrichia (Fig. 21E); legs covered with regular rows of setae, with distinct apical setae on front femora and anteroapical setae on coxae, but no other distinctive hairs or setae; spurs on mid tibia absent; cerci pale brown with grey microtrichia and setae.

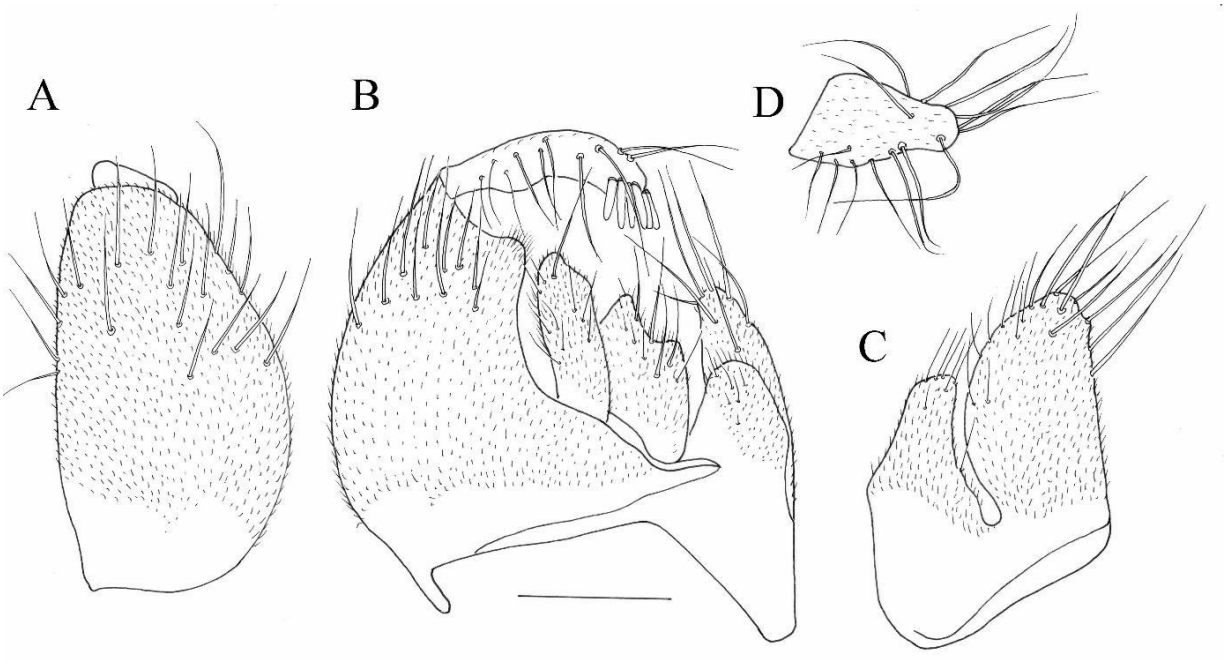

Fig. 13. Terminalia of *Tachydromia semiaptera* (Gil, 1923). A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, right surstylus. The scale bar represents 0.1 mm.

5.2.10. *Tachydromia stenoptera* sp. nov. Gonçalves, Grootaert & Andrade, sp. nov.

Figs 14, 15G–H, 17G–H, 19G, 21F–G

Etymology. The name of this species means "narrow wing" and derives from the combination of two Greek words: the prefix steno- (*stenos*), meaning narrow, with the suffix -ptera (*pterá*), meaning wing. The name was given due to the very narrow lobed distal apex of the male wing.

Material examined

Holotype
PORTUGAL • 1 ♂ Gouveia, São Pedro; 40°29'10.9" N, 7°34'38.5" W; 25 Apr. 2013; A.R. Gonçalves & R. Andrade leg.; RBINS

Paratypes
PORTUGAL • 1 ♂♂, 5 ♀♀; Gouveia, São Pedro; 40°29'10.9" N, 7°34'38.5" W; 25 Apr. 2013; A.R. Gonçalves & R. Andrade leg.
5 ♂♂, 5 ♀♀; Guarda, Panóias de Cima; 40°29'10.2" N, 7°13'18.4" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg; RBINS

Other material
PORTUGAL • 1 ♀; Manteigas, São Pedro; 40º19'07.0'' N, 7º34'37.4'' W; 25 Apr. 2013; A.R. Gonçalves leg. • 2 ♂♂, 9 ♀♀; Gouveia, São Pedro; 40°29'10.9" N, 7°34'38.5" W; 25 Apr. 2013; A.R. Gonçalves

& R. Andrade leg. • 8 ♂♂, 6 ♀♀; Guarda, Panóias de Cima; 40°29'10.2" N, 7°13'18.4" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 4 ♂♂, 1 ♀; Guarda, Adão; 40°27'03.0" N, 7°09'41.8" W; A.R. Gonçalves & R. Andrade leg. • 3 ♂♂, 4 ♀♀; Sabugal, Quadrazais; 40°18'30.6" N, 6°59'08.7" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 1 ♂, 1 ♀; Sabugal, Seixo do Côa; 40°24'48.0" N, 7°01'07.6" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg. • 1 ♂, 4 ♀♀; Sabugal, Fóios; 40°17'43.3" N, 6°52'38.9" W; 1 Apr. 2017; A.R. Gonçalves & R. Andrade leg.

Diagnosis

Small, slender, very dark species. Wing-dimorphic: male is stenopterous; wing with slightly lobed distal apex, no veins distinguishable, dark for the most part and lobe black and white; female is micropterous, wing bilobate, bearing 2 setae on each lobe. Halteres absent. Occiput largely covered by grey microtrichia; one pair of long lateroclinate ocellars; one pair of proclinate verticals; palpi, proboscis and antennae black; pospedicel lanceolate, *ca.* 1.5 times longer than pedicel. Thorax black with part of the prothoracic episternum densely covered with grey microtrichia, appearing sub-triangular in lateral view. Legs almost completely black; male fore tibia with ciliation of long hair-like setae. Abdomen black, tergites mostly glabrous and without grey microtrichia.

Description

Male. Body length: 2.1 - 2.8 mm (measured from frons to end of Terminalia, dry-mounted specimens, n=5). Wing length: 0.8 mm - 0.9 mm (measured from insertion to distal apex of wing, dry-mounted specimens, n=5). **Head.** Globular, black. Face and frons largely glabrous, shining black; clypeus mostly greyish pollinose; ocellar tubercle glabrous. Frons above antennae broad, slightly wider than anterior ocellus, narrowing from its highest part, below the ocellar triangle, towards the insertion of the antennae. Occiput largely covered with grey microtrichia, except for a glabrous triangular patch on upper half and for the lower half, which is shinning black with a narrow middle strip of grey setulae. One pair lateroclinate ocellars, equal length of postpedicel, no posterior ocellars. One pair proclinate verticals, ca. ¾ the length of postpedicel, and about 60 scattered setae on the occiput, in the upper half twice as long as in the lower half; no postocular ciliae present; mouth margin covered by grey microtrichia and with long setulae. **Antenna.** Black, covered by grey microtrichia, including on the insertion. Scape as long as wide; pedicel twice as long as wide with apical circlet of black setae, ventrals longer; postpedicel lanceolate, ca. 2 times as long as pedicel, with numerous pale setulae that are longer dorsally and apically; stylus black, bare and arising dorsoapically, being twice as long as scape, pedicel and postpedicel combined. **Palpus.** Elongate oval, about 3 times as long as wide. Black in ground-colour with grey microtrichia, dorsally clothed in strong, long silvery-white setae, bearing one strong black sub-apical seta which is slightly shorter than palpus; less numerous setae present ventrally with a distinct seta on the ventral apex. **Proboscis.** Black, mostly glabrous, setae present ventrally, slightly larger than palpus. **Thorax.** Elongated, overall black and largely shining, with long setae black, short ones pale. Humeral callus and prothoracic episternum, mesopleuron and sternopleuron, almost undifferentiated, scutellum short; notopleuron, pteropleuron, hypopleuron and postscutellum well developed. Pleura black, overall glabrous with defined patches of grey microtrichia: prosternum and prothoracic episternum partially, but densely, covered, appearing sub-triangular in lateral view; hypopleuron, scutellum and upper part of the metathorax thinly covered. Humeral callus bearing *ca.* 7 setae laterally, no dorsocentrals, ca. 6 acrostichal setae, scattered, biseriate, of equal length; notopleuron with 1 strong, prominent, seta, 6 small setae near mesothoracic spiracle; 4 small, incurvated, prescutellar setae; scutellum bearing 2 long and strong setae medially. **Wing.** Stenopterous. Slightly lobed distal apex. Stalk-like portion dark brown; distal ⅔ of lobed apex black with basal ⅓ translucid (appearing white

under most light conditions). No veins distinguishable. Uniformly covered in microtrichia (Fig. 21F). Halteres absent. **Legs.** Front femur stout and inflated basally, mid femur less so and hind femur least. Overall black; ventral and dorsal posterior surface of coxae, trochanter, basal 0.1 ventral surface of femora and knees, paler. Legs mostly covered with regular rows of numerous black setulae, except for the coxae, trochanters, and the glabrous posteroventral surface of femora; anteroventral surface of coxae, femora (except most of ventral surface) and tibia covered by grey microtrichia; coxae with several downward directed, dorsal anteroapical setae. Femora with a few distinct apical setae. Front tibia bearing a sparse ciliation of hair-like setae (slightly longer than the maximum diameter of the tibia) with curved tips, and a row of long, erect, setae present at its distal ⅓ posterodorsal surface. Tarsomere 1 of fore tarsus with strong and long (twice the tarsomere 1 width) setae; at the posterior distal surface with a row of distinct, short, black setae. Mid tibia at its anteroventral apex has a spur, with a barely noticeable apical elongation, about as long as diameter of tibia at apex (Fig. 19G); the spur possesses several distinct setae and, on the distal half margin, 4 short, stout, curved, spinose setae. **Abdomen.** Black. Tergites with few and scattered setae, mostly present on the posteromarginal surface and near the spiracles; sternites with evenly distributed setae; apical sternites with long, distinct, setae. Tergites with grey microscopic setulae only on anteromarginal margin of 1st tergite and near the spiracles; sternites covered with grey microscopic setulae. **Terminalia** (Fig. 14). Subglobular, mostly covered with grey microtrichia, black. Right epandrial lamella convex, globose, with irregularly placed long setae and a distinctive patch of long setae on lateral surface. Right surstylus short with anterior margin produced into short slender projection, mostly glabrous, bearing long and strong setae on dorsal margins, with several (*ca.* 7) distinct denticles, most of which aggregated on distal margin. Cerci enclosed in epandrial lamellae, with different length and shape, right longer and subcylindrical, left narrowing distally. Long setae of unequal length present on the apical half of right cercus; long and stronger setae on apical margin of left cercus. Left epandrial lamella ⅔ the length of the left cercus, bilobed, with a few long setulae on apical margin. Left surstylus sub-oval with rounded apical margin, twice as long as wide, longer than cerci, densely covered with minute pale setulae and with equally long setulae on apical margin.

Female. Similar to male, except for the following features: micropterous, with wings round, brownish, wings, bilobate, bearing 2 setae on the apical margin of each lobe, covered by grey microtrichia (Fig. 21G); legs covered with regular rows of setae, with distinct apical setae on front femora, but no other distinctive hairs or setae; spur on mid tibia absent; cerci pale brown with grey microtrichia and setae; no longer setae on apical sternites.

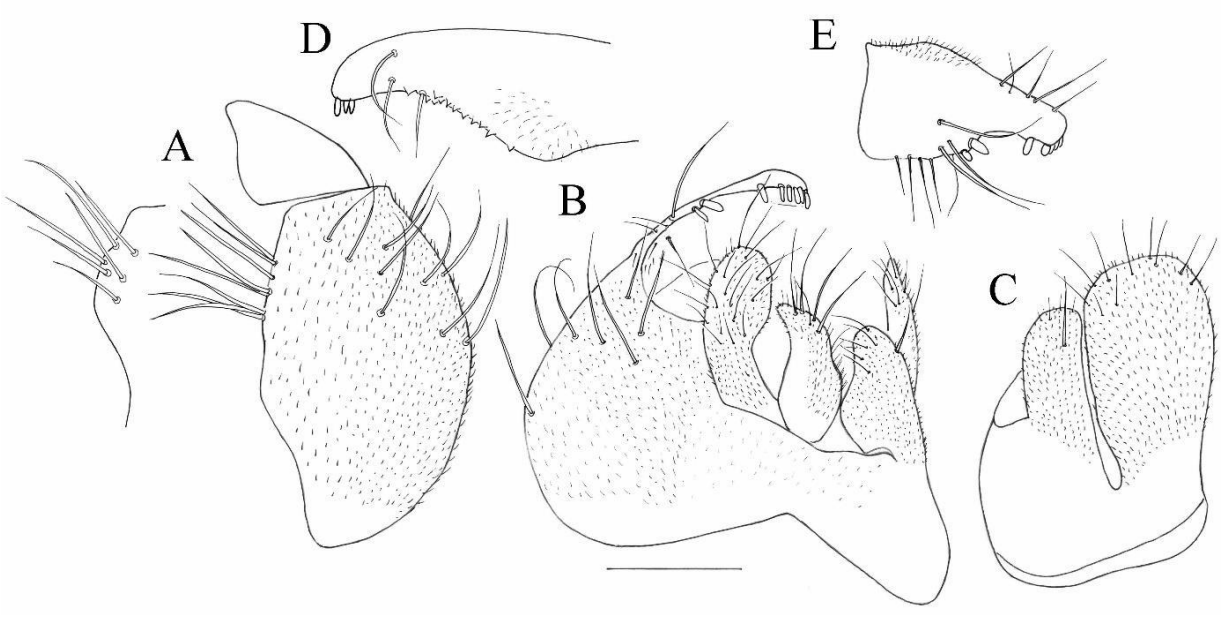

Fig. 14. Terminalia of *Tachydromia stenoptera* sp. nov. A, right surstylus and right epandrial lamella, B, epandrium with cerci, C, left epandrial lamella and left surstylus, D, E, right surstylus. The scale bar represents 0.1 mm.

6. Observations on mating behaviour of *T. semiaptera* and *T. iberica*

Successful mating attempts have only been observed in both species when the male was in possession of a prey. The movements performed by a male interested in copulating, as described below, are the same even if it does not hold a prey, with the additional fact that it chases the female. Usually, the female attempts to steal the prey and either the male allows it and immediately mounts her or, showing no interest in copulating, flees away with the prey. In the last scenario, the male may, at most, briefly move its stretched abdomen vertically. In the case of *T. iberica,* it may also move the mid legs sideways, as observed when it is confronted with another male or any perceived danger.

When the female approaches the male to steal the prey, the male - interested in copulating - stretches its abdomen and waves it up vertically but, additionally, moves its forelegs sideways. The male offers no resistance when the female removes the prey from it and, instead, readily mounts its back, beginning the mating attempt. Instantly, the male places its forelegs above female's eye level, while mid legs are placed in the female's waist in an attempt to grab it. At the beginning of the copula, the male moves its forelegs sideways at female's eye level, while, with its posterior legs, rubs its own last tergite and twitches it. The forelegs' movement is very similar to the wing' movement described for *T. lusitanica* Andrade (2011). When their genitalia seem to be locked, the male keeps itself very still, always with its forelegs above female's eye level. The female then may move around - sometimes very fast -, with the male on top, while keeping the prey. Frequently, in *T. iberica* the male lets itself be carried away by the female in a tail-to-tail position. When the specimens disconnect, usually both vigorously fight for the prey.

7. Discussion

7.1. Phylogenetic relationships and systematics

The phylogenetic relationships within Tachydromiinae have been studied previously but mostly at tribe and generic level (e.g. Nagy et al. 2013), with the specific diversity within genus yet to be studied in further detail. This is the first time that all the Iberian flightless species ("*Ariasella*" + "*Pieltainia*") were sequenced, and their phylogenetic relationship analysed but only a small number of full winged *Tachydromia* could be included at the present time. Nonetheless, the integrative taxonomic approach here employed, by combining the analysis of molecular and morphological data, allows for robust and congruent inferences regarding the evolutionary relationships among the Iberian species and with the genus *Tachydromia* itself.

The results based on molecular characters showed an obvious synonym between genera *Ariasella* and *Pieltainia* and with *Tachydromia*. The evolutive position of the type species of *Ariasella* as sister taxon to remaining *Ariasella* species and *Pieltainia* offers support to the lack of phylogenetic value of the absence of functional wings. The placement of the two genera within the clade of *Tachydromia* provided robust support to synonymize them as suggested by Shamshev & Grootaert (2018): *Pieltainia* and *Ariasella* are in synonymy to *Tachydromia*, with the following combinations: *Tachydromia iberica* (Arias, 1919); *T. lusitanica* (Grootaert *et al*., 2009); *T. pandellei* (Séguy, 1941); *T. pieltaini* (Gil, 1936); *T. semiaptera* (Gil, 1923).

The Iberian ant-like *Tachydromia* form an evolutionary close related group and the recovered phylogenetic relationships are in accordance with the morphological characters. Hence, *T. semiaptera* is the least related species of the Iberian cluster as its peculiar morphology suggests. This taxon possesses a combination of characters in the male (e.g., two projections on mid tibia, lack of ciliation of long hair-like setae on the fore tibia, roundish postpedicel and malleable wing) that is not found in any other species. The spur-like structure (Fig. 19) in the mid tibia of the male is present in all Iberian species (also in *T. apterygon*), with the exception of *T. iberica*, which completely lacks it. As described in the morphological analysis, this structure is, for most species, composed of a basal section bearing denticles and a side projection. In the case of *T. semiaptera*, it seems that the basal section and projection are totally separated or, at least, that their separation is much deeper and originating closer to the tibia than in other species. To our knowledge, this is the only case in genus *Tachydromia* where this deep or total separation occurs, and it may be a synapomorphy. Furthermore, this structure likely has evolutionary relevance, as the male of the species in which mating was observed, grab the females by using the mid legs.

Tachydromia iberica is a species that lacks many of the characters with taxonomic relevance found in other species as it is apterous in both sexes, doesn't have spur-like structures nor characteristic setae (e.g. ciliation of long hair-like setae on the fore tibia). Hence, the only morphological relevant character that allows species distinction seems to be the male terminalia. Some differences at the terminalia were found among three geographically distant populations and, thus, a specimen of each of these was included as well in the molecular analysis to obtain a more robust inference on species status. The differences regarding the terminalia are mainly found in the right surstylus, however, these are differences of degree rather than differences in the basic form and have less taxonomic relevance. Additionally, the specimens sequenced are still very much related as recovered in the phylogenetic analysis, where they are separated by short branches.

Furthermore, and even though we conducted a total evidence phylogenetic analysis, it seems relevant to comment on the COI p-divergences as this gene is often used to distinguish species, despite some

recognized issues. While the genetic diversity at species level within Hybotidae remains scarcely studied, large intraspecific COI p-divergences are not uncommon in Hybotidae (see Nagy *et al*. 2013). In the mentioned study, for example, an intraspecific divergence of up to 5.48% was found within the studied *Tachydromia*. In the specific case of *T. iberica*, the high divergence found at interpopulational level (4% - 5%) may be representative of vicariance processes, especially as no significant morphological or ecological differences were found to substantiate the elevation to species level.

Concerning the sister-species *T. pieltaini* and *T. pandellei*, these two species are morphologically similar but with very clear differences that allow for straightforward species separation. In this case, the two more relevant characters are perhaps both the wing pattern and shape as well as the terminalia (in males), which are very different. These morphological differences seem to be highly congruent with the molecular data, as these species are separated by relatively long branches in the inferred phylogenetic tree.

The four newly described species belong all to the same cluster, in which *T. lusitanica* is placed, being closely related. In simple terms, these species are characterized by an overall blackish with yellow/brown patterns semblance and sub-conical or lanceolate postpedicel. With the exception of the micropterous *T. ebejeri*, the male wing is stenopterous with its lobed tip divided into a white/transparent basal portion and black distally, but it is in *T. lusitanica* where it presents a more remarkable shape by bearing a long distal projection. *T. nigrohirta* sp. nov. is the most related species to *T. lusitanica* phylogenetically and this pair has less morphological differences when in comparison with, for example, *T. pieltaini* and *T. pandellei*, however, those are robust and congruent as well.

Regarding the placement of the Iberian species within the species-groups previously defined for *Tachydromia*, it is possible to associate them more closely with the *arrogans* species-group, however, it is not possible to undoubtedly place these species due to the lack of developed wings and other morphological characteristics which do not fit into the group definition. Concerning the Italian *Tachydromia apterygon*, it seems that its morphological characters - despite the lack of developed wings - would largely place it within *terricola* species-group, as suggested by Plant & Deeming (2006), however, the molecular data clearly places it as being much more related to the *arrogans* species-group than to the latter. Therefore, it would be important to further assess this matter and also to study the species-groups phylogenetic relationships based on molecular data.

Symballophthalmini being recovered as sister group to the remaining Tachydromiinae agrees with the inference made by Sinclair & Cumming (2006) and differs from the results obtained by Wahlberg & Johanson (2018), which placed Symballophthalmini as sister group to Tachydromiini. The lack of support regarding most phylogenetic relationship of the other Hybotidae subfamilies most likely results from lack of amplification success. Concerning the slight differences of the support values between the trimmed and non-trimmed datasets, it is likely that it represents the fact that 28S, as a Rrna gene, is evolving more slowly than protein-encoding genes and, thus, it is especially useful for providing resolution at the deeper nodes (Patwardhan *et al*. 2014).

7.2. Wing reduction/modification in *Tachydromia* and its evolutionary significance

As mentioned before, the lack of functional wings is a common phenomenon in Diptera and several hypotheses for flightlessness in insects have been discussed, with habitat persistence and cryptic behaviours among these (Hackman 1964). Roff (1990) concluded that the loss of wings is common in spatially and temporally homogeneous environments and that there is considerable evidence for an

increase in flightlessness with altitude. The Iberian species are mostly present at mountains and in deciduous forests, which are known to be stable habitats (Roff 1990), their microhabitat being restricted to the leaf litter. The lack of halteres and functional wings in *Tachydromia apterygon* is very likely a case of convergent evolution, especially as it occupies similar habitats (i.e. leaf litter of deciduous forests at high altitude mountains) to those of the Iberian ant-like *Tachydromia*. Overall, the evolution of flightlessness in *Tachydromia* may have occurred several independent times and it is currently known from 13 species (including the Iberian spp.), out of slightly more than 100 species. It is expected that more cases may be discovered, especially if searched for at high altitude in temperate environments.

Regarding the evolution of the wings in the Iberian ant-like *Tachydromia*, it appears that two processes took place: one is the wing reduction itself, which may happen in both sexes, and other is the wing modification with a sexual signalling function, which only happens in the male. Therefore, in some species only wing reduction occurred, which was at different degrees, from the stenopterous species to the apterous, with the micropterous in-between. In certain cases, the female is stenopterous without a distinct lobed tip on its wing, while the male wing has a very distinct and large lobed tip (see: *Tachydromia pieltaini*, *Tachydromia pandellei*, Figs 21A–C). However, more commonly, a more distinct sexual dimorphism is present, where the female is micropterous and the male is stenopterous.

Most interestingly is that the sexual signalling process does not happen the same way in all species, as observed in the mating behaviour of *T. semiaptera*. Contrary to *T.* the male of this species does not wave its wings during the copula, nor they seem to have any other function. Moreover, its wings are much less rigid than the ones from all the remaining related species, so it is possible that the stiffness of the stalk-like portion of the wing is an important part of the wing modification process, making them easier to wave. Hence, while the wings of the stenopterous species may look similar at a first glance, they do differ in their morphology and do have a very different function.

It can be said that in *T. semiaptera* only a process of wing reduction seems to be taking place. Nonetheless, this species has a very distinct feature, which has the same signalling function as that of the wing in *T. lusitanica*. Its fore tibia - which is black, stout and inflated - is waved during the copula in front of the female's eyes. A similar signalling method is used by *T. iberica* (waving the forelegs) but this species, instead of a modified tibia, simply has a yellow and black foreleg. Furthermore, both species lack the ciliation of hair-like setae on the foreleg, present in *T. lusitanica* that was observed to intertwine its forelegs with those of the female (Andrade 2011). This behaviour was not observed in these two species.

Taking all information into account, at least in the case of *T. lusitanica*, wing reduction seems to be coupled with an important modification of sexual relevance, and it appears that both ecological and sexual pressures took place in driving its evolution. It is most likely the case in the other species that present a distinct sexual dimorphism regarding the wing, but it remains to be tested.

7.3. Updates on the habitat, distribution and Conservation implications

As previously mentioned, the Iberian ant-like *Tachydromia* species occur in regions of temperate and supra-mediterranean bioclimatic influence. In these bioclimates, the potential natural vegetation is composed of broad-leaved deciduous or marcescent species and, often, the forests are dominated by oak trees, such as *Quercus robur* and *Q. pyrenaica* (Fig. 25) (Costa et al. 2005). At higher altitude, the oak trees are substituted by *Fagus sylvatica*, a deciduous tree that forms monospecific stands with a very closed canopy, which blocks the penetration of sunlight and creates very shaded environments (Costa et al. 2005). The soil of these deciduous and marcescent forests tends to have a deep layer of leaf litter. This layer of leaf litter, with some degree of light penetration, humidity and rich in organic matter, is

the typical micro-habitat where the scope taxa is to be found. With exception of the species occurring at alpine altitude, the adults of Iberian ant-like *Tachydromia* become active at the beginning of the spring, when the trees are still without leaves, and the sunlight can directly reach the ground. These species can be found both inside and on the forest edge.

T. pandellei and *T. pieltaini* occur in habitats where the forest is often dominated by *Fagus sylvatica*, and are probably only found on the edge of the forest because it provides the presence of leaf litter combined with a higher light penetration than under the dense canopy. The leaf litter itself is a humid, stable environment and likely offers shelter from predators and the elements (e.g. wind, rain) as well as it is the habitat for prey, as these species mainly feed on saprophilous flies (very dependent on organic matter).

In general, at a macroclimate scale, the ant-like *Tachydromia* species seem to have relatively similar ecological requirements and some species do co-occur in the same habitats (Fig. 2). This is, for example, the case of *T. semiaptera*, which co-occurs with *T. ebejeri* sp. nov., *T. nigrohirta* sp. nov. and *T. iberica*; however, this species seems to become active later in the year than the first two species and it does not overlap at a temporal scale with *T. ebejeri* in the same habitats. Regarding the geographic distribution, when further fieldwork is carried out, it will likely be possible to greatly expand the knowledge on the extent of occurrence of most species, especially of *T. semiaptera*, *T. cantabrica* sp. nov., *T. pandellei* and *T. pieltaini*. However, and given the previous sampling efforts, *T. stenoptera* sp. nov. and *T. nigrohirta* sp. nov. appear to be genuinely restricted to a smaller area of occurrence.

Furthermore, given their occurrence in fresh and humid forested habitats, most of the populations of the Iberian ant-like *Tachydromia* exist in highly fragmented landscapes. This is especially true for the species with populations in the southern half of the Iberian Peninsula, where they are circumscribed to small supra-mediterranean bioclimatic islands surrounded by a dry and hot environment. This is the case for the southernmost populations of *T. iberica*, *T. semiaptera* and *T. ebejeri*. However, while occurring in fresh and humid ecosystems, most of the populations in the northern half also occur in fragmented habitats. The habitat fragmentation is often caused by disturbances of anthropic origin, such as the artificialization of the land, agricultural intensification, substitution of the natural vegetation by monocultures and invasive species, as well as induced fires. The climate is also expected to become warmer, greatly reducing the area occupied by temperate and supra-mediterranean trees, as *Q. robur* and *Q. pyrenaica* (Garzón et al. 2008). Climate change, coupled with habitat fragmentation, will almost certainly imperil the conservation of the Iberian ant-like *Tachydromia*.

7.4. A hypothesis on the evolution of the Iberian ant-like *Tachydromia*

A hypothesis on the evolution of these species can be proposed based on the recovered phylogenetic relationships, knowledge about the behaviour and habitat. While the divergence events are not dated, the short internodes in the cluster of the Iberian *Tachydromia* may very well reflect that a rapid succession of splitting processes took place. It is possible that geographically separated populations adapted differently to similar ecological conditions, hence, simultaneously evolving different sexual signalling strategies. Thus, different biological entities may have arisen following vicariance processes and are now separated by the distinct strategies each followed, blocking the gene flow between them even when secondary contact happens.

8. References*

Andrade R. 2011. Observations on the behaviour of *Ariasella lusitanica*, Grootaert *et al*., 2009 (Diptera, Hybotidae, Tachydromiinae) from Portugal. *Bulletin de la Société royale belge d'Entomologie, S.R.B.E./K.B.V.E.*, 147: 241–250.

Arias J. 1919. Descripción preliminar de un nuevo Émpido de España. *Boletin de la Real Sociedad Española de Historia Natural* 19: 479–481.

Benito Garzón M., Sánchez de Dios R. & Sainz Ollero H. 2008. Effects of climate change on the distribution of Iberian tree species. *Applied Vegetation Science* 11: 169–178. https://doi.org/10.3170/2008-7-18348

Bertone M.A., Courtney G.W. & Wiegmann B.M. 2008. Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. *Systematic Entomology* 33 (4): 668–687. https://doi.org/10.1111/j.1365-3113.2008.00437.x

Chvála M. 1970. Revision of Palaearctic species of the genus *Tachydromia* Meig. (= *Tachista* Loew) (Diptera, Empididae). *Acta Entomologica Musei Nationalis Pragae* 38 (1969): 415–524.

Chvála M. 1975. The Tachydromiinae (Dipt. Empididae) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica* 3: 1–336.

Costa M., Morla C. & Sainz H. 2005. *Los Bosques Ibéricos: Una Interpretación Geobotánica*. 4ª edición. Editorial Planeta, Barcelona.

Darriba D., Taboada G.L., Doallo R. & Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. https://doi.org/10.1038/nmeth.2109

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.

Gibson J.F., Kelso S., Jackson M.D., Kits J.H., Miranda G.F., & Skevington J.H. 2011. Diptera-specific polymerase chain reaction amplification primers of use in molecular phylogenetic research. *Annals of the Entomological Society of America* 104 (5), 976–997. https://doi.org/10.1603/AN10153

Grootaert P. & Shamshev I. 2008. Notes on the halobiont genus *Chersodromia* (Diptera: Hybotidae) from Tunisia with the description of a new brachypterous species and notes on brachyptery in empidoids. *Bulletin de la Société royale belge d'Entomologie, S.R.B.E./K.B.V.E.*, 144: 57–63.

Hackman W. 1964. On reduction and loss of wings in Diptera. *Notulae Entomologicae* 44: 73–93.

Huelsenbeck J.P. & Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

JEMU. 2011. D1.Ft. Available from http://jemu.myspecies.info/d1ft [accessed 20 Sep. 2017].

JEMU. 2011. D2.Rt. Available from http://jemu.myspecies.info/d2rt [accessed 20 Sep. 2017].

Katoh K. & Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30 (4): 772–780. https://doi.org/10.1093/molbev/mst010

Katoh K., Kuma K., Toh H. & Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33 (2): 511–518. https://doi.org/10.1093/nar/gki198

Katoh K., Asimenos G. & Toh H. 2009. Multiple alignment of DNA sequences with MAFFT. *In*: Posada D. (ed.) *Bioinformatics for DNA Sequence Analysis* Methods in Molecular Biology 537: 39–64. https://doi.org/10.1007/978-1-59745-251-9_3

Kjer K.M. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. *Molecular Phylogenetics and Evolution* 4 (3): 314–330. https://doi.org/10.1006/mpev.1995.1028

Kumar S., Stecher G. & Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Mace G.M. 2004. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B* 359: 711–719. https://doi.org/10.1098/rstb.2003.1454

Madden T. 2013. The BLAST Sequence Analysis Tool. *In*: The NCBI handbook. Bethesda, MD: National Center for Biotechnology Information.

Marshall S.A. 2012. *Flies: the natural history and diversity of Diptera*. Firefly Books, Buffalo, New York.

McAlpine J.F. 1981. Morphology and terminology – adults. *In*: McAlpine J.F., Peterson B.V., Shewell G.E., Teskey H.J., Vockeroth J.R. & Wood D.M. (eds) *Manual of Nearctic Diptera*: 9–63. Agriculture Canada, Ottawa.

McLean F.G. 2000. Beneficial Diptera and their role in decomposition. *In*: Papp L. & Darvas B. (eds) *Contributions to a manual of Palaearctic Diptera (with special reference to flies of economic importance), Vol. 1*: 491–517. Science Herald Press, Budapest.

Miller M.A., Pfeiffer W. & Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: *Proceedings of the Gateway Computing Environments Workshop (GCE)*: 1–8. New Orleans.

Nagy Z.T., Sonet G., Mortelmans J., Vandewankel C. & Grootaert P. 2013. Using DNA barcodes for assessing diversity in the family Hybotidae (Diptera, Empidoidea). *ZooKeys* 365: 263–278.

Patwardhan A., Ray S. & Roy A. 2014. Molecular Markers in Phylogenetic Studies – A Review. *Phylogenetics & Evolutionary Biology* 2 (2): 1–9. https://doi.org/10.4172/2329-9002.1000131

Posada D. & Buckley T. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53 (5): 793–808. https://doi.org/10.1080/10635150490522304

Rambaut A. 2009. *FigTree: tree figure drawing tool*. Version 1.3.1. Available from http://tree.bio.ed.ac.uk/software/figtree/ [accessed 30 May 2016]

Regier J.C., Shultz J.W., Ganley A.R., Hussey A., Shi D., Ball B., Zwick A., Stajich J.E., Cummings M.P., Martin J.W. & Cunningham C.W. 2008. Resolving arthropod phylogeny: Exploring phylogenetic signal within 4 kb of protein-coding nuclear gene sequence. *Systematic Biology* 57: 920–938. https://doi. org/10.1080/10635150802570791

Roff D.A. 1990. The evolution of flightlessness in insects. *Ecological Monographs* 60: 389–421. https://doi.org/10.2307/1943013

Roháček J. 2012. Wing polymorphism in European species of Sphaeroceridae (Diptera). *Acta Entomologica Musei Nationalis Pragae* 52 (2): 535–558.

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Samways M.J. 1993. Insects in biodiversity conservation: some perspectives and directives. *Biodiversity and Conservation* 2: 258–282. https://doi.org/10.1007/BF00056672

Shamshev I.V. & Grootaert P. 2018. Proposed changes in systematics and status of some genera of Tachydromiini (Diptera: Hybotidae: Tachydromiinae), with description of a new species of *Tachypeza* Meigen from Canada and USA. *Russian Entomological Journal* 27 (4): 425–434. https://doi.org/10.15298/rusentj.27.4.10

Simon C., Frati F., Beckenbach A., Crespi B.J., Liu H. & Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651–701. https://doi.org/10.1093/aesa/87.6.651

Sinclair B.J. & Cumming J.M. 2006. The morphology, higher-level phylogeny and classification of the Empidoidea (Diptera). *Zootaxa* 1180: 1–172. https://doi.org/10.11646/zootaxa.1180.1.1

Stark A. & Doczkal D. 2017. *Tachydromia wendti* spec. nov. (Diptera, Empidoidea, Hybotidae) from riverbeds at the Northern slope of the Alps and its forelands in Germany. *Mauritiana (Altenburg)* 34: 481–498.

Stuckenberg B.R. 1999. Antennal evolution in the Brachycera (Diptera), with a reassessment of the terminology relating to the flagellum. *Studia dipterologica* 6: 33–48.

Troudet J., Grandcolas P., Blin A., Vignes-Lebbe R., & Legendre F. 2017. Taxonomic bias in biodiversity data and societal preferences. *Scientific Reports* 7 (1): 9132. https://doi.org/10.1038/s41598-017-09084-6

Wahlberg E. & Johanson K.A. 2018. Molecular phylogenetics reveals novel relationships within Empidoidea (Diptera). *Systematic Entomology* 43: 610–636. https://doi.org/10.1111/syen.12297

Wiegmann B.M., Trautwein M.D., Winkler I.S., Barr N.B., Kim J.W., Lambkin C., Bertone M.A., Cassel B.K., Bayless K.M., Heimberg A.M., Wheeler B.M., Peterson K.J., Pape T., Sinclair B.J., Skevington J.H., Blagoderov V., Caravas J., Kutty S.N., Schmidt-Ott U., Kampmeier G.E., Thompson F.C., Grimaldi D.A., Beckenbach A.T., Courtney G.W., Friedrich M., Meier R. & Yeates D.K. 2011. Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences* 108 (14): 5690–5695. https://doi.org/10.1073/pnas.1012675108

Zwickl D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD thesis, The University of Texas, Austin.

** References follow the guidelines of the European Journal of Taxonomy (ISSN 2118-9773)*

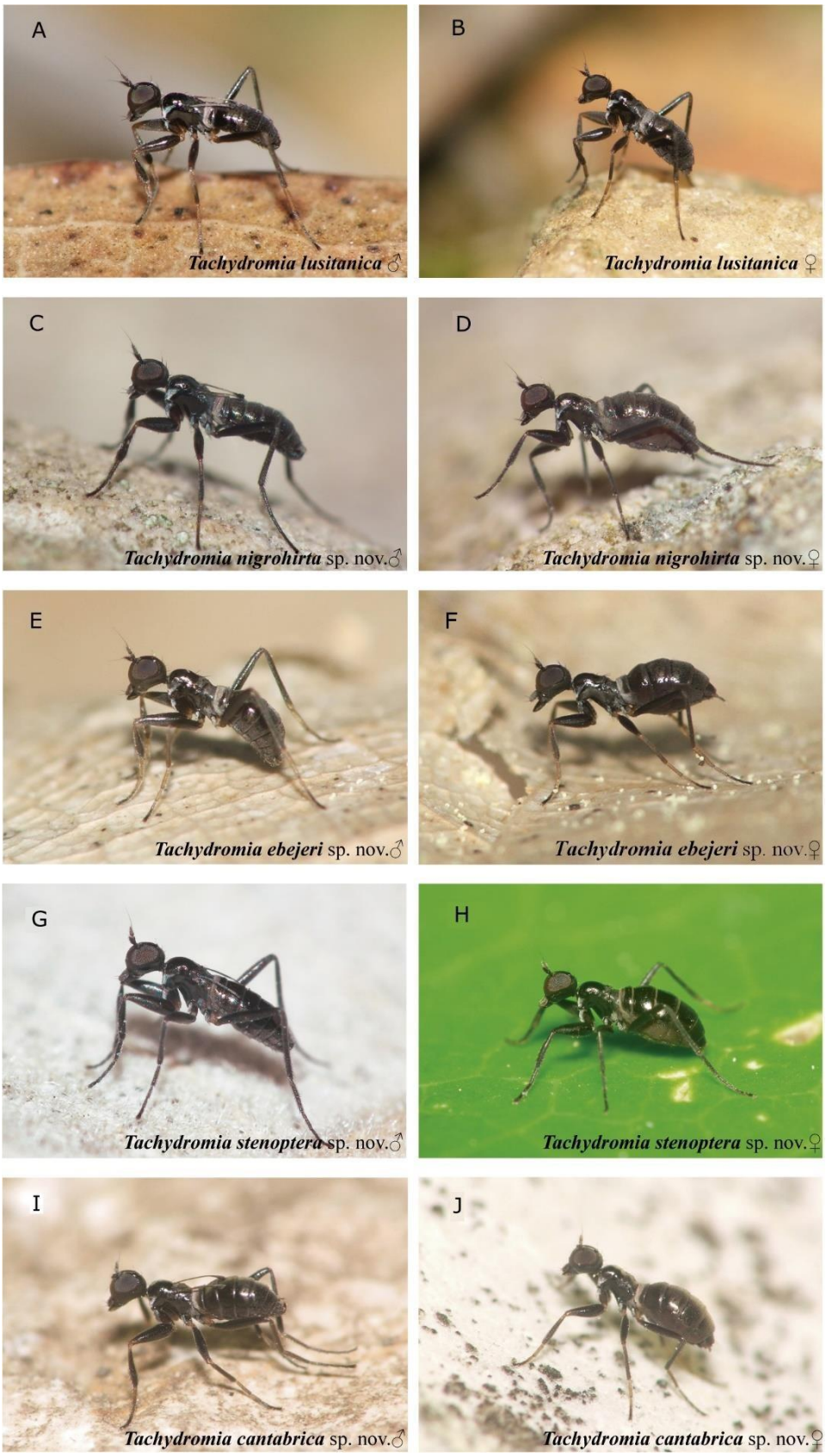

Fig. 15. Habitus of live specimens of the Iberian ant-like *Tachydromia*. Males are pictured on the left column, females on the right. A, B) *T. nigrohirta;* C, D) *T. nigrohirta* sp. nov.; E, F) *T. ebejeri* sp. nov.; G, H) *T. stenoptera* sp.nov.; I, J) *T. cantabrica* sp.nov.

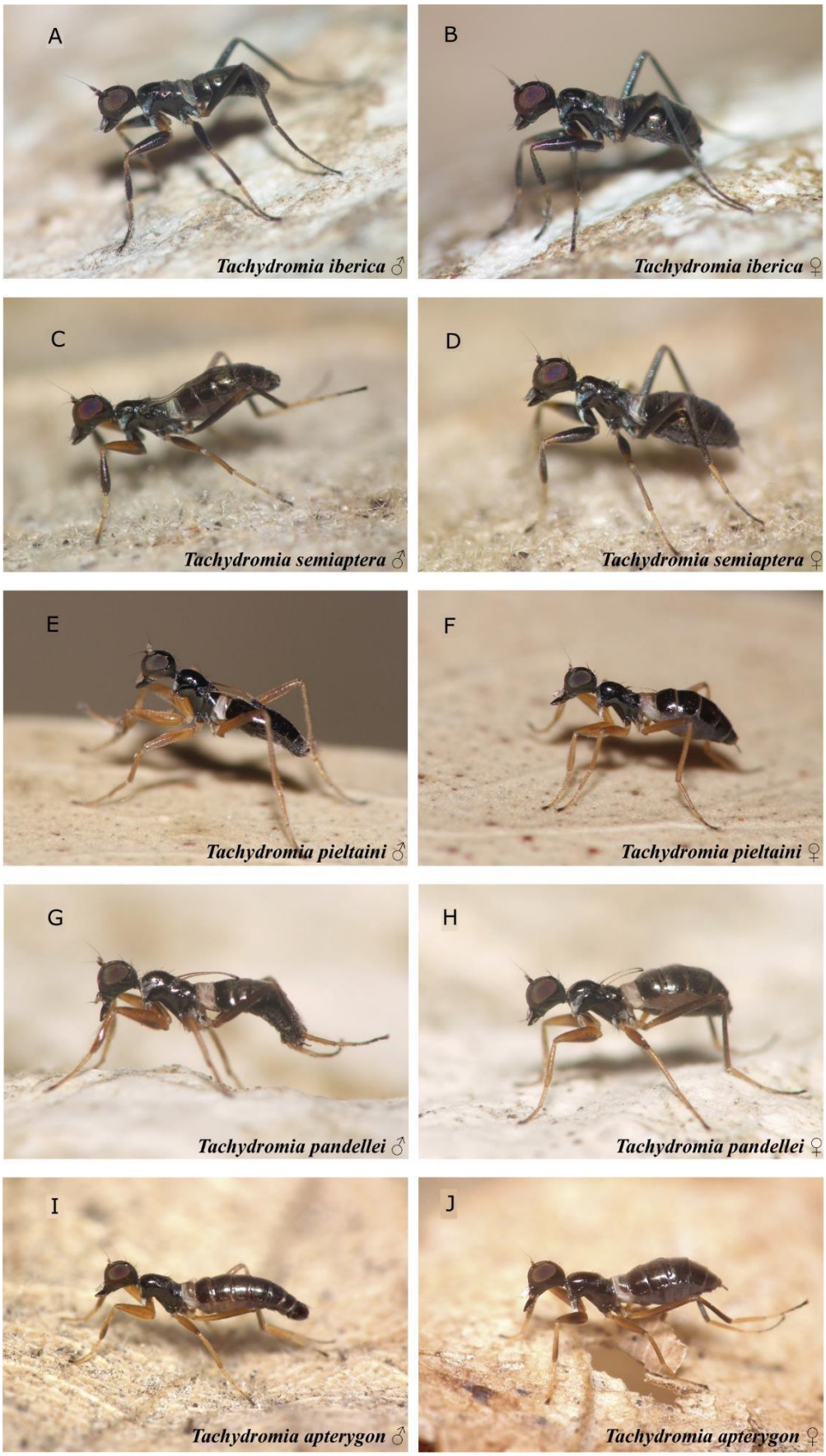

Fig. 16. Habitus of live specimens of the Iberian ant-like *Tachydromia* and the Italian flightless *Tachydromia*. Males are pictured on the left column, females on the right. A, B) *T. iberica*; C, D) *T. semiaptera*; E, F) *T. pieltaini*; G, H) *T. pandellei*; I, J) *T. apterygon* (Italy)

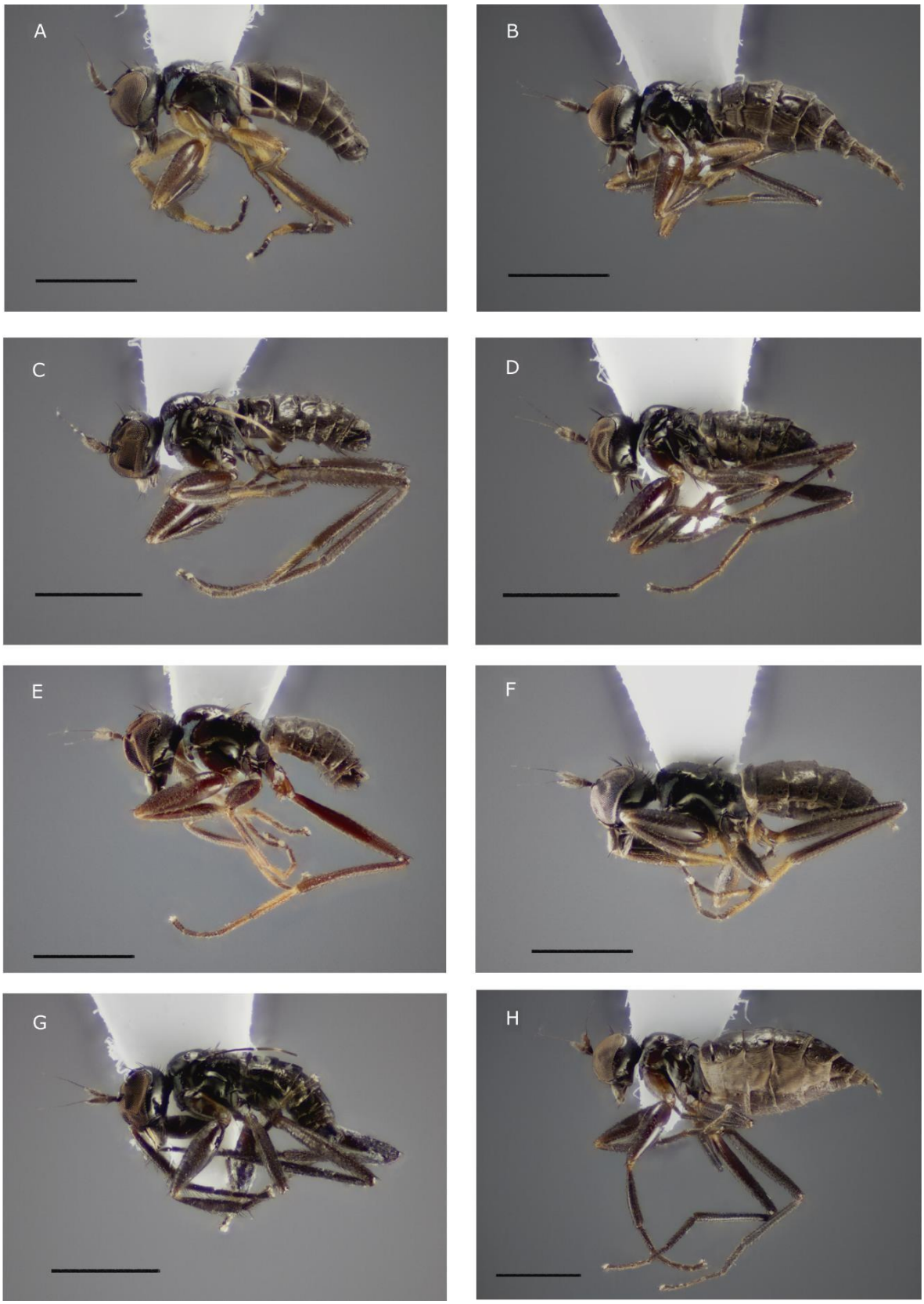

Fig. 17. Mounted specimens of the Iberian ant-like *Tachydromia*. The scale bar under each image represents 1 mm. Males are pictured on the left column, females on the right. A, B) *T. nigrohirta*; C, D) *T. nigrohirta* sp. nov.; E, F) *T. ebejeri* sp. nov.; G, H) *T. stenoptera* sp. nov.

Fig. 18. Mounted specimens of the Iberian ant-like *Tachydromia*. The scale bar under each image represents 1 mm. Males are pictured on the left column, females on the right. A, B) *T. cantabrica* sp. nov.; C, D) *T. iberica*; E, F) *T. semiaptera*; G, H) *T. pieltaini*; I, J) *T. pandellei*.

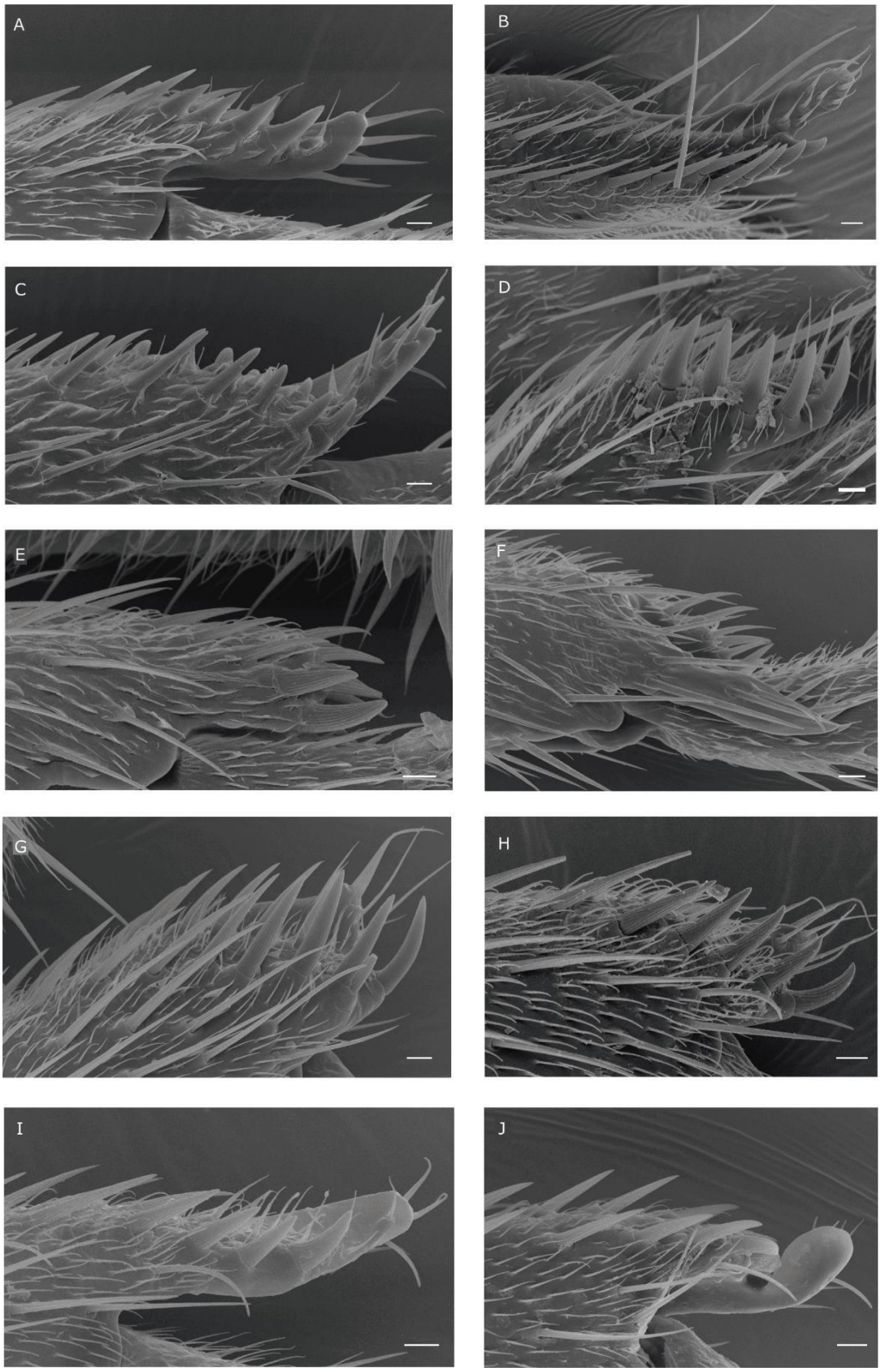

Fig. 19. Images obtained by scanning electron microscopy of the spur-like structures present on the apical portion of the male mid tibia. The scale bar under each image represents 10 µm. A) *T. nigrohirta*; B) *T. pandellei*; C) *T. pieltaini*; D) *T. ebejeri* sp. nov.; E, F) *T. semiaptera*; G) *T. stenoptera* sp. nov.; H) *T. cantabrica* sp. nov.; I) *T. nigrohirta*; J) *T. apterygon*.

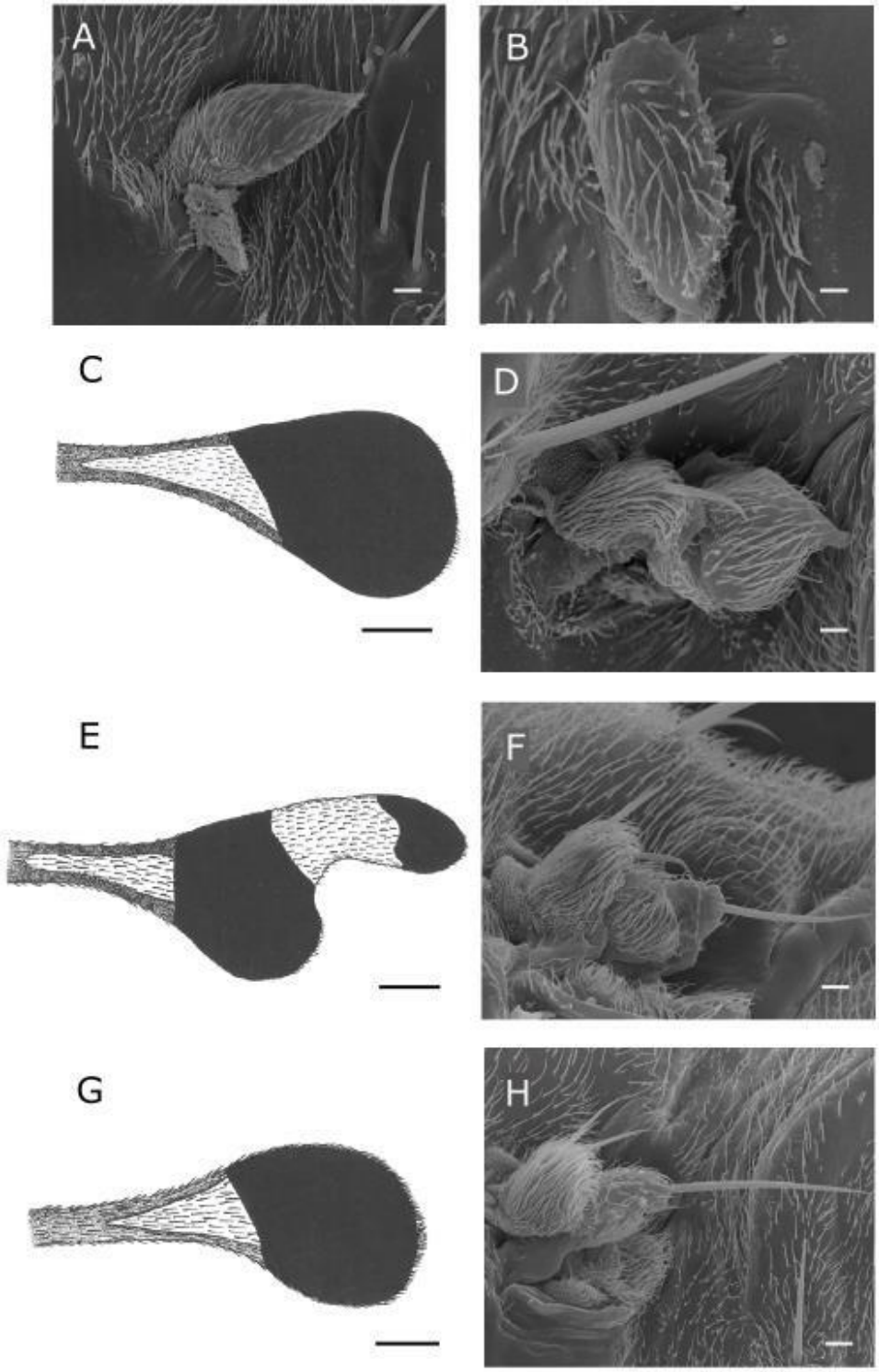

Fig. 20. Drawings of the tip of stenopterous wings and images obtained by scanning electron microscope (SEM) of the micropterous wings. The scale bar under each drawing represents 50 µm, while the scale bar under each SEM image represents 10 µm. Excepting B), males are pictured on the left column, females on the right. A, B) *T. ebejeri* sp. nov.; C, D) *T. cantabrica* sp. nov.; E, F) *T. nigrohirta*; G, H)*T. nigrohirta* sp. nov.

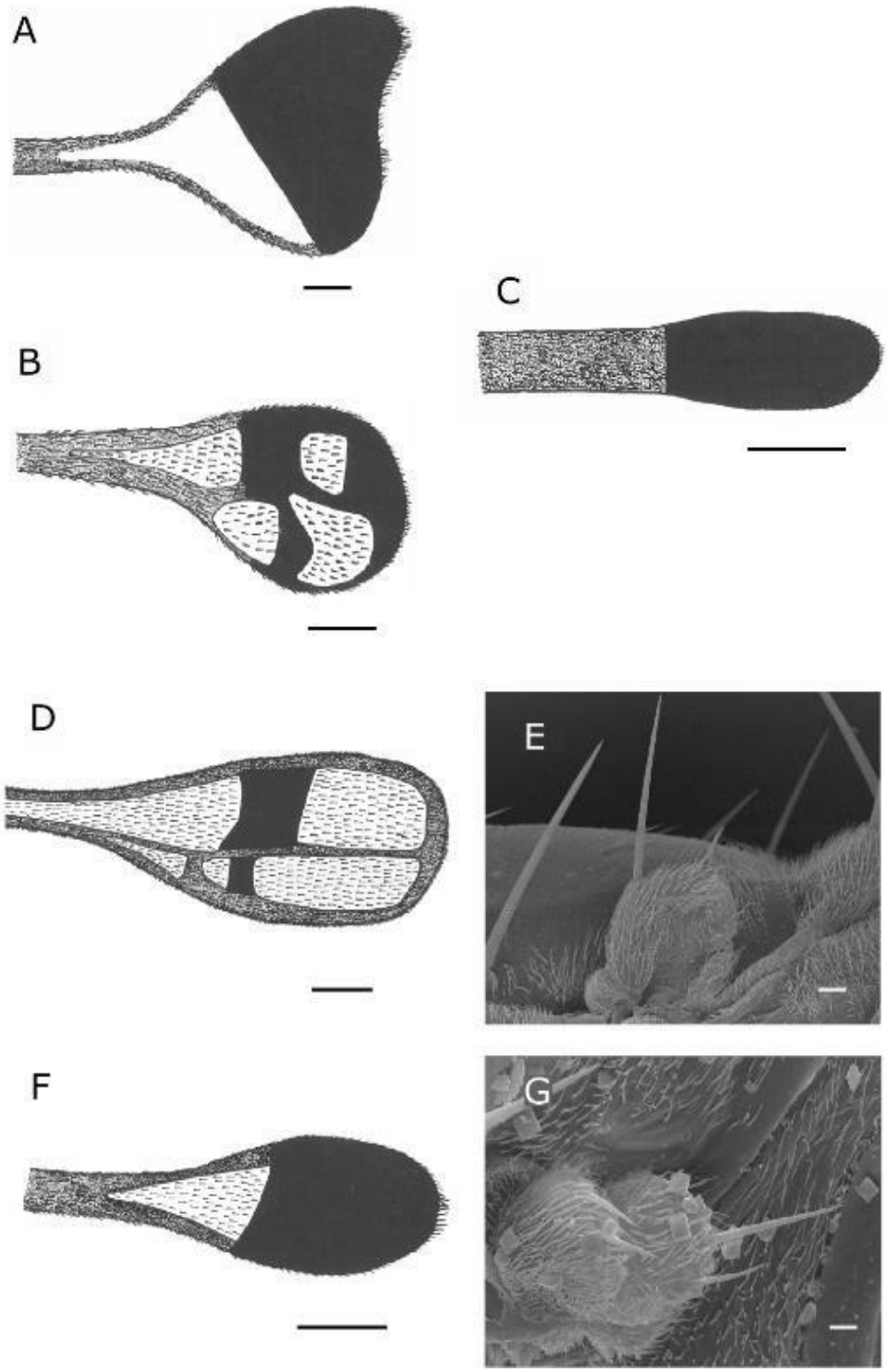

Fig. 21. Drawings of the tip of stenopterous wings and images obtained by scanning electron microscope (SEM) of the micropterous wings. The scale bar under each drawing represents 50 µm, while the scale bar under each SEM image represents 10 µm. Males are pictured on the left column, females on the right. A, C)*T. pandellei*; B, C) *T. pieltaini*; D, E) *T. semiaptera*; F, G) *T. stenoptera* sp. nov.

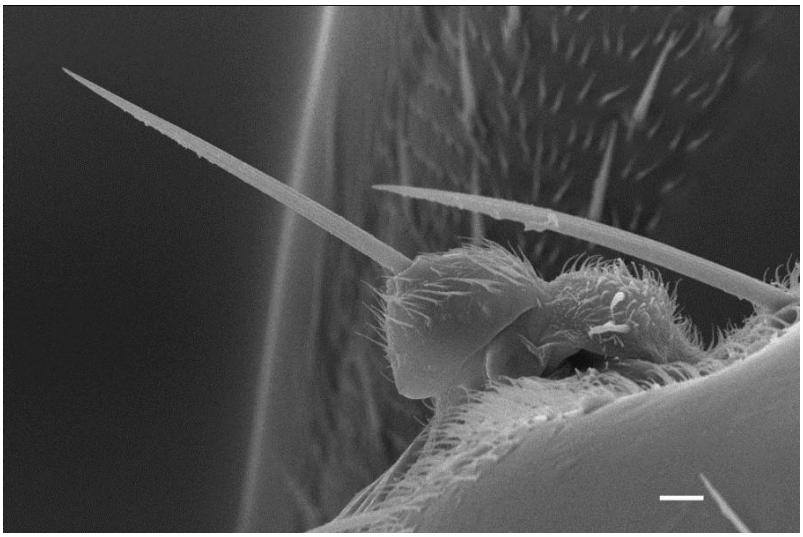

Fig. 22. Image obtained by scanning electron microscope (SEM) of the micropterous wing of a male of *Tachydromia apterygon*. The scale bar represents 10 µm.

Fig. 23. Distribution of *Tachydromia apterygon* Plant & Deeming, 2006 in Italy. Nine localities are currently known.

Fig. 24. ***Fagus sylvatica*** forest in the Apennine Mountains, where ***Tachydromia apterygon*** can be found.

Fig. 25. Habitat of ***Tachydromia ebejeri*** sp. nov. in a ***Quercus pyrenaica*** forest in PORTUGAL, Arganil, Benfeita (Mata da Margaraça), a region under supra-mediterranean influence.

Table 1 Taxon sampling used in the molecular analysis. The cross (X) denotes success in obtaining a sequence of the respective molecular marker.

Species	DNA voucher ZMFK	Label information	GBOL catalog number	COI-5'	COI-3'	28S	AATS	12S	PGD
Tachydromia ebejeri	AR01	Portugal: Sabugal, 01.iv.2017. Leg: A. Gonçalves & R. Andrade	-	X	X	X	X	X	X
Tachydromia stenoptera	AR02	Portugal: Guarda, Adão, 01.iv.2017. Leg: A. Gonçalves & R. Andrade	-	X	X	X	X	X	X
Tachydromia nigrohirta	AR03	Spain: Salamanca, El Maíllo, 25.iv.2017. Leg: A. Gonçalves	-	X	X	X	X	X	-
Tachydromia lusitanica	AR04	Portugal: Fafe, Felgueiras, 09.iv.2017. Leg: R. Andrade	-	X	X	X	X	X	X
Tachydromia pandellei	AR05	France: Hautes-Pyrénées, Arrens-Marsous, Arrens, Route d'Aste, 19.vi.2017. Leg: A. Gonçalves & E. Marabuto	-	X	X	X	X	X	X
Tachydromia semiaptera	AR06	Spain: Ávila, El Barraco, 13.iv.2016. Leg: A. Gonçalves & R. Andrade	-	X	X	X	X	X	X
Tachydromia iberica	AR07	Spain: Madrid, Arroyo de la Fuentecilla (La Hiruela), 23.iv.2017. Leg: A. Gonçalves	-	X	X	X	X	X	X
Tachydromia iberica	AR08	Portugal: Porto de Mós, Arrimal, 11.iii.2017. Leg: A. Gonçalves & F. Barros	-	X	X	X	X	X	X
Tachydromia iberica	AR09	Spain: Badajoz, Tentudía, 03.iv.2015. Leg: A. Gonçalves & R. Andrade	-	X	X	X	X	X	X
Tachydromia apterygon	AR10	Italy: Frosinone, San Donato Val di Comino, 26.vi.2017. Leg: A. Gonçalves & R. Andrade	-	X	X	-	-	X	X
Empis tessellata	AR11	Portugal: Lisboa, Monsanto, 01.iii.2017. Leg: A. Gonçalves & S. Martins	-	X	X	X	X	X	-
Hybos femoratus	AR12	Netherlands. Leg. Ruud van der Weele	-	X	X	X	X	X	-
Drapetis ephippiata	AR13	Netherlands. Leg. Ruud van der Weele	-	X	X	X	X	-	X
Stilpon sp.	AR14	Portugal: Aveiro, Salreu. Leg. R. Andrade	-	X	X	X	X	X	-
Symballophthalmus fuscitarsis	AR15	Belgium. Leg. P. Grootaert	-	X	X	X	-	X	X
Tachydromia cantabrica	AR16	Spain: Palencia, Río Carrión, 14.v.2014. Leg: A. Gonçalves & R. Andrade	-	X	X	X	X	X	-
Tachydromia pieltaini	AR17	Spain: Asturias, Cangas de Onís, 09.iv.2016. Leg: A. Gonçalves	-	X	X	X	X	X	X
Tachydromia connexa	AR18	United Kindgom	-	X	X	X	X	X	X
Tachydromia arrogans	AR19	Belgium. Leg. P. Grootaert	-	X	X	X	X	X	X
Tachydromia umbrarum	AR20	Netherlands. Leg. Ruud van der Weele	-	X	X	X	X	X	X
Tachydromia umbrarum	AR21	Netherlands. Leg. Ruud van der Weele	-	X	X	X	X	X	X
Oropezella sphenoptera	AR22	Norway	ZFMK-TIS-2547243	X	X	X	-	-	-
Oedalea hybotina	AR24	Finland	ZFMK-TIS-2580838	X	X	X	-	-	-
Bicellaria intermedia	AR25	Finland	ZFMK-TIS-2580874	X	X	X	X	X	X
Platypalpus pectoralis	AR26	Finland	ZFMK-TIS-2580880	X	X	X	X	X	X
Tachypeza truncorum	AR28	Finland	ZFMK-TIS-2586890	X	X	X	-	-	-
Trichina clavipes	AR29	Germany	ZFMK-TIS-2593538	X	X	X	-	-	X
Tachydromia terricola	AR30	Finland	ZFMK-TIS-2593583	X	X	X	X	X	X
Tachypeza nubila	AR31	Netherlands. Leg. Ruud van der Weele	-	X	X	X	X	X	-
Trichina bilobata	AR32	Germany	ZFMK-TIS-2513222	X	X	X	X	X	X
Platypalpus pseudofluvipes	AR33	Finland	ZFMK-TIS-2593700	X	X	X	X	X	X
Bicellaria sulcata	AR34	Germany	ZFMK-TIS-2601004	X	X	X	X	-	-
Oedalea zetterstedti	AR35	Germany	ZFMK-TIS-2595873	X	X	X	-	X	X
Tachypeza truncorum	AR36	Finland	ZFMK-TIS-2586890	X	X	X	-	-	X
Crossopalpus sp.	AR37	Portugal: Aveiro, Salreu. Leg. R. Andrade	-	X	X	X	X	-	X

Table 2 Primers used for amplifying and sequencing the molecular

Gene	Primer's name	Sequence	Reference
COI-5'	LCO-1490	GGTCAACAAATCATAAAGATATTG	Folmer et al., 1994
COI-5'	HCO-2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994
COI-3'	COI-780-F	CARCAYYTATTYTGATTTTTTGG	Gibson et al, 2008
COI-3'	TLS-N-3014 (Pat)	TCCAATGCACTAATCTGCCATATTA	Simon et al., 1994
PGD	PGD-2F	ATHGARTAYGGNGAYATGCA	Regier, 2008
PGD	PGD-3R	GTRTGNGCNCCRAARTARTC	Bertone et al., 2008
28s	28s-D1Ft	TGTAAAACGACGGCCAGTCCCCCTGAATTTAAGCATAT	Jemu
28s	28s-D2Rt	CAGGAAACAGCTATGACGTTAGACTCCTTGGTCCGTG	Jemu
12S	12S bi	AAGAGCGACGGGCGATGTGT	Simon et al., 1994
12S	12S ai	AAACTAGGATTAGATACCCTATTAT	Simon et al., 1994
AATS	AATS-1F40	GNATGAAYCARTTYAARCCNAT	Wiegmann et al., 2011
AATS	AATS-1R244	CATNCCRCARTCNATRTGYTT	Wiegmann et al., 2011

	reactions of 20 µl
H_2O	2,3
Q-Solution	2
Qiagen Multiplex-Mix	10
Primer A (10 pmol/µl)	1,6
Primer B (10 pmol/µl)	1,6
DNA	2,5

COI			
PCR protocol	Temperature (ºC)	Time (minutes)	Cycles
Initial step	95	15:00	
Denaturation	94	00:35	25
Annealing	55	01:30	25
Elongation	72	01:30	25
Final Elongation	72	10:00	
Cooling	10	∞	

PGD			
PCR protocol	Temperature (ºC)	time (minutes)	Cycles
Initial step	95	15:00	
Denaturation	95	00:30	36
Annealing	50	01:00	36
Elongation	72	01:00	36
Final Elongation	72	10:00	
Cooling	10	∞	

28S			
PCR protocol	Temperature (ºC)	time (minutes)	Cycles
Initial step	95	15:00	
Denaturation	94	00:35	40
Annealing	60	01:30	40
Elongation	72	01:30	40
Final Elongation	72	10:00	
Cooling	10	∞	

AATS			
PCR protocol	Temperature (ºC)	time (minutes)	Cycles
Initial step	95	15:00	
Denaturation	95	00:30	38
Annealing	50	01:00	38
Elongation	72	01:00	38
Final Elongation	72	10:00	
Cooling	10	∞	

12S			
PCR protocol	Temperature (ºC)	time (minutes)	Cycles
Initial step	94	01:30	
Denaturation	94	00:45	38
Annealing	50	01:00	38
Elongation	74	02:00	38
Final Elongation	74	05:00	
Cooling	10	∞	

Table 4 Selected evolutionary model to best fit each data partition using jModelTest 2.1.5 under the Akaike Information Criterion.

Genetic marker	**Selected evolutionary model**		
12S	TIM3+G		
28S	**trimmed**	**non-trimmed**	
	GTR+I+G	TVM+I+G	
	1st codon position	**2nd codon position**	**3rd codon position**
COI	TIM2+I+G	TVM+I+G	TIM2+G
AATS	TPM3uf+I+G	GTR+I	TVM+I
PGD	GTR+G	GTR+I	TIM2+I+G

Table 5 Mean sequence divergence (uncorrected p-distances) of COI, between pairs of species of the Iberian ant-like *Tachydromia* (calculated in MEGA7). Standard error estimates are shown above the diagonal

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	*Tachydromia ebejeri*		0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
2	*Tachydromia stenoptera*	0,10		0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
3	*Tachydromia nigrohirta*	0,10	0,09		0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
4	*Tachydromia lusitanica*	0,10	0,09	0,08		0,01	0,01	0,01	0,01	0,01	0,01	0,01
5	*Tachydromia pandellei*	0,11	0,10	0,10	0,10		0,01	0,01	0,01	0,01	0,01	0,01
6	*Tachydromia semiaptera*	0,12	0,11	0,11	0,11	0,12		0,01	0,01	0,01	0,01	0,01
7	*Tachydromia iberica* [Spain, Madrid]	0,11	0,10	0,11	0,10	0,10	0,10		0,00	0,01	0,01	0,01
8	*Tachydromia iberica* [Portugal, Leiria	0,10	0,09	0,10	0,09	0,10	0,09	0,04		0,01	0,01	0,01
9	*Tachydromia iberica* [Spain, Huelva]	0,10	0,10	0,10	0,10	0,10	0,10	0,05	0,05		0,01	0,01
10	*Tachydromia cantabrica*	0,10	0,08	0,09	0,09	0,11	0,11	0,10	0,10	0,10		0,01
11	*Tachydromia pieltaini*	0,11	0,10	0,10	0,09	0,10	0,12	0,10	0,10	0,11	0,10	

9. Supplementary material

Fig. 1. Maximum-likelihood tree based on the combined dataset (COI, trimmed 28S,12S, AATS and PGD) using Garli v.2.01.1067 and the structural alignment for 28S. Bootstrap support values are depicted at the nodes (only >50 or >0.5, respectively). BS = Bootstrap support values. A grey-scale is used to highlight the ingroup, where the darkest shade of grey highlights the Iberian flightless ant-like *Tachydromia* species, followed by a lighter shade which includes *T. apterygon*, hence representing all the flightess species occurring in southern Europe and, finally, the lighter shade covers all *Tachydromia* analysed, including the macropterous species assigned to different species-groups sensu Chvála (1970).

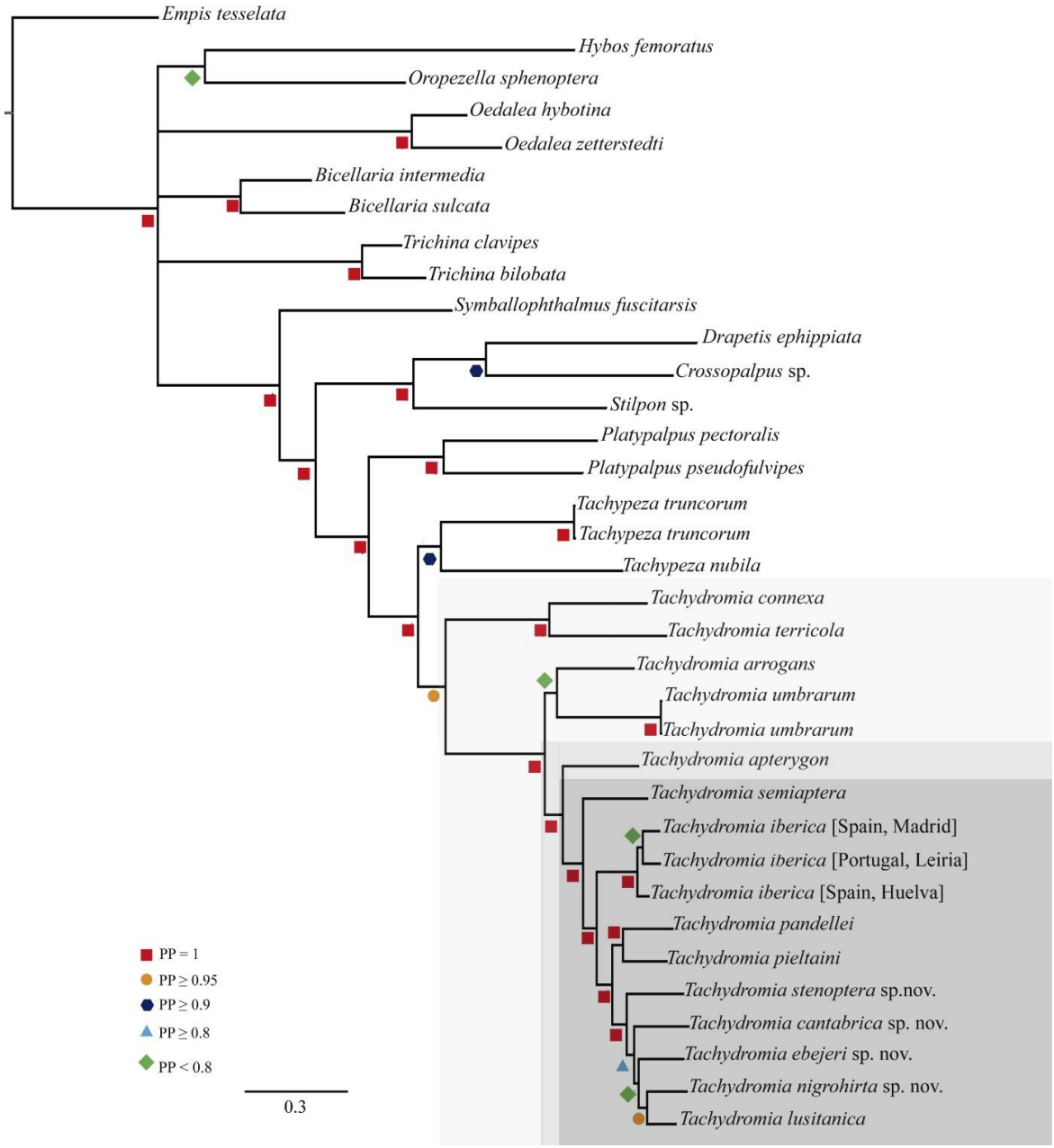

Fig. 2. Bayesian Inference tree based on the combined dataset (COI, trimmed 28S,12S, AATS and PGD) using MrBayes 3.2.6 and the structural alignment for 28S. Bayesian posterior probabilities are depicted at the nodes (only >50 or >0.5, respectively). PP = Bayesian posterior probabilities. A grey-scale is used to highlight the ingroup, where the darkest shade of grey highlights the Iberian flightless ant-like *Tachydromia* species, followed by a lighter shade which includes *T. apterygon*, hence representing all the flightess species occurring in southern Europe and, finally, the lighter shade covers all *Tachydromia* analysed, including the macropterous species assigned to different species-groups sensu Chvála (1970).

10. Supporting information

File S1 (.docx) contains the structural alignment of 28S before trimming.
File S2 (.docx) contains the structural alignment of 28S after trimming.
File S3 (.nexus) contains the full alignment of all sequences included in this study, containing the non-trimmed 28S.
File S4 (.nexus) contains the full alignment of all sequences included in this study, containing the trimmed 28S.