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Reclustering the cluster flies (Diptera: Oestroidea, Polleniidae)

PIERFILIPPO CERRETTI[1], JOHN O. STIREMAN III[2], DAVIDE BADANO[3], SILVIA GISONDI[1,4], KNUT ROGNES[5], GIUSEPPE LO GIUDICE[6] and THOMAS PAPE[4]

[1]Dipartimento di Biologia e Biotecnologie 'Charles Darwin', Sapienza Università di Roma, Rome, Italy, [2]Department of Biological Sciences, Wright State University, Dayton, OH, U.S.A., [3]Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Università degli studi di Genova, Genoa, Italy, [4]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark, [5]Faculty of Arts and Education, Department of Early Childhood Education, University of Stavanger, Stavanger, Norway and [6]CUTFAA, Raggruppamento Carabinieri Biodiversità, Verona, Italy

Abstract. A phylogenetic analysis of selected oestroid taxa based on 66 morphological traits and sequences from three nuclear protein-coding genes (*CAD*, *MAC*, *MCS*) resolved the composition and phylogenetic position of the former subfamily Polleniinae of the Calliphoridae – here resurrected at family rank as Polleniidae Brauer & Bergenstamm, 1889 **stat. rev.** Six species are transferred from the family Rhinophoridae to the Polleniidae: the Palaearctic genus *Alvamaja* Rognes, along with its single species *Alvamaja chlorometallica* Rognes, and five Afrotropical species comprising the *carinata*-group formerly in the genus *Phyto* Robineau-Desvoidy but here assigned to genus *Morinia* Robineau-Desvoidy, i.e. *M. carinata* (Pape, 1987) **comb.n.**, *M. lactineala* (Pape, 1997) **comb.n.**, *M. longirostris* (Crosskey, 1977) **comb.n.**, *M. royi* (Pape, 1997) **comb.n.** and *M. stuckenbergi* (Crosskey, 1977) **comb.n.** The Polleniidae are monophyletic and, in agreement with most recent phylogenetic reconstructions, sister to the Tachinidae. The female of *A. chlorometallica* and a new species of *Morinia* of the *carinata*-group (*Morinia tsitsikamma* **sp.n.** from South Africa) are described.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:25B0C220-DEE4-4B0C-88EA-35FDE298EBC5.

Introduction

Darwin (1881), in his last book, on the habits of earthworms and their role in soil formation, described the last moments of dying earthworms, leaving the protection of their burrows to die under the sun, being 'affected by the parasitic larvae of a fly'. This passing mention tells us little about the identity of the culprit observed by the great naturalist, but this might be the earliest report of maggots of the genus *Pollenia* Robineau-Desvoidy, specialized parasitoids or predators of earthworms. With *c*. 100 described species in Nearctic, Palaearctic, Oriental and Australasian regions, *Pollenia* is the most diverse genus of a small clade of flies that also includes *Dexopollenia* Townsend (21 species, Oriental region), *Melanodexia* Williston (eight species, Nearctic region), *Morinia* Robineau-Desvoidy (12 species, Palaearctic and Afrotropical regions; see later), *Xanthotryxus* Aldrich (seven species, east Palaearctic and Oriental regions) and *Nesodexia* Villeneuve (one species, endemic to Corsica, Palaearctic region), plus the tentatively assigned *Anthracomyza* Malloch (one species, Australia, Australasian region) and *Wilhelmina* Villeneuve [one species, Oriental region; a second species, *W. indica* Sidhu & Singh from India, has been described on a single female (Sidhu & Singh, 2005) but the genus level affiliation is uncertain] (Rognes, 1991, 1998; S. Gisondi *et al*., unpublished data). Evidence for monophyly of this 'calliphorid' clade is based on a combination of character states as discussed by Rognes (1991, 1997, 1998) (see diagnosis to the family Polleniidae later).

The adults of *Pollenia* species, commonly known as cluster flies, are often abundant, especially in the western Palaearctic.

Correspondence: Pierfilippo Cerretti, Dipartimento di Biologia e Biotecnologie 'Charles Darwin', Sapienza Università di Roma, Piazzale A. Moro 5, 00185 Rome, Italy. E-mail: pierfilippo.cerretti@uniroma1.it

They are frequently observed on flowers, suggesting that they are important pollinators, particularly in urban and agricultural ecosystems (Szpila, 2003; Grzywacz *et al.*, 2012; Jewiss-Gaines *et al.*, 2012; Marshall, 2012). In autumn, these flies can become a nuisance, gathering en masse in enormous clusters in buildings to overwinter, hence their common name. Under such conditions, *Pollenia* species are often considered as pests, although they do not have any significant economic importance (Yahnke & George, 1972). Despite the early report by Darwin, we know surprisingly little about the larval biology of *Pollenia*; our knowledge of this group is largely limited to a handful of species, mostly belonging to the *P. rudis* species-group (Rognes, 1987).

As already mentioned, cluster fly larvae develop on earthworms, and rearing observations suggest that they do not accept other food sources, although other hosts (e.g. insect larvae) are occasionally reported (Yahnke & George, 1972; Jewiss-Gaines *et al.*, 2012). The first-instar larva usually penetrates the host dorsally, near the clitellum – although it can attack the earthworm from any position – and begins to consume it from the interior, near the point of penetration, with only the spiracles visible from the outside. When the larva reaches the third instar, it often leaves the moribund earthworm to feed externally on its remains (Keilin, 1915; Yahnke & George, 1972; Szpila, 2003). The biology of the related genera mentioned earlier remains unknown (Rognes, 1998) with the noteworthy exception of the Oriental *Wilhelmina nepenthicola* Schmitz & Villeneuve, which might be related to *Pollenia* and relatives or to Phumosiinae (calliphorids). The unusual larva of this species, characterized by the presence of elongate body protrusions and proleg-like protuberances, lives within the pitchers of the carnivorous plants of the genus *Nepenthes*, where it feeds on the dead insects and other invertebrates that have fallen into the pitfall trap formed by a modified leaf (Schmitz & Villeneuve, 1932; Kurahashi & Omar, 2007).

Before the advent of molecular phylogenetic techniques, comparative morphology and morphological phylogenetics were the main approaches to unravel the relationships among organisms and reconstruct their evolutionary history. Even today, in the era of phylogenomics, morphology retains its standing as an extraordinary source of information, providing clues about phylogenetic affinities and often strengthening the conclusions of molecular-based phylogenetic analyses (Assis, 2009; Giribet, 2009; Wanninger, 2015). However, morphological evidence alone can sometimes be deceiving, particularly when dealing with large taxonomic groups that have undergone rapid radiations. The oestroid flies (Fig. 1) is one such group, a megadiverse clade of Diptera and one of the dominant insect groups in terrestrial environments, whose diversity is parallelled by astoundingly disparate life histories (Pape, 1992; Marshall, 2012; Cerretti *et al.*, 2017; Courtney *et al.*, 2017; Kutty *et al.*, 2019). *Pollenia* and relatives were traditionally grouped with the blow flies, Calliphoridae. Despite considerable attention due to their ecological, medical, veterinary and forensic relevance, very few attempts have been made at providing a definition of Calliphoridae based on strict monophyly (Kutty *et al.*, 2019). Hennig (1973) suspected that the blow flies in their traditional composition were nonmonophyletic, a hypothesis later elaborated on and strongly corroborated by Rognes (1997), based on morphology, and by Kutty *et al.* (2010), based on molecular data. Two blow fly subfamilies have been raised to the rank of full families, Mesembrinellidae (Guimarães, 1977; Marinho *et al.*, 2017) and Rhiniidae (Kutty *et al.*, 2010), and as will be argued later, the cluster flies, including *Pollenia* and closely related genera, also warrant recognition as a separate family.

The Rhinophoridae, the only known insects that parasitize crustaceans (specifically woodlice), is another particularly difficult oestroid group; only characters of their larvae, which remain inadequately known, strongly support their monophyly (Crosskey, 1977; Pape, 1986; Pape, 1992; Pape & Arnaud Jr., 2001; Cerretti & Pape, 2012; Cerretti *et al.*, 2014). The adults are notoriously difficult to sort out from other oestroid flies because they lack unique autapomorphies. It is, in fact, common to find specimens belonging to this family mixed up among unsorted 'calliphorid' or tachinid specimens in museum collections around the world. As a consequence of the lack of adult unique autapomorphies, over the last few decades the family Rhinophoridae has gained and lost taxa based on the re-evaluation of key adult morphological characters and the discovery and study of preimaginal stages (Table 1). Recently, molecular phylogenetic reconstructions carried out using multiple genes have converged towards a monophyletic Rhinophoridae, generally confirming family reassignments made on morphological grounds (Winkler *et al.*, 2015; Cerretti *et al.*, 2017; Kutty *et al.*, 2019; but see Kutty *et al.*, 2010).

The present study was triggered by the finding of a 'weird-looking' oestroid fly (Fig. 2) in West Coast National Park of the Western Cape of South Africa, during a field expedition carried out in October 2012 by JOS, James O'Hara (CNC, Canada), Isaac Winkler (Cornell College, Iowa, USA), Ashley Kirk-Spriggs (National Museum Bloemfontein, South Africa) and PC, as part of a research project focused on understanding the phylogeny and evolution of World Tachinidae (Diptera) (Cerretti *et al.*, 2013). The only collected specimen was a female with an overall *Pollenia*-like habitus except for the tongue-like lower calypteres. The specimen was identified by PC as a '*Pollenia*-like fly disguised as a rhinophorid' and set apart for further investigations. Afterwards, three legs were removed and preserved in 95% ethanol for DNA extraction and the voucher specimen was sent to KR for further study. KR identified the specimen as an undescribed species of the *carinata* species-group (hereafter '*carinata*-group') of the rhinophorid genus *Phyto* Robineau-Desvoidy (Crosskey, 1977; Pape, 1987; Pape, 1992; Pape, 1997) and he provided a suite of images and a detailed list of key character states in support of his conclusion. These traits included a slender body shape, occiput with black setulae only, tongue-shaped lower calypter, small metathoracic spiracle fringed with two lappets subequal in size, strong first postsutural supra-alar seta, and hind tibia with equally strong anterodorsal, dorsal and posterodorsal preapical setae. This combination of traits has led several taxonomists to include the *carinata*-group within the Rhinophoridae. At that time (2013), the Stireman laboratory was busy obtaining DNA sequences from hundreds of oestroid taxa, including the fresh material collected in South Africa. The results of a preliminary

Fig. 1. Diversity of Oestroidea. (A) *Mesembrinella randa* (Walker) (Mesembrinellidae), Ecuador: Yasuni National Park; (B) *Sarcophaga* sp. (Sarcophagidae), Madagascar: Ranomafana National Park; (C) *Cuterebra austeni* Sabrosky (Oestridae, Cuterebrinae), U.S.A.: New Mexico, Gila National Forest; (D) *Adejeania* sp. (Tachinidae), Peru: Wayqecha; (E) *Chaetodexia* sp. (Tachinidae), Madagascar: Andasibe; (F) *Pollenia* sp. (Polleniidae), Canada: Wellington County, Fergus; (G) *Morinia doronici* (Scopoli) (Polleniidae), Europe: Finland, Jyväskylä; (H) *Cynomya cadaverina* (Robineau-Desvoidy) (Calliphoridae, Calliphorinae), Canada: Ontario, Dunks Bay; (I) *Chrysomya putoria* Wiedemann (Calliphoridae, Chrysomyinae), Tanzania: Magombera; (J) *Bengalia* sp. (Calliphoridae, Bengaliinae), Tanzania: Iringa; (K) *Stomorhina* sp. (Rhiniidae), Mauritius; (L) *Silbomyia* sp. (Calliphoridae, Ameniinae), Vietman: Cat Ba; (M) *Bixinia winkleri* Cerretti, Lo Giudice & Pape (Rhinophoridae), Australia, Queensland. Photographs by: Steve Marshall (A–F, H–K); Raimo Peltonen (G), PC (M). [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Family reassignments in Rhinophoridae since Crosskey (1977).

Genus	Former family	Current status
Angioneura Brauer & Bergenstamm, 1893	Rhinophoridae	Valid genus in the family Calliphoridae, *teste* Pape (1986), subfamily Melanomyinae, *teste* Rognes (1991, 1997, 2011)
Melanomya Rondani, 1856	Rhinophoridae	Valid genus in the family Calliphoridae, *teste* Pape (1986) and Rognes (1986), see also Downes (1986), subfamily Melanomyinae, *teste* Rognes (1991, 1997, 2011)
Morinia Robineau-Desvoidy, 1830	Rhinophoridae	Valid genus in the family Calliphoridae, *teste* Pape (1986), subfamily Polleniinae, *teste* Rognes (1991, 1997, 2011), family Polleniidae, *teste* this work.
Termitoloemus Baranov, 1936	Rhinophoridae	Valid genus in the family Calliphoridae, *teste* Pape (1986), subfamily Bengaliinae, *teste* Rognes (2011), Singh & Rognes (2015)
Axinia Colless, 1994	Axiniidae	Valid genus in the family Rhinophoridae, *teste* Pape (1998)
Bezzimyia Townsend, 1919	Tachinidae	Valid genus in the family Rhinophoridae, *teste* Pape (1998)
Malayia Malloch, 1926	Tachinidae	Valid genus in the family Rhinophoridae, *teste* Pape & Shima (1993)
Shannoniella Townsend, 1939	Tachinidae	Valid genus in the family Rhinophoridae, *teste* Pape (1998)
Trypetidomima Townsend, 1935	Tachinidae	Valid genus in the family Rhinophoridae, *teste* Pape (1998)

Fig. 2. Female habitus of *Morinia* sp. (undescribed species of the *carinata*-group) (Polleniidae), South Africa, Western Cape Province, West Coast National Park. [Colour figure can be viewed at wileyonlinelibrary.com].

phylogenetic reconstruction based on three protein-coding nuclear genes (*CAD*, *MCS*, *MAC*; see Winkler *et al.*, 2015; Blaschke *et al.*, 2018; Stireman *et al.*, 2019) mostly focused on tachinids were circulated privately for a few months after the South African field expedition. The undescribed species of the *carinata*-group clustered with a species of the genus *Pollenia* with strong statistical support, and not with the several 'other' rhinophorids included in that analysis, which formed a well-supported monophyletic group not closely related to the '*Pollenia* clade.' A sketch of this cladogram was later presented at the 8th International Congress of Dipterology in Potsdam (Germany) (Stireman *et al.*, 2014).

Nothing further happened until June 2015, when a series of male and female specimens of another undescribed species of the *carinata*-group were recognized by TP from a collection of Afrotropical rhinophorids collected by Ashley Kirk-Spriggs. We examined both sexes of this undescribed species, along with several other representatives of the *carinata*-group housed in the Natural History Museum of Denmark in Copenhagen, in light of the molecular results obtained from the West Coast specimen.

Spurred by the fact that the genus *Alvamaja* Rognes, which was recently described in the Rhinophoridae (Rognes, 2010), shares several character states with Polleniidae, we extended our studies to include this taxon as well. *Alvamaja* was erected by Rognes (2010) to accommodate a single previously undescribed species (*Alvamaja chlorometallica* Rognes) known from three male specimens found among a small collection of oestroids from the Balkans (Serbia). *Alvamaja chlorometallica* is remarkable as its general appearance recalls that of the rhinophorid genus *Phyto* in sharing an elongated but relatively robust abdomen, very similar wing venation, tongue-shaped lower calypter, antennal arista with long trichia, head profile not receding and first postsutural supra-alar seta well developed. However, in contrast to all known rhinophorids, *A. chlorometallica* is characterized by a shiny metallic green integument and by setulose postalar wall. Rognes (2010) thoroughly described the new taxon, compared it with other oestroids and assigned *Alvamaja* to the rhinophorids pending examination of female specimens. In the autumn of 2014, a female specimen of *A. chlorometallica* was finally collected in Romania and recently made available to us for further examination. A total evidence phylogenetic analysis, combining molecular and morphological data, was performed in order to investigate the affinities of this taxon, as well as other enigmatic rhinophorid-like flies.

As a result of these studies, we have gathered overwhelming evidence supporting the view that both *A. chlorometallica* and species of the *carinata*-group belong to the monophyletic oestroid family-group taxon Polleniidae, which we resurrect here at full family status, as originally proposed by Brauer & von Bergenstamm (1889).

Specifically, the aims of this paper are: (i) to recognize Polleniidae as a separate calyptrate family; (ii) to provide phylogenetic evidence for a family-level reassignment of the taxa mentioned (*carinata*-group and *Alvamaja*) to Polleniidae; (iii)

to support with a phylogenetic framework the genus-level inclusion of the *carinata*-group in the polleniid genus *Morinia*; (iv) to describe a new species of the polleniid *carinata*-group from South Africa; and (v) to describe the female of *A. chlorometallica*.

Material and methods

Specimen preparation for morphological analyses and photography

Pinned specimens were examined using a MZ12.5 stereoscopic microscope (Leica, Wetzlar, Germany) and a TM1000 environmental scanning electron microscope (ESEM, Hitachi, Tokyo). Composite 'all-in-focus' images were produced from stacked images captured using a DS-L1 digital camera (Nikon, Tokyo, Japan) mounted on the MZ12.5 and processed with COMBINEZM by Alan Hadley (http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm, 26 April 2008) and PHOTOSHOP CS6 (1990-2012 Adobe Systems Inc., San Jose, CA, U.S.A.).

Male and female terminalia were dissected and prepared for examination according to the procedures described by Cerretti & Shima (2011) and Cerretti & Pape (2012). After study and documentation, the male terminalia were rehydrated and preserved in glycerine inside a small plastic vial pinned together with the source specimen, and the abdomen was glued back on the voucher or on a separate label pinned with it.

Label data

Label data are given verbatim using the following symbols:
/ end of a line and beginning of the next;
// end of a label and beginning of the next (from top to bottom on the same pin).

Abbreviations of depositories

Depositories for material studied or otherwise referred to are given with the following abbreviations: NHMUK, Natural History Museum [formerly British Museum (Natural History)], London, U.K.; MZUR, Museum of Zoology, Sapienza Università di Roma, Rome, Italy; NHMD, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark; NMB, National Museum, Bloemfontein, South Africa.

Terminology

Terminology of adult morphology follows Cumming & Wood (2018). Measurements and ratios of the head follow Cerretti (2010).

Genetic data and molecular phylogeny

To evaluate the position of the *carinata*-group and its relationships with other oestroid lineages, we used a combination of published and novel sequence data for three nuclear coding genes, *CAD* (carbamoyl-phosphate synthetase 2), *MAC* (methyl-accepting chemoreceptor), and *MCS* (molybdenum cofactor sulfurase) (see Winkler *et al.*, 2015; Blaschke *et al.*, 2018; Stireman *et al.*, 2019). The total dataset consists of 29 ingroup taxa including nine Rhinophoridae (sensu stricto), six 'Calliphoridae' (including *Bengalia* Robineau-Desvoidy and *Rhynchomya* Robineau-Desvoidy), five Tachinidae, three Sarcophagidae, two Oestridae, one Mesembrinellidae, the only known Ulurumyiidae (*Ulurumyia macalpinei* Michelsen & Pape), a species of *Pollenia*, and an undescribed species of *Morinia* from South Africa (belonging to the *carinata*-group) (Table 2). *Musca domestica* Linnaeus was used as outgroup. Published sequences were derived primarily from the study of Winkler *et al.* (2015) on the affinities of Tachinidae and relatives.

New sequence data (primarily of Rhinophoridae and Calliphoridae; Table 2) were obtained from alcohol-preserved legs of vouchered specimens. Three legs were removed from fresh or ethanol-preserved flies and stored in 95% ethanol until the extraction procedure. DNA extraction and PCR amplification were performed using the methods and primers described in Winkler *et al.* (2015; see also Blaschke *et al.*, 2018; Stireman *et al.*, 2019). Sanger sequencing was performed by the University of Arizona Genetics Core (uagc.arl.arizona.edu).

Sequences were edited using CODONCODE ALIGNER 3.5 (CodonCode Corporation, Dedham, MA, U.S.A.) and aligned using the L-INS-i algorithm of MAFFT v.7 (Katoh & Standley, 2013). An intron of length *c*. 50–80 bp was omitted from the *CAD* gene and 5′ and 3′ ends were trimmed from all sequences. Concatenation of the partial sequences resulted in a total aligned sequence length of 5246 bp (*CAD*, 1626 bp; *MCS*, 2072 bp; *MAC*, 1548 bp). Sequence coverage was not complete for all taxa (see Table 2), but except for *Melanomya bicolor* (Coquillett) (*MAC* only), all were represented by at least partial sequences from two loci.

Maximum likelihood (ML) phylogenetic analyses were conducted in RAXML v.8 (Stamatakis, 2014) implemented via the CIPRES Science Gateway (Miller *et al.*, 2010; https://www.phylo.org) using a GTR + I + G model of nucleotide substitution with sites partitioned by gene and codon position within loci. We also performed an ML analysis of the translated amino acid alignment using a JTT matrix model of amino acid substitution. Trees were evaluated with 1000 bootstrap replicates. A parallel ML analysis (employing a GTR + G model) was performed using IQ-TREE (Nguyen *et al.*, 2015) in order to evaluate branch support using ultrafast bootstraps (UFboot; Hoang *et al.*, 2017) and Shimodaira–Hasegawa approximate likelihood ratio (SH-aLRT) and aBayes tests (a Bayesian-like modification of aLRT; 1000 replicates; Anisimova *et al.*, 2011). Bayesian analysis was conducted with MRBAYES v.3.2.6 (Ronquist *et al.*, 2012), also partitioned by gene and codon position (i.e. nine partitions). The dataset was analysed under a GTR + I + G model. Two runs

Table 2. Oestroid DNA sequence data and GenBank accession numbers from the loci *CAD*, *MAC* and *MCS* used in the current phylogenetic analysis.

Family	Subfamily	Species		MAC	MCS	CAD
Muscidae	Muscinae	*Musca domestica* Linnaeus	U.S.A.	KP722541	XM005183808	AY280689
Calliphoridae	Ameniinae	*Amenia* sp.	Australia	**MK093925**	**MK093916**	**MK093902**
Calliphoridae	Bengaliinae	*Bengalia* sp.	Vietnam	**MK093931**	**MK093913**	KY928446
Calliphoridae	Luciliinae	*Lucilia sericata* (Meigen)	U.S.A.	MF480017	KP686290	KP973901
Calliphoridae	Melanomyinae	*Melanomya bicolor* (Coquillett)	U.S.A.	**MK093929**	–	–
Calliphoridae	Melanomyinae	*Angioneura abdominalis* (Reinhard)	U.S.A.	KP722526	KP686276	KP973909
Mesembrinellidae	–	*Mesembrinella* sp.	Ecuador	**MK093923**	KP686293	KP973922
Oestridae	Cuterebrinae	*Cuterebra austeni* Sabrosky	U.S.A.	KP722532	KP686282	KP973914
Oestridae	Oestrinae	*Cephenemyia jellisoni* Townsend	U.S.A.	**MK093926**	KP686280	KP973912
Polleniidae	–	*Pollenia pediculata* (Macquart)	U.S.A.	KP722544	KP686296	KP973925
Polleniidae	–	*Morinia* sp.	South Africa	**MK093920**	**MK093908**	**MK093903**
Rhiniidae	–	*Rhyncomya* sp.	South Africa	**MK093932**	**MK093914**	KY928447
Rhinophoridae	–	*Axinia* sp.	Australia	MF414455	MF480066	MF479951
Rhinophoridae	–	*Bezzimyia* sp.	Ecuador	–	**MK093918**	KY928451
Rhinophoridae	–	*Oplisa* sp. [cf. *tergestina* (Schiner)]	Italy	**MK093933**	**MK093917**	KY928448
Rhinophoridae	–	*Rhinomorinia sarcophagina* (Schiner)	Italy	**MK093921**	**MK093909**	KY928449
Rhinophoridae	–	*Rhinomorinia* sp.	South Africa	KP722546	KP686298	KP973926
Rhinophoridae	–	*Stevenia deceptoria* (Loew)	Italy	**MK093928**	**MK093910**	**MK093904**
Rhinophoridae	–	*Paykullia maculata* (Fallén)	Italy	**MK093927**	**MK093911**	**MK093905**
Rhinophoridae	–	*Phyto* sp.	Italy	**MK093924**	**MK093912**	**MK093906**
Rhinophoridae	–	*Melanophora roralis* (Linnaeus)	U.S.A.	KP722540	KP686292	KP973907
Sarcophagidae	Miltogramminae	*Macronychia* sp.	U.S.A.	KP722539	KP686291	KP973921
Sarcophagidae	Sarcophaginae	*Lepidodexia* sp.	Ecuador	**MK093934**	–	**MK093907**
Sarcophagidae	Sarcophaginae	*Helicobia rapax* (Walker)	U.S.A.	**MK093922**	–	KP973918
Tachinidae	Dexiinae	*Voria ruralis* (Fallén)	U.S.A.	KP722553	KP686305	KP973904/ KP973898
Tachinidae	Exoristinae	*Hyphantrophaga hyphantriae* (Townsend)	U.S.A.	KP722537	KP686288	KP973919
Tachinidae	Exoristinae	*Lespesia aletiae* (Riley)	U.S.A.	KP722538[a]	KP686289[a]	KP973920
Tachinidae	Phasiinae	*Trichopoda pennipes* (Fabricius)	U.S.A.	KP722551	KP686303	MF414561
Tachinidae	Tachininae	*Epalpus signifer* (Walker)	U.S.A.	KP722534	KP686284	KP973899
Ulurumyiidae	–	*Ulurumyia macalpinei* Michelsen & Pape (McAlpine's fly)	Australia	**MK093930**	**MK093919**	KY92845

[a]These sequences from GenBank may be from *Lespesia datanarum* (Townsend) and not *L. aletiae*.
Most sequences are from Winkler *et al.* (2015), new sequences are marked in bold.

of four Markov chain Monte Carlo (MCMC) chains were done for 20 million generations and sampled every 1000 generations, with the first 25% of trees discarded as burn-in.

Morphological data and total evidence phylogeny

A total evidence data matrix (File S1) was produced by scoring 66 morphological traits (see Cerretti *et al.*, 2017, and literature therein), for all the 30 taxa of the molecular dataset plus *Morinia doronici* (Scopoli) (type species of genus *Morinia*) (based on two males and one female from Italy, MZUR), *Morinia tsitsikamma* **sp.n.** (based on type material, see below), *Morinia carinata* (based on one male paratype, NHMD, and original description) and *A. chlorometallica* (based on one female from Romania, NHMUK, and original description) for which molecular data were not available. In the case of the following terminal taxa, morphological characters have been scored from congeners as specified in Cerretti *et al.* (2017): *Amenia* sp., *Axinia* sp., *Bengalia* sp., *Bezzimyia* sp., *Mesembrinella* (*Huascaromusca*) sp., *Lepidodexia* sp., *Macronychia* sp., *Oplisa* sp., *Phyto* sp., *Rhinomorinia* sp. and *Rhynchomya* sp. The two sets of characters (molecular and morphological) have been concatenated and treated as stemming from single operational taxonomic units in our analysis. Bayesian analyses were performed using MRBAYES (see earlier), partitioned by gene, codon position and morphology (i.e. 10 partitions). The dataset was analysed under a combination of GTR + I + G model (all nine partitions of DNA data) and Mk1 model (morphology). Two runs of four MCMC chains were done for 10 million generations and sampled every 1000 generations, with the first 25% of trees discarded as burn-in. The average standard deviation of split frequencies was approximately 0.001 after 10 million generations. The character state optimization was performed on the topology obtained from the Bayesian analysis of the combined dataset using both the 'unambiguous character state changes' (Fig. S4) and 'fast optimization' (ACCTRAN) (Fig. S5) algorithms in WINCLADA v.1.00.08 (Nixon, 2002).

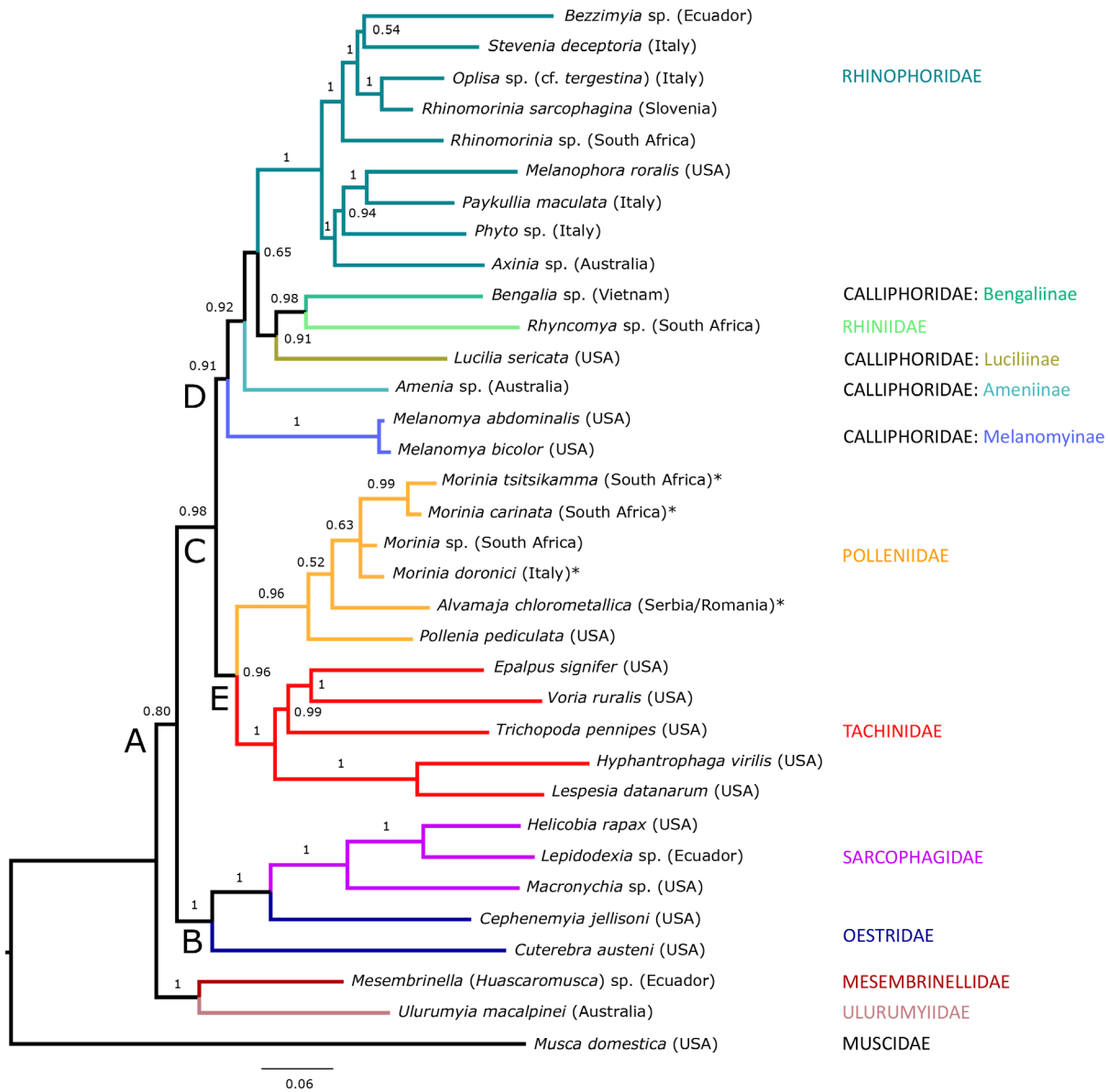

Fig. 3. Total evidence phylogeny (*CAD* + *MCS* + *MAC* + *morphology*) of Oestroidea as inferred from Bayesian analysis using MRBAYES 2.6. Numbers above branches indicate posterior probability values. Asterisks indicate taxa for which molecular data are not available. [Colour figure can be viewed at wileyonlinelibrary.com].

Results

Phylogeny

The ML and Bayesian phylogenetic analyses performed on both the molecular (Figs S1, S2) and the combined morphological and molecular datasets (total evidence) agree in reconstructing *Mesembrinella* Giglio-Tos as sister to *Ulurumyia* with strong statistical support, with this combined clade in turn as sister to all the remaining oestroid taxa (Fig. 3). The only exception to this is the ML analysis of the amino acid dataset, which places *Ulurumyia* as sister to all other Oestroidea and *Mesembrinella* as sister to all Oestroidea except *Ulurumyia* with weak support (Fig. S3). Clade A (Fig. 3) is resolved into two clades: clade B, which reconstructs Oestridae (*Cuterebra* Clark and *Cephenemyia* Latreille) as paraphyletic with respect to Sarcophagidae; and clade C, which is in turn divided into two subclades, D and E. Within clade D, the calliphorid taxa form a paraphyletic grade including both the rhiniid *Rhynchomya* and a well-supported Rhinophoridae (Fig. 3). Clade E contains the polleniid taxa *Pollenia*, *Morinia* and *Alvamaja* (i.e. Polleniidae) as sister to a monophyletic Tachinidae, with strong statistical support. The included species of *Morinia* form a weakly supported clade sister to *Alvamaja*.

The molecular evidence provides absolute support for the association of the South African *Morinia* specimen (= *Phyto carinata* group) with *Pollenia* [bootstrap (BS), 100; UFboot, 100; SH-aLRT, 100; aBayes, 1.0], as well as strong support for a Polleniidae + Tachinidae clade (BS, 81; UFboot, 78; SH-aLRT, 96; aBayes, 1.0) (Figs S1–S3). The IQ-TREE DNA and RAXML amino acid analyses depart slightly from this arrangement, with *Rhyncomya* + *Bengalia* joining clade E (tachinids, polleniids) rather than D (calliphorids and rhinophorids), albeit with low support (UFboot, 32; BS, 49; Fig. S3). In the latter case, the polleniids are reconstructed as sister to *Rhyncomya* + *Bengalia* rather than to Tachinidae.

Systematics

Family Polleniidae Brauer & Bergenstamm, 1889 ***stat. rev.***

Diagnosis. Small to medium-sized oestroid flies varying from yellow to black in ground colour. Facial sclerite at least weakly carinate [with few exceptions, e.g. *Pollenia griseotomentosa* (Jacentkovský)]. Stem vein bare dorsally. Anal vein not reaching wing margin. Posterodorsal margin of hind coxa bare. Prosternum and proepisternal depression bare. Postalar wall setose (occasionally bare in small specimens of *Morinia*). Female: ovipositor sclerite length moderate; sternite 8 of ovipositor elongate with apex entire; cerci long and narrow. Male: ventral and ventrolateral surface of distalmost parts of distiphallus smooth.

Genus Morinia *Robineau-Desvoidy.*

Morinia Robineau-Desvoidy, 1830: 264. Type species: *Morinia velox* Robineau-Desvoidy, 1830 (= *Musca doronici* Scopoli, 1763), by subsequent designation (Rondani, 1862: 159).

Diagnosis. Small to medium-sized, relatively slender flies, black in ground colour, rarely with metallic bronze reflections (Fig. 4A) or evenly covered with grey microtomentum (Fig. 2). Outer posthumeral seta and presutural intra-alar setae absent. Metathoracic spiracle small and circular; anterior and posterior lappets of metathoracic spiracle subequal in size (posterior lappet slightly larger) and more or less outwardly directed. Lower calypter narrow (i.e. tongue-shaped as in Rhinophorids) and diverging from scutellum. Node at base of R_{4+5} bare. Bend of vein M obtuse [vein M_1 vanishing in the membrane in *M. lactineala* (Pape)]. Hind tibia with equally strong dorsal, anterodorsal and posterodorsal preapical setae.

New combinations.

Morinia carinata (Pape, 1987) (*Phyto*) **comb.n.** Formerly in Rhinophoridae.

Morinia lactineala (Pape, 1997) (*Phyto*) **comb.n.** Formerly in Rhinophoridae.

Morinia longirostris (Crosskey, 1977) (*Phyto*) **comb.n.** Formerly in Rhinophoridae.

Morinia royi (Pape, 1997) (*Phyto*) **comb.n.** Formerly in Rhinophoridae.

Morinia stuckenbergi (Crosskey, 1977: 44) (*Phyto*) **comb.n.** Formerly in Rhinophoridae.

Morinia tsitsikamma ***sp.n.***

(Figs 4–6)

http://zoobank.org/urn:lsid:zoobank.org:act:3896A907-0722-4200-9C8E-CFF38BC01BFF.

Material examined: Holotype ♂: Malaise trap / indigenous / forest // RSA: Tsitsikamma N. P. / Bloukrans Pass. Platbos al: / 33°56.558'S, 23°37.566′E / 22–25.i.2009 / A. Kirk-Spriggs, S. Otto // Entomology Dept. / National Museum / PO Box 266 / Bloemfontein 9300 / South Africa // BMSA(D) / 05392 (NMB).

Paratypes: 19 ♂♂, same data as holotype but numbered 05381–05386, 05388–05391, 05393–05396, 05398, 05400–05403 (and 05403 with: Sweeping / forest paths / indigenous /forest); 2 ♀♀, same data as holotype 05399 but 05404 (Sweeping / forest paths / indigenous /forest) (all in NMB, except 1♂ in MZUR and and 1♂ in NHMD).

Etymology. A noun in apposition. Named for the type locality.

Diagnosis. *Morinia tsitsikamma* is distinguishable from Palaearctic [*M. doronici* (Scopoli), *M. crassitarsis* (Villeneuve), *M. nigerrima* (Herting), *M. piliparafacia* Fan, *M. proceripenisa* Feng, *M. skufyini* Khitsova] and Oriental [*M. argenticincta* (Senior-White)] congeners by the following combination of character states: thorax, legs and abdomen black in ground colour with bronze metallic reflections; both males and females with one to two proclinate and one to two upper lateroclinate orbital setae; parafacial covered with short setulae (parafacial is usually bare in Palaearctic species but setulose *M. piliparafacia*, *M. skufyini*); face with a small though visible median carina. The other named Afrotropical species of *Morinia* (i.e. *M. carinata*, *M. lactineala*, *M. longirostris*, *M. royi*, *M. stuckenbergi*) share a long and narrow prementum, which is 1.0–1.3× as long as head height, and relatively narrow labella. *Morina tsitsikamma* is instead characterized by prementum shorter than head height, i.e. 3–4× as long as wide, and labella normally developed. In addition, the newly described species has the notopleuron bare, except for the two notopleural setae, the arista with long trichia and the anterior margin of metathoracic spiracle with two to five relatively long setae (Fig. 5C).

Description. (Values in square brackets refer to the holotype.)

Body length: ♂, 5.8–7.1 (7.0) mm; ♀, 6.6–7.8 mm.

Coloration (Figs 4, 5). Ground colour of head blackish except for lower part of parafacial and anterior part of frons, which are dark brown; head entirely covered with whitish or silver reflecting microtomentum. Antenna and mouthparts brownish-black. Thorax, legs and abdomen black in ground colour with bronze metallic reflections; thorax dorsally covered with whitish, sparse microtomentum except for two, barely visible, presutural dark vittae located between rows of dorsocentral and acrostichal setae. Abdomen thinly covered with whitish microtomentum (barely visible only in posterodorsal view). Tegula brownish, basicosta yellow. Wing membrane yellowish.

Head (Fig. 4). Frons 1.3–1.5 (1.4)× (male), 1.6–1.7× (female) as wide as compound eye in dorsal view. Frontal vitta 2.5–(3.0)× (male), 2.5–3.0× (female) as wide as fronto-orbital plate. Inner and outer vertical setae well developed. One or two proclinate and one to two upper lateroclinate orbital setae (usually two in male, one in female). Scattered short setulae

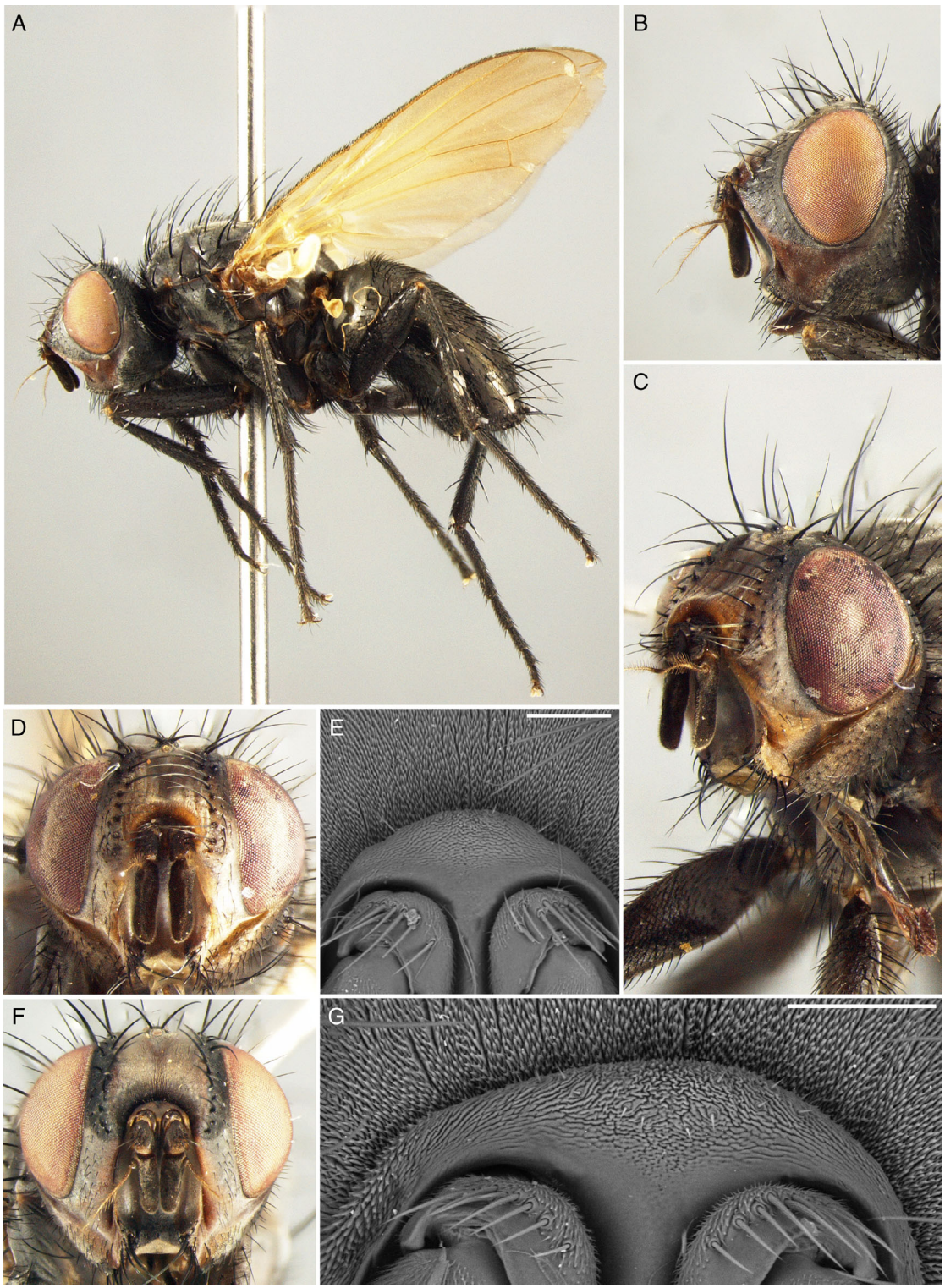

Fig. 4. *Morinia tsitsikamma* **sp.n.** (A) Habitus in lateral view, holotype 05392, male; (B) head in lateral view, holotype 05392, male; (C) head in frontolateral view, male paratype 05403, head; (D) head in frontal view, male paratype 05403; (E) lunule in frontal view, male paratype 05383 (scale 100 μm); (F) head in frontal view, female paratype 05404; (G) lunule in frontal view, female paratype 0540 (scale 100 μm). [Colour figure can be viewed at wileyonlinelibrary.com].

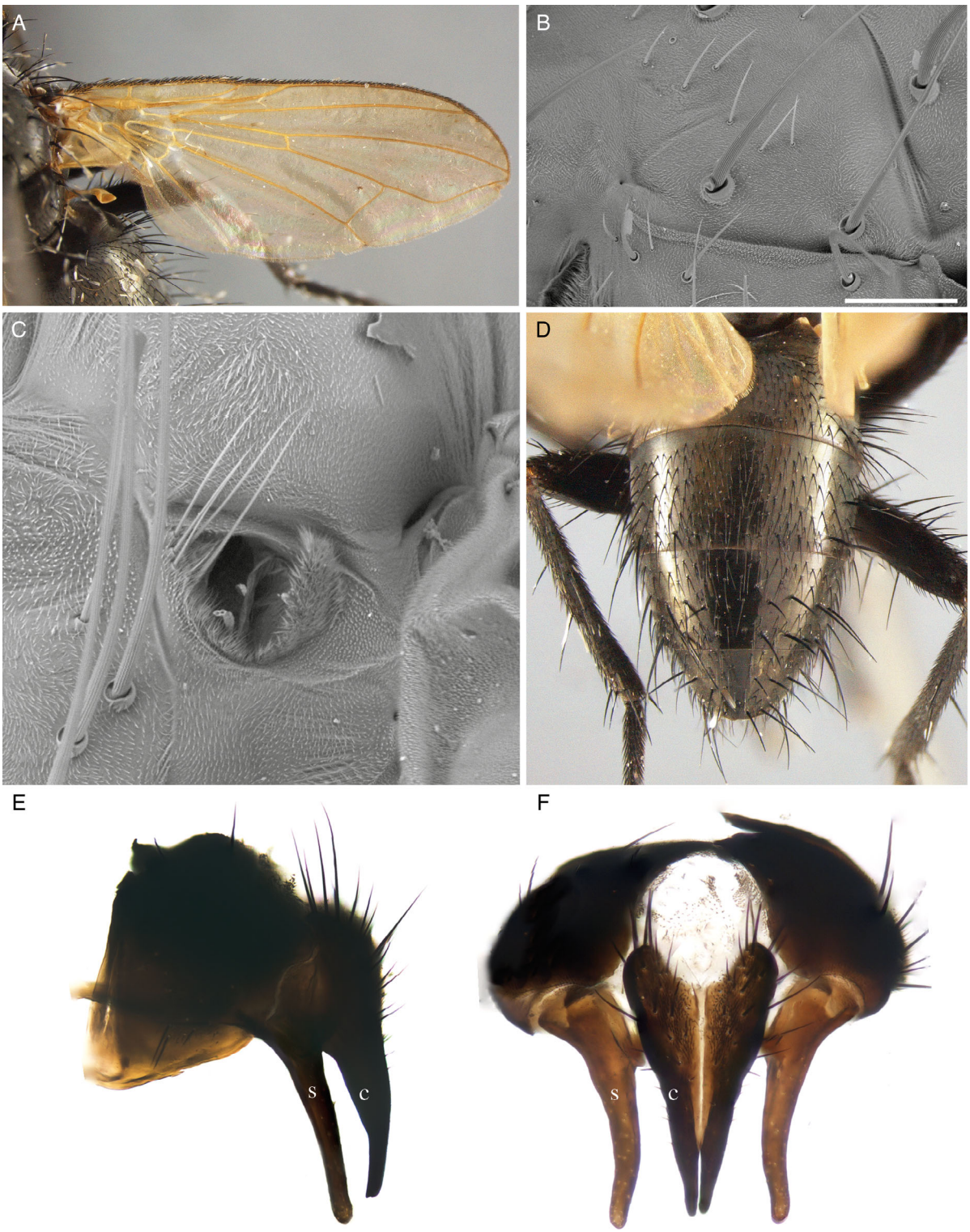

Fig. 5. *Morinia tsitsikamma* **sp.n.** (A) Right wing in dorsal view, male paratype 05385; (B) Notopleuron in lateral view, holotype 05392 (scale 200 μm); (C) Left metathoracic spiracle, male paratype 05403, male (scale 200 μm); (D) Abdomen in dorsal view, holotype 05392; (E) Epandrial complex in lateral view, male paratype 05390; (F) Epandrial complex in caudal view, male paratype 05390. c, cercus. **s**, surstylus. [Colour figure can be viewed at wileyonlinelibrary.com].

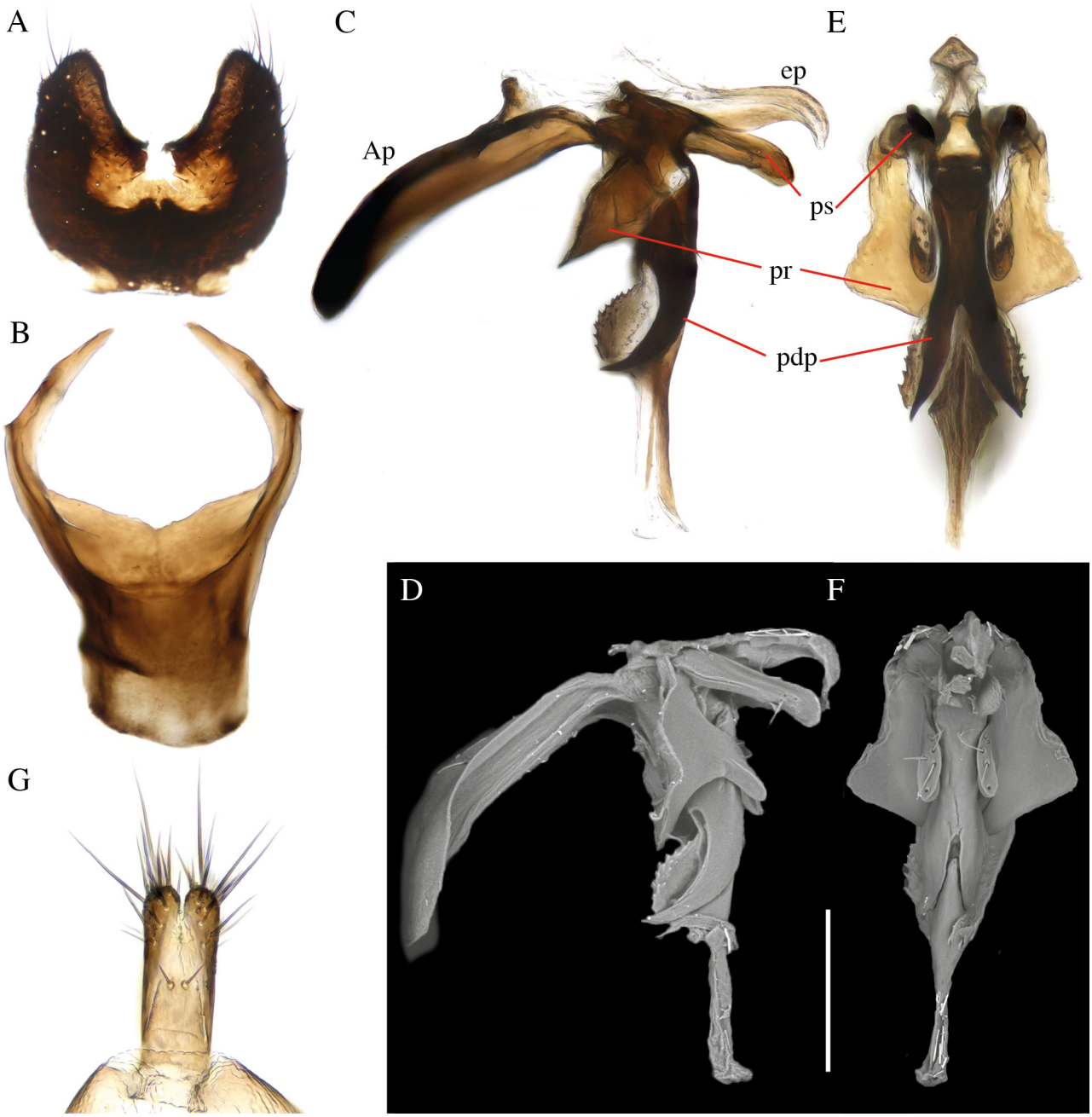

Fig. 6. *Morinia tsitsikamma* **sp.n.** (A) Sternite 5, male paratype 05390; (B) hypandrium in dorsal view, male paratype 05390; (C, D) phallic complex in lateral view, male paratype 05390 (scale, 200 µm); (E, F) phallic complex in dorsal view, male paratype 05390 (scale, 200 µm); (G) apical portion of oviscapt. ap, phallic apodeme; ep, epiphallus; hya, hypandrial arm; pdp, phallic dorsolateral process; pr, pregonite; ps, postgonite. [Colour figure can be viewed at wileyonlinelibrary.com].

dispersed between orbital setae and inner edge of compound eye. Ocellar and postocellar setae well developed, proclinate. Seven to eight frontal setae, descending to upper margin of pedicel. Lunula virtually bare, with only minute setulae, which are only visible under high magnification (Fig. 4E, G). Face concave, with a small and slightly visible median carina. Facial ridge concave with one to two setulae above vibrissa. Vibrissa arising above level of lower facial margin. Subvibrissal setae well developed, as long as vibrissa. Face and lower facial margin not visible in lateral view. Parafacial wide, about twice as wide as postpedicel (both measured at middle) and covered with short setulae. Genal dilation well developed. Gena (0.5)–0.6× (male), 0.6–0.7× (female) as high as compound eye. Postpedicel 2.2–2.6 (2.4)× (male), 1.8–2.2× (female) times as

long as pedicel. Arista short plumose (longest trichia about 2× proximal diameter of arista) and thickened on proximal 1/7–1/8. Second aristomere about as long as wide. Prementum shorter than head height, about 3–4× as long as wide. Labella normally developed. Palpus slightly clavate, about twice as long as labellar lobes.

Thorax (Fig. 5B, C). Two postpronotal setae. One posthumeral seta. One presutural, three postsutural supra-alar setae (first postsutural supra-alar seta longer and stronger than notopleural setae). Two postsutural intra-alar setae (presutural intra-alar setae absent). Two presutural, three postsutural dorsocentral setae. One presutural, one postsutural acrostichal setae. Notopleuron bare except for the two notopleural setae. Metathoracic spiracle small and rounded; posterior lappet of metathoracic spiracle slightly larger than anterior lappet and outwardly directed; anterior margin of metathoracic spiracle with two to five relatively long setae (Fig. 5C). Two pairs of marginal scutellar setae (basal seta about as long as crossed, horizontal apical setae) and one pair of discal scutellar setae. Two katepisternal setae. Three or four strong anepisternal setae. Anepimeron with a patch of slender setulae below lower calypter. Postalar wall with setulae.

Wing (Fig. 5A). Costal spine at most weakly differentiated from regular row of costal setulae. Second costal sector cs_2 bare ventrally. Base of R_{4+5} bare. Section of M_1 between crossveins r-m and dm-m longer than the section between dm-m and bend of M_1. Wing cell r_{4+5} open at wing margin. Bend of M_1 rounded.

Legs. Preapical posterodorsal seta of fore tibia varying from slightly shorter to as long as preapical dorsal seta. Midtibia with one anterodorsal seta. Preapical anteroventral seta on hind tibia about as long and robust as preapical posteroventral seta. Hind tibia with two anterodorsal and two anteroventral setae. Hind tibia with equally strong dorsal, anterodorsal and posterodorsal preapical setae.

Abdomen (Figs 4A, 5D). Mid-dorsal depression on syntergite 1 + 2 confined to anterior third; syntergite 1 + 2 and tergite 3 without median marginal setae. Tergites 4 and 5 with a row of marginal and median discal setae. Tergite 5, measured mid-dorsally, 0.6–(0.8)× (male), 0.7–0.9× (female) as long as tergite 4.

Male terminalia (Figs 5E, F, 6). Connection between tergite 6 and syntergosternite 7 + 8 membranous. Cerci nearly straight in lateral view, only slightly curved on distal fourth and subequal in length to surstyli, not fused medially. Surstylus long and narrow in lateral view, with short and scattered setulae distally. Surstylus freely articulated with epandrium. Bacilliform sclerite joining surstylus at the anterobasal corner of surstylus (seen in lateral view). Hypandrium wide, hypandrial arms well developed, converging posteriorly but not touching each other. Pregonite subtriangular, with three to four thin setulae on the pointed distal part. Postgonite with a short seta arising at mid-length (slightly beyond) on lower surface. Postgonal apodeme present. Epiphallus well developed, weakly sclerotized and hook-shaped. Apical part of dorsolateral processes of distiphallus (pdp) separate from phallus wall (i.e. with free tip). Median process of ventral sclerotization of distiphallus long, narrow and not fused to base of ventral sclerotization. Ventrolateral lobe semicircular, about as long as median process of ventral sclerite, fringed with robust, scale-like spinulae. No armature on ventral and ventrolateral surface of distalmost parts of distiphallus, i.e. smooth and without small teeth (Fig. 6C, D).

Female terminalia (Fig. 6G). Length of sclerites 7–8 moderate (sternites 7 and 8 about 2× as long as wide). Tergite 8 divided into two broad hemitergites. Sensilla trichodea (alpha setae) present on sternites. Epiproct lobe-shaped, without microtrichosity, without sensilla trichodea, with two setae apically. Hypoproct with ligulae. Cerci narrow and elongate, about twice as long as epiproct, with setae but without sensilla trichodea and without microtrichosity.

Distribution. Afrotropical – South Africa (Eastern Cape).

Alvamaja chlorometallica *Rognes, 2010*

(Fig. 7)

Description of female.

Material examined: 1 ♀: Romania – Moldova / Comăneşti – Bacău county / 13.07.2014 / 46.27356/26.434257 / Leg. Pintilioaie Alex (NHMUK).

Female differs from male (see Rognes, 2010) as follows:

Body length: 7.5 mm.

Frons 1.1× as wide as compound eye in dorsal view. Fronto-orbital plate with two proclinate orbital setae and one upper reclinate orbital seta. Outer vertical seta well developed. Terminalia (Fig. 7C–E): Sclerites 7–8 and intersegmental membrane very long (sternite 7 about 3× as long as wide, sternite 8 about 4× as long as wide). Tergite 8 divided into two long and narrow hemitergites (almost stick-like, but with an enlarged anterior end). Sternite 8 elongate with entire apex. Sensilla trichodea (alpha setae) present on sternites. Epiproct lobe-shaped, without microtrichosity, with several sensilla trichodea apically, with seven setae apically. Hypoproct with well-developed ligulae. Cerci moderately elongated, i.e. slightly longer than epiproct, with several sensilla trichodea on apical third, with short setae apically and without microtrichosity.

Distribution. Palaearctic – Romania, Serbia.

Discussion

The total evidence and nearly all the molecular-based phylogenetic analyses retrieved members of the former calliphorid subfamily Polleniinae as sister to Tachinidae, corroborating the results by Singh & Wells (2013), Winkler *et al.* (2015), Cerretti *et al.* (2017), Blaschke *et al.* (2018), Stireman *et al.* (2019) and Kutty *et al.* (2019). This clade is also consistently reconstructed as distinct from the nonpolleniine calliphorid clades with strong statistical support. Hence, we propose to resurrect Polleniidae at the family rank. Using the optimization algorithm for unambiguous character state changes, we found no morphological support for the position of the polleniids as sister to the tachinids (Fig. S4) (see also Cerretti *et al.*, 2017). However, enforcing the ACCTRAN character state optimization, a sister-group relationship between these two families is supported by one nonhomoplastic synapomorphy: ventral and ventrolateral surface of distalmost parts of distiphallus smooth,

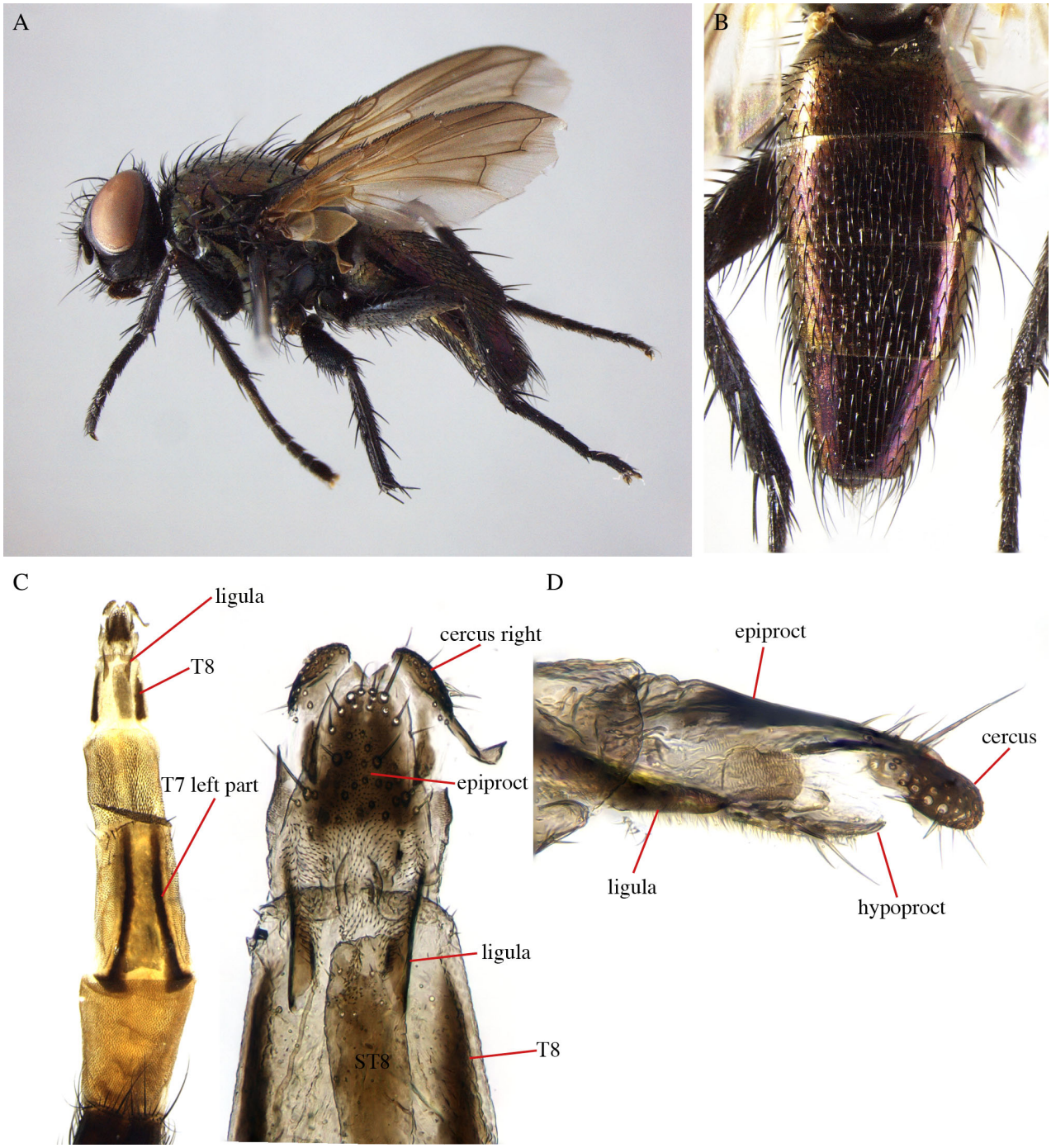

Fig. 7. *Alvamaja chlorometallica* Rognes, female. (A) Habitus in lateral view; (B) abdomen in dorsal view; (C, D) oviscapt in dorsal view: (C) posterior tip of tergite 6, tergites 7 and 8, epiproct and cerci; (D) detail of tergite 8, epiproct and cerci; (E) tip of oviscapt in left lateral view. T, tergite. [Colour figure can be viewed at wileyonlinelibrary.com].

i.e. without small teeth-like sclerotizations (42:1) (Figs 6C, D, S5). Our analyses also revealed that the species of the *carinata*-group, originally assigned to the rhinophorid genus *Phyto* (Crosskey, 1977; Pape, 1987; Pape, 1997), and *A. chlorometallica*, originally assigned to the Rhinophoridae, actually belong, and are here assigned, to the oestroid family Polleniidae. The family, as currently re-circumscribed, is widespread through the Holarctic and Palaeotropical realms, including the Australasian region, and includes seven genera (*Alvamaja*, *Dexopollenia*, *Melanodexia*, *Morinia*, *Nesodexia*, *Pollenia*, *Xanthotryxus*) and

145 species (S. Gisondi *et al*., unpublished data). Our results indicate that Polleniidae are also present in the Afrotropical region, represented by species of the genus *Morinia*.

The morphological character state optimization, using the unambiguous changes algorithm and carried out on the total evidence tree, revealed that polleniid monophyly relies on one homoplastic synapomorphy: ovipositor sclerite length moderate (45:1). Applying the ACCTRAN algorithm recovers other two homoplastic synapomorphies supporting polleniid monophyly: facial sclerite at least weakly carinate (3:1) and sternite 8 of ovipositor elongate with apex entire (48:2). In conclusion, we have been unable to identify unique morphological synapomorphies of adult Polleniidae, making it difficult to distinguish them from other oestroids except by exclusion. Moreover, several adult polleniids (e.g. *Alvamaja*, *Morinia*) are morphologically similar to rhinophorids in sharing a slender body shape, a tongue-shaped lower calypter and slightly elongated cerci in female (see Rognes, 2010 for a thorough discussion). This notable morphological similarity explains why species of the *carinata*-group and *A. chlorometallica* were originally assigned to Rhinophoridae. The current study revealed that, despite their strong resemblance to rhinophorids, the presence of a few fine setulae on the postalar wall is a reliable clue to support their exclusion from the 'true' members of this family. These setulae possibly characterized the early oestroid ancestor but were independently lost in several oestroid lineages. As pointed out by Crosskey (1977), rhinophorids invariably have a bare postalar wall with no known exceptions. The species of the *carinata*-group, the undescribed *Morinia* from Western Cape and *M. tsitsikamma* **sp.n.** have been assigned here to this genus, despite weak statistical support, due to their sharing with the Palaearctic type species, *M. doronici*, the probably derived character states of a bare node at the base of vein R_{4+5} (30:0) and the outer posthumeral and presutural intra-alar setae absent (neither character was included in our morphological dataset due to strong homoplasy across oestroid clades). The genus *Morinia* was previously known from seven species from the Palaearctic and Oriental regions (S. Gisondi *et al*., unpublished data), and it was classified in the family Rhinophoridae until Pape (1986) moved it to the Calliphoridae (Table 1) (see also Tschorsnig, 1985). The weak statistical support for *Morinia* monophyly in our analyses stems from the lack of molecular data for *M. tsitsikamma* **sp.n.** and *M. carinata* and the highly incomplete set of morphological characters scored for *Morinia* sp. from Western Cape due to the lack of a male specimen for study. Also, no molecular data were obtained from the type species, *M. doronici*, which is also the only non-Afrotropical representative of the genus included in the present analysis. This lack of information in the current dataset impaired the resolution of the intrageneric relationships, preventing assessment of whether the southern African *carinata*-group and the Palaearctic-Oriental species of *Morinia* constitute separate monophyletic groups or if one represents a paraphyletic grade relative to the other. For these reasons, we have chosen not to erect a new genus for the *carinata*-group.

The total evidence analysis retrieved the monotypic *Alvamaja* as sister to *Morinia*. At present, we choose not to consider *Alvamaja* as a junior synonym of *Morinia* because of its highly autapomorphic appearance. Finally, *A. chlorometallica* does not share any of the derived, although not unique, character states supporting *Morinia* monophyly.

As mentioned in the introduction, very little is known about the ecology and reproductive strategies of the polleniids except for a few *Pollenia* species that are predators/parasitoids of earthworms. Our results hint that species of both *Alvamaja* and the *carinata*-group are unlikely to be parasitoids of woodlice and may be parasitoids/predators of earth-inhabiting invertebrates such as earthworms or insect larvae.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Phylogeny of Oestroidea as inferred from ML analysis of DNA data using RAXML. Numbers indicate bootstrap values.

Figure S2. Phylogeny of Oestroidea as inferred from Bayesian analysis of DNA data using MRBAYES. Numbers above branches indicate posterior probability values.

Figure S3. Phylogeny of Oestroidea as inferred from ML analysis of the translated amino acid alignment using a JTT matrix model of amino acid substitution. Numbers indicate bootstrap values.

Figure S4. Phylogeny of Oestroidea as inferred from from Bayesian analysis of DNA + morphological data using MRBAYES, with morphological character state changes optimized using the unambiguous changes algorithm in WINCLADA v.1.00.08. Black squares indicate nonhomoplasious apomorphies, and white squares indicate homoplasious apomorphies. Numbers above squares indicate characters, and numbers below squares indicate character states.

Figure S5. Phylogeny of Oestroidea as inferred from from Bayesian analysis of DNA + morphological data using MRBAYES, with morphological character state changes optimized using fast optimization (ACCTRAN) algorithm in WINCLADA v.1.00.08. Black squares indicate nonhomoplasious apomorphies, and white squares indicate homoplasious apomorphies. Numbers above squares indicate characters, and numbers below squares indicate character states.

File S1. Characters and character state description, and data matrix.

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