European Journal of Taxonomy 532: 1–26
https://doi.org/10.5852/ejt.2019.532
ISSN 2118-9773
www.europeanjournaloftaxonomy.eu
2019 · Yassin A. *et al.*

Research article
urn:lsid:zoobank.org:pub:1301752E-3FD5-4F3E-A4F3-6766D18C709A

Resolving the synonymy and polyphyly of the '*Drosophila bakoue* species complex' (Diptera: Drosophilidae: '*D. montium* species group') with descriptions of two new species from Madagascar

Amir YASSIN[1,*], Arnaud SUWALSKI[2] & Lala H. RAVELOSON RAVAOMANARIVO[3]

[1,2]Institut de Systématique, Évolution, Biodiversité (ISYEB), CNRS, EPHE, MNHN, Sorbonne Université, Univ. des Antilles, 57 rue Cuvier, CP 50, 75005 Paris, France.
[3]Département d'Entomologie, Faculté de Sciences, Univ. d'Antananarivo, Antananarivo, Madagascar.

[*]Corresponding author: yassin@mnhn.fr
[2]Email: suwalski@mnhn.fr
[3]Email: lravaomanarivo@gmail.com

urn:lsid:zoobank.org:author:981F1D4F-BE5B-4F04-AA7C-B5FA30D0A857
urn:lsid:zoobank.org:author:E053BA9E-6371-4461-AE9D-FD5C823B9F2D
urn:lsid:zoobank.org:author:838B66C9-CF3A-45EE-B28A-B0FE0BBE49B6

Abstract. The '*D. bakoue* species complex' Rafael, 1984 ('*D. montium* species group' Da Lage *et al.*, 2007) comprises seven Afrotropical species. Using complete mitochondrial genome sequences and detailed morphological analysis, we revised the phylogenetic relationships between these species including two new ones. We found the '*D. bakoue* species complex' to be a junior synonym of the '*D. seguyi* species complex' Lachaise, 1971 and its seven species polyphyletic. We thus classified the species into three complexes, the '*D. seguyi* species complex' comprises *D. seguyi* Smart, 1945, *D. malagassya* Tsacas & Rafael, 1982, *D. curta* Chassagnard & Tsacas, 1997 and *D. chocolata* sp. nov., the new '*D. tsacasi* species complex' comprises *D. tsacasi* Bock & Wheeler, 1972 and *D. seguyiana* Chassagnard & Tsacas, 1997, and the new '*D. vulcana* species complex' comprises *D. vulcana* Graber, 1957 and *D. mylenae* sp. nov. *Drosophila bakoue* Tsacas & Lachaise, 1974 could not be assigned to any of the defined complexes. The two new species are endemic to Madagascar and we report the presence of *D. seguyi* and *D. curta* in Mayotte and Madagascar, respectively. The results hence represent a significant step towards understanding the diversity and evolution of this species group in Africa and the islands of the Western Indian Ocean.

Keywords. Africa, integrative taxonomy, mitogenomics, phylogenetic classification.

Yassin A., Suwalski A. & Raveloson Ravaomanarivo L.H. 2019. Resolving the synonymy and polyphyly of the '*Drosophila bakoue* species complex' (Diptera: Drosophilidae: '*D. montium* species group') with descriptions of two new species from Madagascar. *European Journal of Taxonomy* 532: 1–26. https://doi.org/10.5852/ejt.2019.532

Introduction

The '*D. montium* species group' Da Lage *et al.*, 2007 is the largest clade of the subgenus *Sophophora* Sturtevant, 1939. It contains 71 Oriental and Australian species and 23 Afrotropical species (Toda 2018) and is divided into seven subgroups (Yassin 2018). Of these, the '*D. seguyi* species subgroup' Yassin, 2018 comprises the Afrotropical species along with two Asian species (Yassin 2018). The earliest attempt to classify the Afrotropical species into species complexes was made by Lachaise (1971) who erected the '*D. seguyi* species complex' Lachaise, 1971 for four species from Ivory Coast. These species turned out to be three new species, with none corresponding to *D. seguyi* Smart, 1945 (Tsacas & Lachaise 1974). Tsacas & Lachaise (1974) classified seven of the then eight described African species of the '*D. montium* species group' into two complexes. The first complex comprised two species with a row of dorsal pegs on the male surstylus and very small phallic anterior parameres (outer paraphyses): *D. bocqueti* Tsacas & Lachaise, 1974 and *D. burlai* Tsacas & Lachaise, 1974. Tsacas (1979) later called this complex the '*D. bocqueti* species complex' Tsacas, 1979 and then added to it a third species, *D. chauvacae* Tsacas, 1984, described from Comoros (Tsacas 1984). The second complex was suggested to be called '*D. seguyi* species complex' by Tsacas & Lachaise (1974) and comprised five species with no dorsal pegs on the surstylus and with distinct anterior parameres: *D. bakoue* Tsacas & Lachaise, 1974, *D. greeni* Bock & Wheeler, 1972, *D. seguyi*, *D. tsacasi* Bock & Wheeler, 1972 and *D. vulcana* Graber, 1957. Rafael (1984) then added a sixth species, *D. malagassya* Tsacas & Rafael, 1982, to this complex and changed its name into the '*D. bakoue* species complex' Rafael, 1984. Chassagnard *et al.* (1997) excluded *D. greeni* from the '*D. bakoue* species complex', while adding to it two species: *D. curta* Chassagnard & Tsacas, 1997 and *D. seguyiana* Chassagnard & Tsacas, 1997.

Several taxonomic problems arise when dealing with the '*D. bakoue* species complex'. First, although a species complex is not a taxonomic category subject to the rules of the International Code of Zoological Nomenclature (ICZN), application of the priority principle of the ICZN should consider the '*D. bakoue* species complex' Rafael, 1984 a junior synonym for the '*D. seguyi* species complex' Lachaise, 1971. Second, the two traits that were used to define this complex, i.e., the absence of surstylus pegs and the distinction of anterior parameres, are ancestral and shared by most other species of the '*D. montium* species group' (i.e., symplesiomorphies). Third, while molecular phylogenetic studies support the monophyly of African species belonging to the '*D. montium* species group', they produce conflicting results considering the relationships between the species of the complex (Yassin *et al.* 2016). Indeed, these studies used six out of its seven species, but type strains of only two species were used (*D. malagassya* and *D. tsacasi*), shedding doubts on the identification of laboratory strains of the other species.

Here, we attempt to resolve these problems using complete mitochondrial genome sequences and a detailed morphological analysis of the available strains or from the taxonomic literature. We split the '*D. bakoue* species complex' into three complexes, namely '*D. seguyi* species complex' Lachaise, 1971, and the two new '*D. tsacasi* species complex' and '*D. vulcana* species complex' after considering '*D. bakoue* species complex' a junior synonym of '*D. seguyi* species complex'. During this revision, we describe two new species from Madagascar (*D. chocolata* sp. nov. and *D. mylenae* sp. nov.). We report *D. seguyi* and *D. curta* from the islands of the Western Indian Ocean for the first time and discuss the impact of our findings on understanding the evolution of the '*D. montium* species group' in Africa.

Material and methods

Table 1 shows the source for the different strains used in molecular and morphological analyses. For mitochondrial genomes, we generated complete genome sequences for all the above mentioned strains (except for *D. bakoue*) within our ongoing project to determine the genetic basis of a particular female-limited color dimorphism that has evolved in several species of the '*D. montium* species group'. The genomes of three of these species have already been published (*D. burlai*, *D. kikkawai* Burla, 1954

Table 1. Strains of *Drosophila* Fallén, 1823 used in the current study, with GenBank accession numbers of their assembled mitogenomes.

Species	Locality	Date	Collector(s)	Donor/stock	GenBank accession no.
D. bocqueti Tsacas & Lachaise, 1974	Andasibe, Madagascar	2008	J.R. David and A. Yassin	J.R. David	MK742870
D. cf. *bocqueti* Tsacas & Lachaise, 1974	São Tomé, São Tomé and Príncipe	2014	J.R. David and M. Lang	J.R. David	MK742871
D. burlai Tsacas & Lachaise, 1974	Mount Oku, Cameroon	2008	M. Veuille	J.R. David	MK742872
D. chocolata Yassin & David sp. nov.	Andasibe, Madagascar	2008	J.R. David and A. Yassin	J.R. David	MK742873
D. curta Chassagnard & Tsacas, 1997	Andasibe, Madagascar	2008	J.R. David and A. Yassin	J.R. David	MK742874
D. jambulina Parshad & Paika, 1964	New Delhi, India	1998	J.R. David	J.R. David	MK742875
D. kikkawai Burla, 1954	Chandigarh, India	1998	J.R. David	J.R. David	MK742876
D. leontia Tsacas & David, 1977	Bangalore, India	2000	J.R. David	J.R. David	MK742877
D. malagassya Tsacas & Rafael, 1982	Mandraka, Madagascar	2008	J.R. David and A. Yassin	J.R. David	MK742878
D. mylenae David & Yassin sp. nov.	Nosy Be, Madagascar	2008	J.R. David and M. Dauvergne	J.R. David	MK742879
D. punjabiensis Parshad & Paika, 1964	Kuala Lumpur, Malaysia	1962	M. Wasserman	DSSC (14028–0641.00)	MK742880
D. rufa Kikkawa & Peng, 1938	Ehime, Japan	1991	Unknown	DSSC (14028–0661.03)	MK742881
D. seguyi Smart, 1945	Mayotte Island, France	2013	J.R. David, N. Gidaszewski and V. Debat	J.R. David	MK742882
D. tsacasi Bock & Wheeler, 1972	Ivory Coast	1951	H. Burla	DSSC (14028–0701.00)	MK742883
D. aff. *tsacasi* Bock & Wheeler, 1972	Kenya	2005	Unknown	J.R. David	MK742884
D. aff. *tsacasi* Bock & Wheeler, 1972	Bioko Island, Equatorial Guinea	2013	D. Matute	J.R. David	MK742885
D. vulcana Graber, 1957	Mount Selinda, Zimbabwe	1971	H.E. Paterson	DSSC (14028–0711.00)	MK742886

and *D. leontia* Tsacas & David, 1977) (Yassin *et al.* 2016). For the other species, we used the same protocols for DNA extraction, genome library construction, sequencing on an Illumina HiSeq 2000 platform, and genome alignment given in Yassin *et al.* (2016). However, instead of aligning reads to the reference genome of *D. kikkawai*, we aligned them to the reference mitochondrial genome of *D. yakuba* Burla, 1954 (GenBank accession no. NC_001322). In addition, we used nearly complete mitochondrial genomes of *D. baimaii* Bock & Wheeler, 1972, *D. auraria* Peng, 1937 and *D. barbarae* Bock & Wheeler, 1972 sequenced by O'Grady & DeSalle (2008). For each species, we used a customized perl script to infer the consensus sequence from multiple strains (e.g., from strains with 'light' or 'dark' females). Phylogenetic analysis was conducted after estimating the best substitution model (GTR + G) for the complete mitochondrial sequence using the MEGA7 software package (Kumar *et al.* 2016). A Bayesian phylogeny was then inferred using MrBayes ver. 3.2.4 (Ronquist *et al.* 2012). Two runs of 500000 generations were conducted and sampled every 1000 generations under a strict clock model. We assessed convergence using MrBayes (the average standard deviation of split frequency < 0.01 and the potential scale reduction factor ~1.00). A burn-in period of 25% of samples was used. We also extended our taxonomic scope by analyzing sequences of the ~650 bp-long DNA barcoding region of the *cytochrome oxidase subunit 1* (*COI*) mitochondrial genes for the African species of the '*D. montium* species group' published in Li *et al.* (2012), Chen *et al.* (2013) and Prigent *et al.* (2017), and of the ~1350 bp-long of the nuclear *Amylase-related* (*Amyrel*) gene from Da Lage *et al.* (2007) using MEGA7 and MrBayes as mentioned above.

We have previously found that mitochondrial DNA might fail in identifying closely-related taxa in 68 drosophilid species, most likely due to widespread cytological introgression at shallow systematic levels

(Yassin *et al.* 2010). On the other hand, the divergence in nuclear genomes shows a stronger association with the degree of reproductive isolation (Turissini *et al.* 2018). For the species analyzed here, we have also aligned the nuclear genome on the reference genome of *D. kikkawai* as in Yassin *et al.* (2016) and estimated pairwise genetic divergence (Yassin, in prep.). For three populations of *D. kikkawai* from Brazil, Colombia and India that were used in Yassin *et al.* (2016), we estimated the divergence in the nuclear genome to range from 1.44 to 1.48%. The divergence between the three strains and *D. leontia*, their sister species with whom they can produce fertile females but sterile males, ranged from 2.64 to 2.67%. We therefore compared the genome divergence of the new species from their closest relatives to these values.

For the African species of the '*D. montium* species group', genitalia of ten individuals per sex per strain were dissected, mounted on microscopic slides in DMHF mounting medium (Entomopraxis A9001) and photographed under a Leica light microscope as in Yassin & Orgogozo (2013). A scanning electron microscopic (SEM) analysis was conducted using a Hitachi SU3500 microscope in the SEM Utility of the Muséum national d'Histoire naturelle (MNHN) in Paris on five ethanol-preserved specimens per sex per species. Wings and legs were removed from each specimen. Specimens were then dried at critical point and coated with a gold alloy before imaging. Descriptions of the new species were conducted following standard taxonomic terminology and measurements defined in Bächli *et al.* (2004).

Abbreviations

4C	=	third costal section between R_{2+3} and R_{4+5}/M between r-m and dm-cu
4v	=	M between dm-cu and wing margin/M between r-m and dm-cu
5x	=	CuA_1 between dm-cu and wing margin/dm-cu between M_1 and CuA_1
aap	=	aedeagal apodeme
ac	=	third costal section between R_{2+3} and R_{4+5}/distance between distal ends of R_{4+5} and M
ae	=	aedeagus (penis)
C	=	second costal section between subcostal break and R_{2+3}/third costal section between R_{2+3} and R_{4+5}
cer	=	cercus (anal plate)
CuA	=	anterior branch of cubital vein cubitus plus anal veins
cvl	=	cercal ventral lobe (secondary clasper)
dc	=	anterior/posterior dorsocentral setae length ratio
ep	=	epandrium (genital arch)
H	=	upper/lower postpronotal setae length ratio
hb	=	third costal section between R_{2+3} and R_{4+5} with heavy bristles/third costal section between R_{2+3} and R_{4+5}
hyp	=	hypandrium (novasternum)
hmp	=	hypandrial median process
ip	=	inner paraphysis (posterior paramere)
op	=	outer paraphysis (anterior paramere)
or1	=	proclinate orbital seta
or2	=	anterior reclinate orbital seta
or3	=	posterior reclinate orbital seta
prox.x	=	distance between base of R_{4+5} and r-m/M between r-m and dm-cu
scut	=	basal/apical scutellar setae length ratio
sst	=	surstylus (primary clasper)
T2-T7	=	abdominal tergites 2 to 7
vtm	=	medial vertical seta

Institutional abbreviations

DSSC = *Drosophila* Species Stock Center, Cornell University, USA
MNHN = Muséum national d'Histoire naturelle, Paris, France
ZMUZ = Zoological Museum of the University of Zurich, Zurich, Switzerland
ZUAC = University of Antananarivo, Antananarivo, Madagascar

Results

Split of the bakoue species complex into three species complexes

The Bayesian mitogenomic phylogeny (Fig. 1) reconfirmed the monophyly of African species of '*D. montium* species group' (i.e., the '*D. seguyi* species complex' subgroup) and the polyphyly of the '*D. bakoue* species complex' as was shown in previous phylogenetic studies using nuclear genes (Zhang *et al.* 2003; Da Lage *et al.* 2007; Chen *et al.* 2013; Yassin *et al.* 2016). The latter was split into three

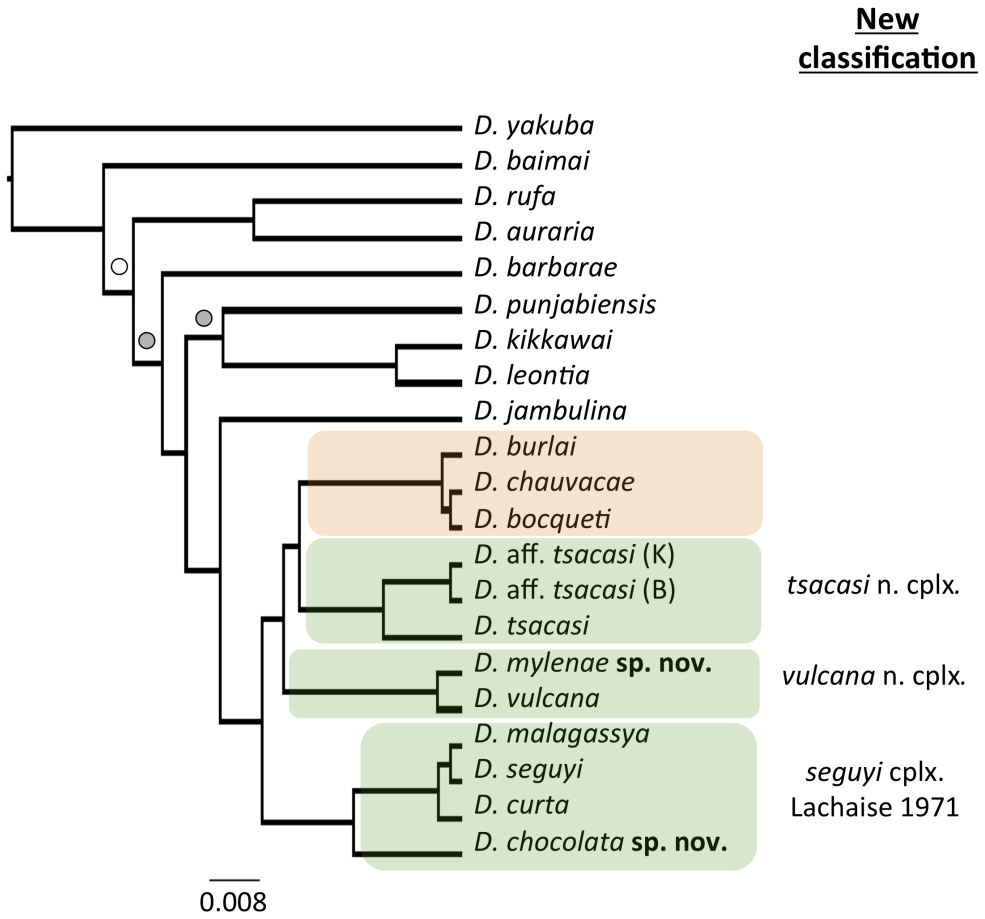

Fig. 1. Bayesian phylogeny of 19 species from the '*D. montium* species group' Da Lage, 2007 inferred from complete mitochondrial genomes. Nodes supported by 100% posterior probability (pp) are not labeled, those with pp > 90% or 60% are labeled with gray and white circles, respectively. Species belonging to the '*D. bocqueti* species complex' and the '*D. bakoue* species complex' are highlighted in orange and green, respectively. 'K' and 'B' refer to the strains of *D.* aff *tsacasi* Bock & Wheeler, 1972 from Kenya and Bioko Island, respectively.

monophyletic clades: '*seguyi*', '*tsacasi*' and '*vulcana*'. The two new Malagasy species, *D. chocolata* sp. nov. and *D. mylenae* sp. nov., are members of the clades '*seguyi*' and the '*vulcana*', respectively.

The monophyly of these clades was also supported in single gene phylogenies, with a larger number of species (Fig. 2). Interestingly, the position of *D. bakoue* greatly differed between the mitochondrial gene *COI* and the nuclear gene *Amyrel*. For the former, flies from Cameroon were distinct from all other species of the former '*D. bakoue* species complex'. For *Amyrel*, a strain from Benin was a part of the clade '*tsacasi*'. However, the relationships between either strains with the true *D. bakoue* described from Ivory Coast remain unclear (see section 'Taxonomy' below).

At the morphological level, the three clades of species mainly differ in the male periphallic organs (Fig. 3). The surstylus (primary clasper) is lobate with long, irregularly spaced prensisetae in species of the '*tsacasi*' clade (Fig. 3B), whereas it is almost quadrate with a regular row of short compact prensisetae in the two other complexes (Fig. 3A, C). The cercal ventral lobe (secondary clasper) is separated from the cercus in the '*vulcana*' clade with the teeth less curved (Fig. 3C), whereas it is fused to the cercus and carries curved teeth arising from long chitinous roots in the two other clades (Fig. 3A–B). Within

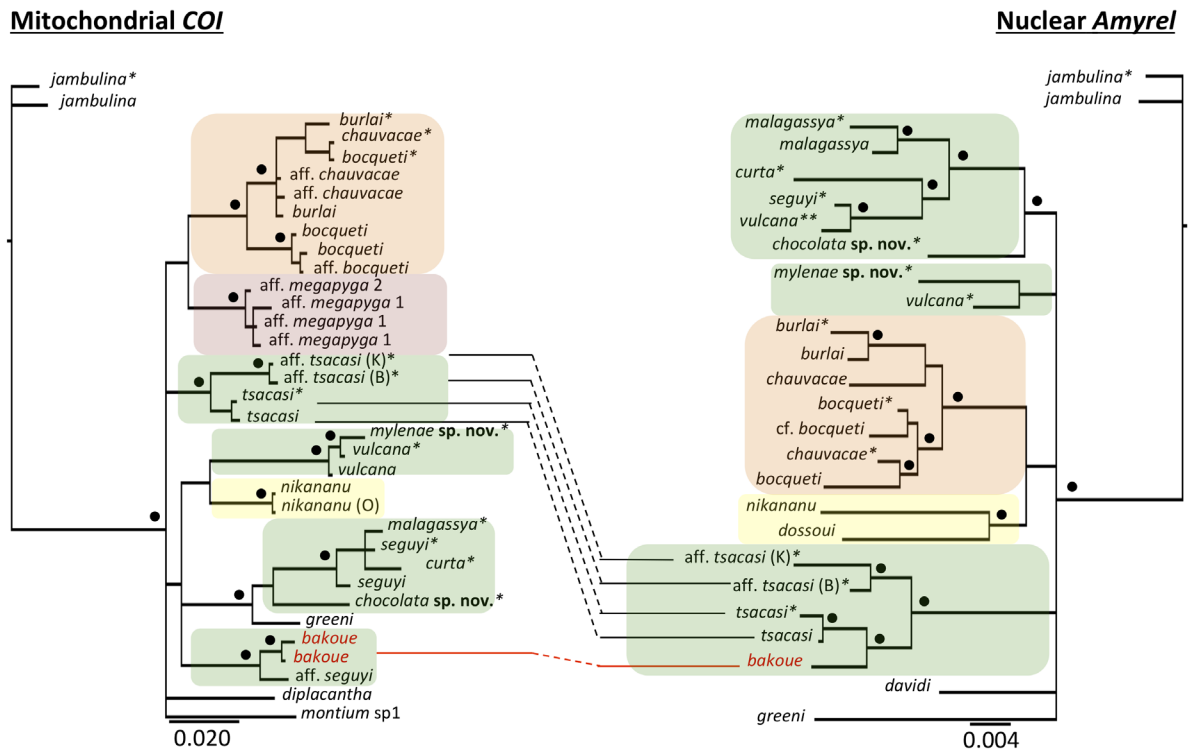

Fig. 2. Comparison of Bayesian phylogenies of the '*D. seguyi* species subgroup' Yassin, 2018 inferred from mitochondrial *COI* (left) and nuclear *Amyrel* (right) genes. Nodes supported by >95% posterior probability (pp) are labeled with black circles. An asterisk refers to species sequenced here, whereas two asterisks refer to the sequence of *D. vulcana* Graber, 1957 of Da Lage *et al.* (2007), which turned out to belong to *D. seguyi* Smart, 1945 from Kenya (see the text). Note the discrepancy in position of different geographical strains of *D. bakoue* Tsacas & Lachaise, 1974 (in red) between the two genes. Species belonging to '*D. bocqueti* species complex' Tsacas & Lachaise, 1974, '*D. bakoue* species complex' Rafael, 1984, '*D. nikananu* species complex' Tsacas & Chassagnard, 1992, and '*D. megapyga* species complex' Lachaise & Tsacas, 2001 are highlighted in orange, green, yellow and red, respectively.

each clade, species mostly differ in their body pigmentation and genital morphology as will be discussed below. Each of these clades was considered a separate 'species complex' within the '*D. seguyi* species subgroup' to resolve the polyphyly of the former '*D. bakoue* species complex' (see section 'Taxonomy' below).

Nuclear genome divergence within each complex

The two geographical strains of *D.* aff. *tsacasi* from Kenya and Bioko Island which shows no reproductive isolation had a nuclear genome divergence of 1.66%, a value approaching 1.44–1.48% between geographical populations of *D. kikkawai* (see section 'Material and methods'). For species pairs with partial reproductive isolation (David *et al.* 2014), the nuclear genome divergence ranged from 2.16% between *D. bocqueti* and *D. chauvacae* in the '*D. bocqueti* species complex', to 3.12–3.30% between the three species of the '*seguyi*' clade, namely *D. seguyi*, *D. malagassya* and *D. curta*. These values agree with the 2.64–2.67% divergence estimate between *D. kikkawai* and *D. leontia*. Together, these results suggest that nuclear genomic divergence in the '*D. montium* species group' ranges from 1.44 to 1.66% among strains belonging to the same species, and from 2.16 to 3.30% among species with partial reproductive isolation.

Within the '*seguyi*' clade, the nuclear genome of *D. chocolata* sp. nov. diverges from the remaining three species of the complex by 4.58–4.84%. Such a significant genomic divergence supports a new specific status and coincides with the distinct morphological characters of *D. chocolata* sp. nov. Within the '*tsacasi*' clade, the nuclear divergence between *D.* aff. *tsacasi* and *D. tsacasi* is 1.81 and 2.05% for the Bioko and Kenyan strains, respectively. Although these values are higher than the intraspecific estimates in the '*D. montium* species group', they are still lower than the interspecific ones. Given the slight morphological differentiation between *D.* aff. *tsacasi* and *D. tsacasi*, as well as the dubious status of other taxa within the '*tsacasi*' clade (see below), further analyses should be undertaken before properly determining the specific status of *D.* aff. *tsacasi*. Within the '*vulcana*' clade, *D. mylenae* sp. nov. diverges from *D. vulcana* by 2.35%, a value falling within the interspecific range. This nuclear divergence, together with additional morphological differences, supports the specific status of *D. mylenae*

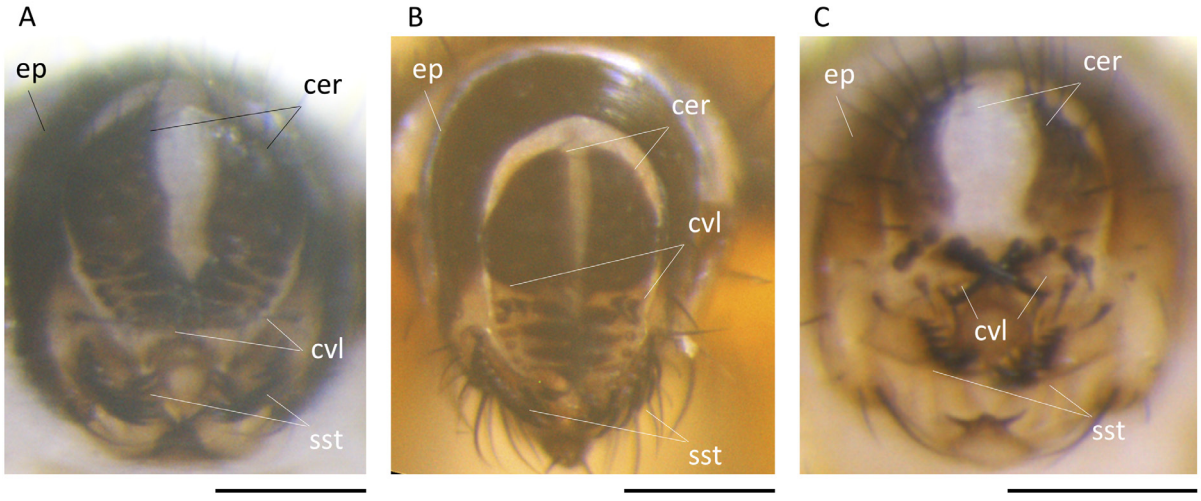

Fig. 3. Photomicrographs of male periphallic organs. **A**. *D. seguyi* Smart, 1945 (MNHN). **B**. *D.* aff. *tsacasi* Bock & Wheeler, 1972 (MNHN). **C**. *D. mylenae* David & Yassin sp. nov. (MNHN). Scale bars = 0.1 mm.

sp. nov., in spite of its low mitochondrial differentiation from its sister species *D. vulcana* (see section 'Discussion' below).

Taxonomy

We follow the scheme of Yassin (2013) of higher order classification of the Drosophilidae.

Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Family Drosophilidae Rondani, 1856
Subfamily Drosophilinae Rondani, 1856
Tribe Drosophilini Okada, 1989
Genus *Drosophila* Fallén, 1823
Subgenus *Sophophora* Sturtevant, 1939
'*D. montium* species group' Da Lage *et al.*, 2007
'*D. seguyi* species subgroup' Yassin, 2018

Drosophila* (*Sophophora*) *bakoue Tsacas & Lachaise, 1974
Fig. 2

Drosophila (*Sophophora*) *bakoue* Tsacas & Lachaise, 1974: 197.

Diagnosis

Male with sex combs on the two first tarsomeres of the foreleg and completely yellow abdominal tergites; surstylus without dorsal tooth-like protuberance.

Type material

Holotype

IVORY COAST • ♂; Lamto; 6°13′ N, 5°02′ W; 22 Dec. 1970; D. Lachaise leg.; MNHN.

Distribution

Ivory Coast (type), Benin, Cameroon, Congo, Gabon, Malawi, Nigeria, and São Tomé Island (new location).

Remarks

D. bakoue resembles the species of the '*D. nikananu* species complex' Tsacas & Chassagnard, 1992 in males having completely yellow abdominal tergites. However, it differs from the species of the '*D. nikananu* species complex' in males having sex combs on the two first tarsomeres of the foreleg (the comb is lost or reduced on the second tarsomere in the '*D. nikananu* species complex') and lacking a dorsal tooth-like protuberance on the surstylus.

Lachaise (1979) attributed a laboratory strain collected from Makoukou (Gabon) to this species, and showed that it could produce fertile F1 females and sterile F1 males when its females were crossed with males of the strain of *D. vulcana* of Bock & Wheeler (1972). Rafael (1984) attributed another strain from Kunden (Cameroon) to *D. bakoue* and showed that it could not hybridize with the same strain of *D. vulcana* or with the strain of *D. tsacasi* of Bock & Wheeler (1972). Intriguingly, Rafael (1984) pointed out that both the Gabonese and Cameroonian strains of *D. bakoue* showed some differences in body size and pigmentation from the type material from Ivory Coast. She also found that the Cameroonian strain hybridized readily with *D. malagassya*, though both F1 sexes were sterile. Kopp (2016) analyzed a strain collected from the island of São Tomé and attributed to *D. bakoue* by J.R. David, and found

that it produced sterile F1 males and females when crossed with the same strain of *D. tsacasi*. Da Lage *et al.* (2007) analyzed the sequence of the nuclear gene *Amyrel* from a strain collected from Benin and found it to be sister to the strain of *D. tsacasi* of Bock & Wheeler (1972). Prigent *et al.* (2017) partially sequenced the mitochondrial gene *COI* from two specimens from Mount Oku in Cameroon and did not recover such affinity (Fig. 2). These results suggest that at least two different species may have been attributed to *D. bakoue*, from which the strains from Benin and São Tomé are closely related to *D. tsacasi*, whereas the strains from Gabon and Cameroon are distant. The relation of these species with the true *D. bakoue* from Ivory Coast needs more investigations.

'*D. seguyi* species complex' Lachaise, 1971

Figs 1–2, 3A, 4, 5E–F, 6

'*D. seguyi* species complex' Lachaise, 1971: 1623.
'*D. bakoue* species complex' Rafael, 1984: 179, syn. nov.

Diagnosis

Male abdominal tergites T2 to T4 yellowish with very distinct black stripes or entirely dark brown, T5 with broader stripe or completely black, T6 shiny black (Fig. 4); cercal ventral lobe (secondary clasper) fused to cerci with two or three very large curved black medial teeth on the internal margin; surstylus quadrate with a lateral row of strong, short prensisetae on the outer and inner margins (with two medial prensisetae in *D. chocolata* sp. nov.), the innermost one significantly long; hypandrial median process tapering; aedeagus hirsute with fine cuticular scales; posterior parameres as long as aedeagus (slightly shorter in *D. chocolata* sp. nov.), with finely serrated margins (Figs 3A, 5). Female abdominal tergites lighter than male's or concolorously dark (Fig. 4).

Remarks

The original '*D. seguyi* species complex' was renamed '*D. bakoue* species complex' without new definition or justification (Rafael 1984). We consider here the '*D. bakoue* species complex' to be a junior synonym for the '*D. seguyi* species complex'. Chassagnard *et al.* (1997) discussed a '*D. seguyi* species complex' comprising *D. curta*, *D. seguyi* and *D. seguyiana* in a table legend, but considered all these species members of the '*D. bakoue* species complex' in the main text. We restrict here the '*D. seguyi* species complex' to four monophyletic species, while placing *D. seguyiana* in the '*D. tsacasi* species complex' (see below). David *et al.* (2014) referred to three of these species as geographical strains of *D. malagassya* with partial reproductive isolation. Closer examination of their material revealed, however, that each strain was a distinct species (namely, *D. seguyi*, *D. malagassya* and *D. curta*).

Taxon content

D. seguyi Smart, 1945.
D. malagassya Tsacas & Rafael, 1982.
D. curta Chassagnard & Tsacas, 1997.
D. chocolata sp. nov.

Drosophila* (*Sophophora*) *seguyi Smart, 1945

Figs 1–2, 3A, 4A–B, 5A, 6A–B

Drosophila (Sophophora) seguyi Smart, 1945: 56.

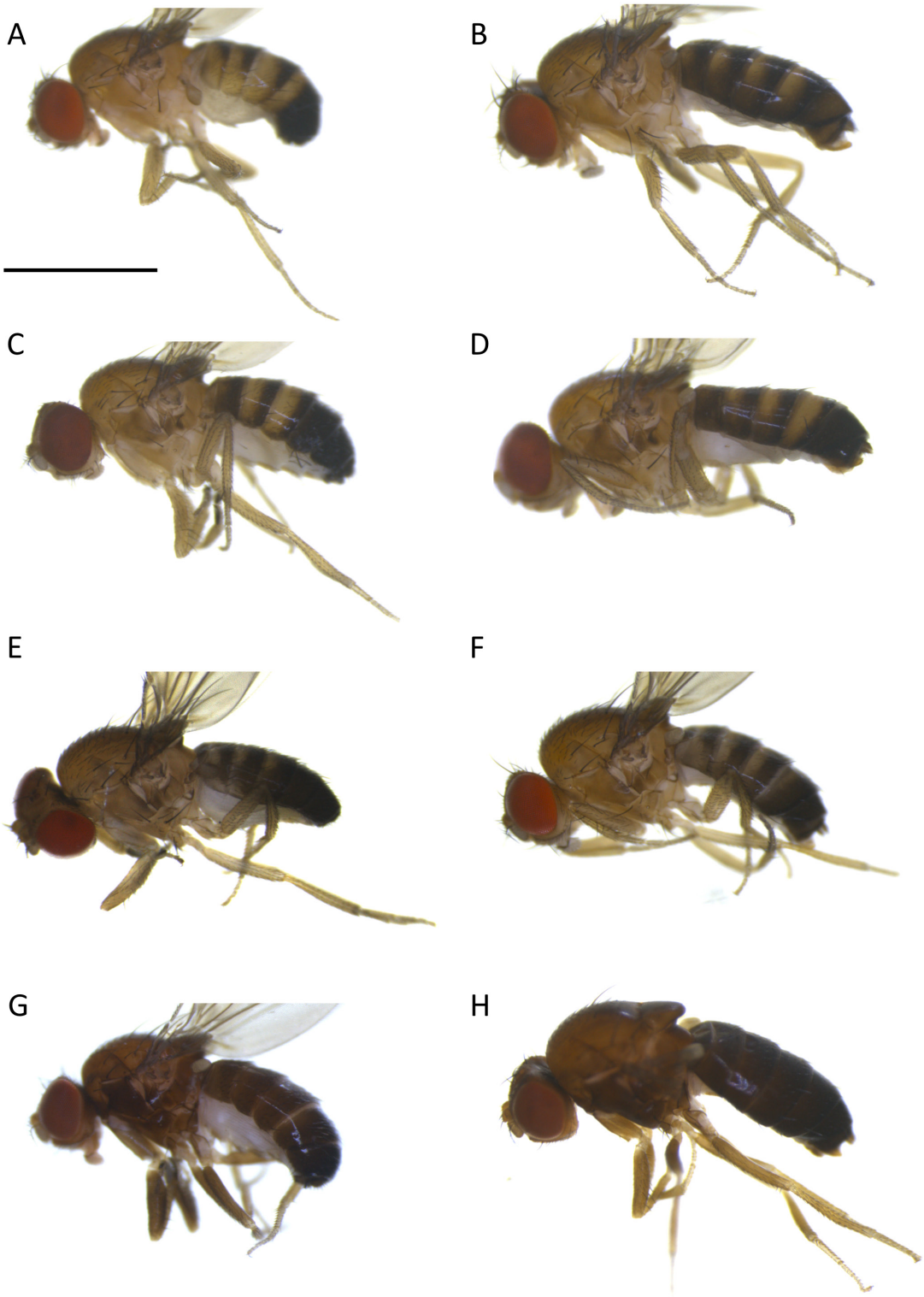

Fig. 4. Adult habitus. **A**–**B**. *D. seguyi* Smart, 1945 (MNHN). **A**. ♂. **B**. ♀. **C**–**D**. *D. malagassya* Tsacas & Rafael, 1982 (MNHN). **C**. ♂. **D**. ♀. **E**–**F**. *D. curta* Chassagnard & Tsacas, 1997 (MNHN). **E**. ♂. **F**. ♀. **G**–**H**. *D. chocolata* Yassin & David sp. nov. (MNHN). **G**. ♂. **H**. ♀. Only dark morphs of females of species with female-limited color dimorphism are shown. Scale bar = 1 mm.

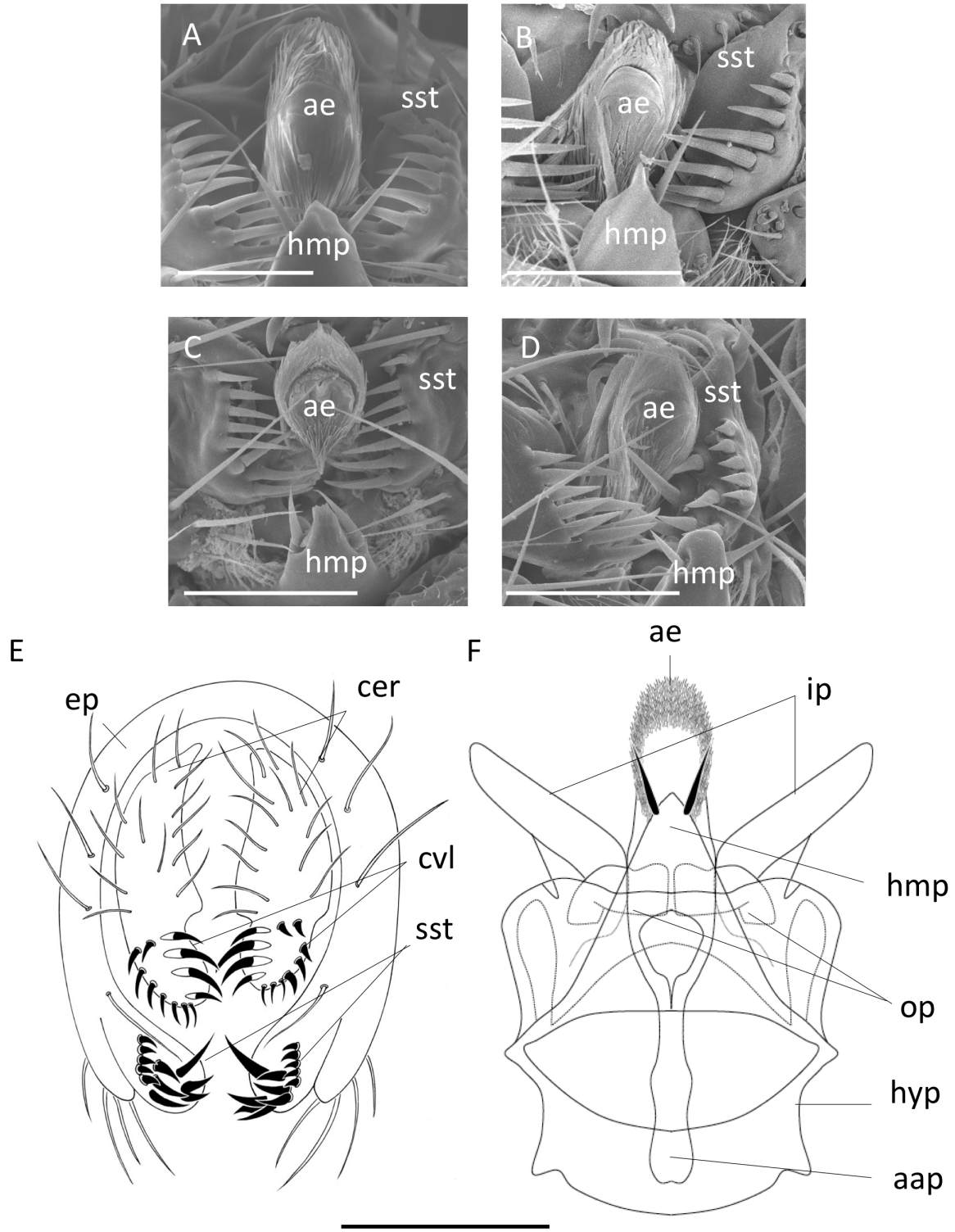

Fig. 5. Male terminalia of the '*D. seguyi* species complex'. **A**. *D. seguyi* Smart, 1945 (MNHN). **B**. *D. malagassya* Tsacas & Rafael, 1982 (MNHN). **C**. *D. curta* Chassagnard & Tsacas, 1997 (MNHN). **D–F**. *D. chocolata* Yassin & David sp. nov. Scale bars: A–D = 50 µm; E–F = 100 µm..

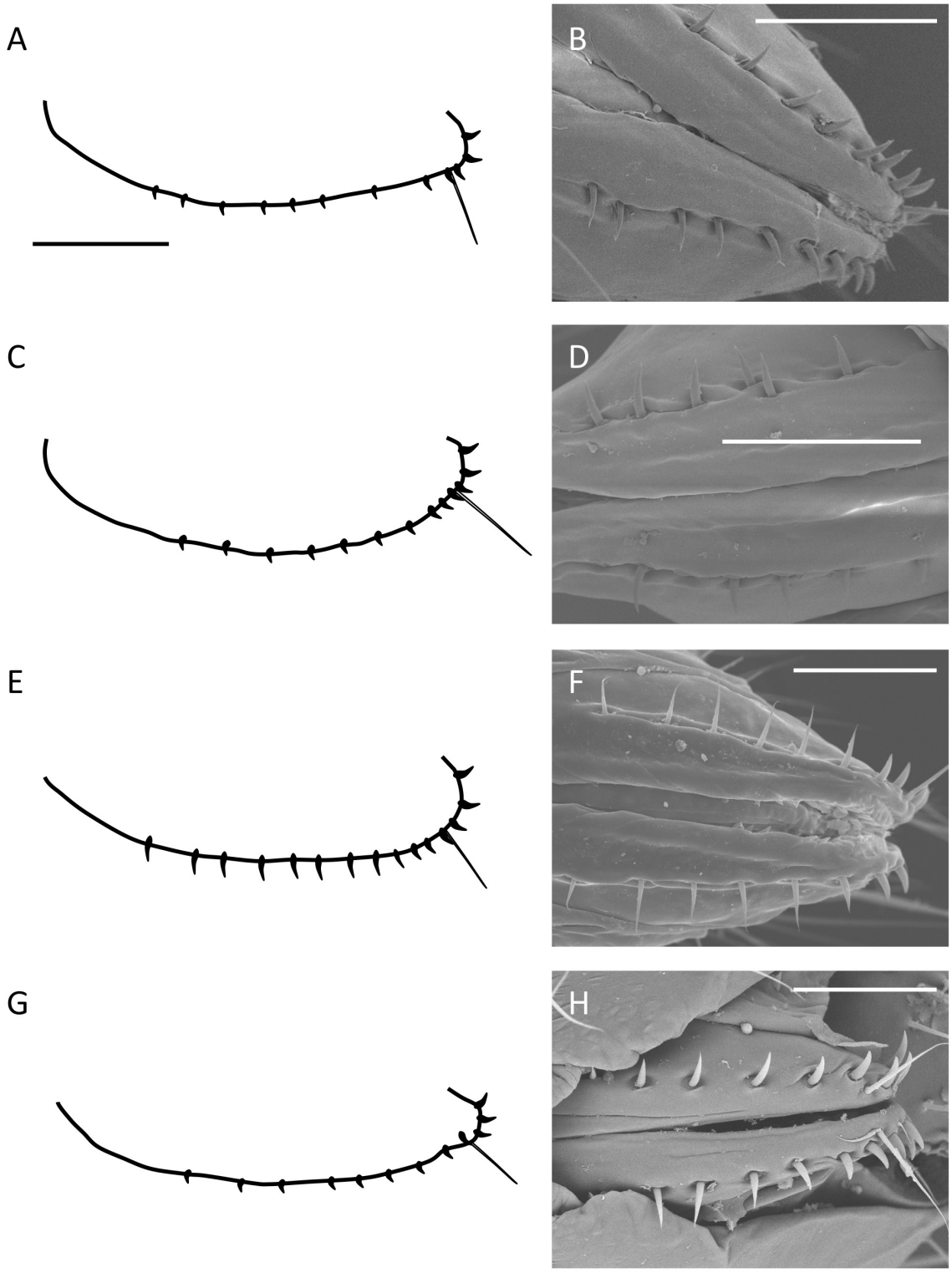

Fig. 6. Female oviscapts of the '*D. seguyi* species complex'. **A**–**B**. *D. seguyi* Smart, 1945 (MNHN). **C**–**D**. *D. malagassya* Tsacas & Rafael, 1982 (MNHN). **E**–**F**. *D. curta* Chassagnard & Tsacas, 1997 (MNHN). **G**–**H**. *D. chocolata* Yassin & David sp. nov. (MNHN). Scale bars: A, C, E, G = 100 µm; B, D, F, H = 50 µm.

Diagnosis

Male and female body pigmentation including halters and legs yellow (Fig. 4A–B); male abdominal tergites T2–T4 with a thin black stripe expanding on the middle and fainting towards the margin, T5 with a broader stripe, T6 completely black (Fig. 4A); dorsalmost surstylus prensiseta on the same axis with remaining prensisetae, hypandrial median process weakly pointed, aedeagus finger-like (Fig. 5A); female T2–T5 with a broad black stripe and an expanding grayish area on the margin (Fig. 4B); oviscapt fourth posterior peg-like outer ovisensillum on the same axis with the third and fifth ovisensilla (Fig. 6A), with anterior ovisensilla short and thick (Fig. 6B).

Type material

Holotype

KENYA • ♂; Mount Elgon; 01°07′ N, 34°31′ E; 2490 m a.s.l.; 1932–1933; R. Jeannel leg.; MNHN.

Distribution

Kenya (type), Cameroon (new record), Malawi, island of Mayotte (new record), Tanzania and Zimbabwe.

Remarks

Two strains (K2 and K59) collected from Mombasa (Kenya) in 1979 and identified as *D. seguyi* by T. Okada were studied in genetic studies (Ohnishi & Watanabe 1984; Zhang *et al.* 2003; Li *et al.* 2012; Chen *et al.* 2013; M. Watada, pers. comm.). These strains were also sent to the laboratory of Gif-sur-Yvette in France, where Léonidas Tsacas and Marie-Thérèse Chassagnard worked. Tsacas & Chassagnard (1992) considered the strain of *D. vulcana* of Bock & Wheeler (1972) to be the true *D. seguyi*. In Tsacas' notebook recording the strains maintained at Gif-sur-Yvette, we found a note by him considering the K59 strain to be the true *D. vulcana*. This strain was used in the revision of the genus *Drosophila* Fallén, 1823 of Da Lage *et al.* (2007), based on the nuclear gene *Amyrel*, although in the publication, Mount Selinda, the locality of the strain of *D. vulcana* of Bock & Wheeler (1972), was mistakenly mentioned as the source of the *Amyrel* sequence (J.-L. Da Lage, pers. comm.). In spite of the suggestion of Tsacas & Chassagnard (1992), we prefer for taxonomic stability to consider both Kenyan strains to belong to the true *D. seguyi* and to preserve the concept of *D. seguyi* of Bock & Wheeler (1972). A strain collected in Cameroon by J. Pool in 2004, and preserved in the Drosophila Species Stock Center, was identified based on the mitochondrial gene *cytochrome oxidase subunit 2* (*COII*) as *D. seguyi*, indicating the broad distribution of this species in Africa.

David *et al.* (2014) mentioned the presence of a strain of *D. malagassya* on the island of Mayotte (discussed under *D. malagassya* above). We have used this strain, as *D.* cf. *malagassya*, in our combined phylogenetic study of the montium clade and showed its close affinity to the Kenyan strains of *D. seguyi* (Yassin *et al.* 2016). Detailed morphological comparisons support the conspecificity of the strains from Kenya and Mayotte. Females of the strain of Mayotte exhibit the characteristic Mendelian color dimorphism, with the black morph being dominant (Yassin *et al.* 2016).

Drosophila* (*Sophophora*) *malagassya Tsacas & Rafael, 1982
Figs 1–2, 4C–D, 5B, 6C–D

Drosophila (*Sophophora*) *malagassya* Tsacas & Rafael, 1982: 86.

Diagnosis

Male and female body pigmentation including halters and legs yellow (Fig. 4C–D); male abdominal tergites T2, T5 and T6 completely black; T3 and T4 with a broad black stripes not expanding towards

the margins (Fig. 4C); dorsalmost surstylus prensiseta on the same axis with remaining prensisetae; hypandrial median process strongly pointed; aedeagus spatulate (Fig. 5B); female T2 almost entirely black, T3 and T4 with a broad black stripe and an expanding grayish area on the margin, T5 with a black stripe but no grayish area (Fig. 4B); oviscapt fourth posterior peg-like outer ovisensillum on the same axis with the third and fifth ovisensilla (Fig. 6C), with anterior ovisensilla short and thick (Fig. 6D).

Type material

Holotype

MADAGASCAR • ♂; Antananarivo, Botanical and Zoological Garden of Tsimbazaza; 18°55′ S, 47°31′ E; Sep. 1980; D. Lachaise leg.; MNHN.

Other material examined

MADAGASCAR • 5 ♂♂, 3 ♀♀; Andasibe; 17°20′ S, 48°54′ E; 16–17 Feb. 2008 (ex-laboratory strain Jul. 2014); J.R. David & A. Yassin leg.; ZUAC.

Description

As in Tsacas & Rafael (1982).

Distribution

Madagascar (endemic).

Remarks

The species was described from a laboratory strain collected from the Tsimbazaza Botanical Park in Antananarivo in 1980. With Jean R. David and Vincent Debat, we collected it in 2008 and 2010 from Andasibe, Mandraka and Ranomafana. Rafael (1984) showed that *D. malagassya* crossed readily with a Cameroonian strain attributed to *D. bakoue*, producing sterile F1 males and females, and to a lesser degree with *D. tsacasi*, producing a few or unviable F1 flies. David *et al.* (2014) suggested the presence of *D. malagassya* on the island of Mayotte (Comoros archipelago), but the Mayotte strain turned out to be *D. seguyi*. The females of *D. malagassya* have two color morphs (Yassin *et al.* 2016).

Drosophila* (*Sophophora*) *curta Chassagnard & Tsacas, 1997
Figs 1–2, 4E–F, 5C, 6E–F

Drosophila (*Sophophora*) *curta* Chassagnard & Tsacas in Chassagnard *et al.*, 1997: 93.

Diagnosis

Male and female body pigmentation including halters and legs yellow (Fig. 4E–F); male abdominal tergites T2–T4 with a broad black stripes expanding towards the margins, T5 and T6 completely black (Fig. 4E); dorsalmost surstylus prensiseta not on the same axis with remaining prensisetae; hypandrial median process truncated and serrated; aedeagus spatulate (Fig. 5C); female T2 with a thin black stripe expanding on the middle and fainting towards the margin, T3–T5 with a broad black stripe and an expanding grayish area on the margin (Fig. 4F); oviscapt fourth posterior peg-like outer ovisensillum on the same axis with the third and fifth ovisensilla (Fig. 6C), with anterior ovisensilla long and thin, almost setiferous (Fig. 6D).

Type material

Holotype

MALAWI • ♂; Cape Maclear; 14°01′ S, 34°51′ E; 30 Mar. 1991; D. Lachaise leg.; MNHN.

Other material

MADAGASCAR • 5 ♂♂, 5 ♀♀; Andasibe; 17°20′ S, 48°54′ E; 16–17 Feb. 2008 (ex-laboratory strain Jul. 2014); J.R. David & A. Yassin leg.; ZUAC.

Description

As in Chassagnard *et al.* (1997).

Distribution

Malawi (type) and Madagascar (new record).

Remarks

Yassin *et al.* (2016) included a Kenyan strain of *D. curta* in their phylogenetic analysis. However, the strain turned out to be a likely new species belonging to the '*D. tsacasi* species complex' depicted in Figs 1–2 as *D.* aff. *tsacasi* (see below). In Madagascar, *D. curta* was collected only from Andasibe during the expedition of 2008. Females have two color morphs (Yassin *et al.* 2016).

Drosophila* (*Sophophora*) *chocolata Yassin & David sp. nov.
urn:lsid:zoobank.org:act:F34BF7C9-1143-4093-80E5-47A239D5C86C
Figs 1–2, 4G–H, 5D–F, 6G–H

Diagnosis

Male and female body pigmentation brown, halters white, with male femurs darker on all legs (Fig. 4G–H); male abdominal tergites T2–T4 entirely dark brown, T5 and T6 entirely black (Fig. 4G); dorsalmost surstylus prensiseta on the same axis with remaining prensisetae (Fig. 5D–E); hypandrial median process lobate; aedeagus spatulate (Fig. 5D, F); female T2–T7 entirely dark brown (Fig. 4H); oviscapt fourth posterior peg-like outer ovisensillum not on the same axis with the third and fifth ovisensilla (Fig. 6G), with anterior ovisensilla short and thick (Fig. 6H).

Etimology

In reference to body color.

Type material

Holotype

MADAGASCAR • ♂; Andasibe; 17°20′ S, 48°54′ E; 16–17 Feb. 2008 (ex-laboratory strain Jul. 2014); J.R. David & A. Yassin leg.; MNHN.

Paratypes

MADAGASCAR • 9 ♂♂, 10 ♀♀; same collection data as for holotype; MNHN.

Other material

MADAGASCAR • 5 ♂♂, 2 ♀♀; same collection data as for holotype; ZUAC.

Description

Male

Head (Fig. 4G). Frons brown, frontal length 0.38 mm; frontal index = 1.00, frontal tapering ratio = 1.47. Frontal triangle concolorous; ocellar triangle slightly darker, about 40% of frontal length. Orbital plates shining, apically slightly diverging from eye margin, about 87% of frontal length. Orbital setae black, distance of or3 to or1 = 67% of or3 to vtm, or1/or3 ratio = 1.29, or2/or1 ratio = 0.33, postocellar setae =

73%, ocellar setae = 47%, vibrissal index = 1.00. Face grayish. Carina flat. Cheek index about 12.50. Eye dark red, eye index = 1.39. Antennae dark brown. Arista with five dorsal, two ventral branches, plus terminal fork. Proboscis brown.

Thorax (Fig. 4G). Length 1.2 mm. Scutum brown, shining, darker before scutellum and having a darker median stripe on dorsocentral region, six rows of acrostichal setulae. H index = 0.50. Transverse distance of dorsocentral setae 160% of longitudinal distance; dc index = 0.88. Scutellum dark brown; scut index = 0.83. Pleura dark brown, shining. Legs dark brown, sex combs on protarsomeres 1 and 2, with about 21 and 16 peg-like setae, respectively. Wing hyaline, veins reddish, length 2.02 mm, length to width ratio = 2.18. Indices: C = 2.20, ac = 2.46, hb = 0.52, 4C = 1.88, 4v = 3.68, 5x = 0.61, M = 1.38, prox. x = 0.94. Haltere white.

Abdomen (Fig. 4G). Entirely brown, shining, tergites T5 and T6 completely black.

Terminalia (Figs. 5D–F). Epandrium black, with six setae, the lower most being particularly long; epandrial ventral lobe black with eight bristles. Cercus black; cercal ventral lobe yellow, partially separated from cercus, with a series of three strong, curved spines on the inner margin, and smaller spines along the ventral, outer and dorsal margins, larger dorsally. Surstylus with a regular row of five short, stout peg-like prensisetae, and a ventromedial cluster of prensisetae, the innermost pointing dorsally. Hypandrium black anteriorly, dark posteriorly, as long as broad, with a lobate medial posterior extension bearing two short, divergent thick bristles; posterior margin microtrichose with long fine hairs. Outer paraphyses large, S-curved, transverse, bearing three minute setulae. Inner paraphyses as long as aedeagus, swollen medially, broad and lobate apically. Aedeagus broad, hirsute, subapically narrowed. Aedeagal apodeme black anteriorly.

Female

Habitus (Fig. 4H). Similar to male but with no sex combs on protarsi.

Terminalia (Fig. 6G–H). Valve of oviscapt mediodorsally mostly membranous, posteriorly rounded, ventrally slightly concave, with no discal and twelve marginal, peg-like, pointed-tipped, short and thick ovisensilla on the outer surface and one long, straight, subterminal and three tiny (microscopic) trichoid-like ovisensilla on the inner surface. The fourth peg-like ovisensillum characteristically dorsally positioned in respect to the main axis of ovisensilla insertion on the ventral margin of the oviscapt valve.

Distribution

Madagascar (endemic).

Remarks

Specimens of this species were collected from different localities in Madagascar: Andasibe (800–1200 m a.s.l.), Antananarivo (1300 m a.s.l.) and Mandraka (1400 m a.s.l.) during the 2008 expedition by J.R. David and A. Yassin. It was also collected from Ranomafana (600 m a.s.l.) in 2009 by J.R. David, V. Debat and A. Yassin. This indicates that *D. chocolata* sp. nov. is widespread and that, unlike *D. ifestia* Tsacas, 1984, is not mountainous. The species can be maintained on a 'standard *Drosophila* medium' in the laboratory. A mutant strain from Antananarivo was established having a light abdomen in both sexes, but both the mutant and the original strains were subsequently lost.

The species resembles *D. ifestia*, a species endemic to high mountains in East Africa, in males having entirely dark abdomen. However, *D. ifestia* differs from *D. chocolata* sp. nov. in the color of the thorax being lighter with the halters reddish brown (Fig. 7A–B), which are white in *D. chocolata* sp. nov. (Fig. 6A–B), in the lack of long innermost prensiseta on the surstylus (present in all species of the

'*D. seguyi* species complex'), and in the shape of the hypandrial median process being broadly truncated in *D. ifestia* with the hypandrial bristles extending in parallel to each other (divergent in all species of the '*D. seguyi* species complex'). We therefore concur with the conclusion of Tsacas (1984) that *D. ifestia* does not belong to any of the defined species complexes of the '*D. seguyi* species subgroup'.

'*D. tsacasi* species complex' new complex
Figs 1–2, 3B

Diagnosis

Male abdominal tergites T2–T5 yellowish with usually distinct black stripes, T6 usually black; cercal ventral lobe (secondary clasper) fused to cerci with four or five very large curved black medial teeth arising from long dark chitinous roots on the internal margin; surstylus lobate with a lateral row of long, irregularly spaced prensisetae on the outer margin and a cluster of prensisetae on the inner margins (no medial prensisetae), the innermost one significantly long (Fig. 3B). Female abdominal tergites lighter than male's or concolorously light.

Remarks

This complex contains two described species, *D. tsacasi* and *D. seguyiana*, and two putatively new species, *D.* aff. *tsacasi* (see above under *D. curta*) and *D.* aff. *bakoue* (see above under *D. bakoue*). The molecular, morphological and reproductive isolation between these species requires further investigation.

Taxon content

D. tsacasi Bock & Wheeler, 1972.
D. seguyiana Chassagnard & Tsacas, 1997.

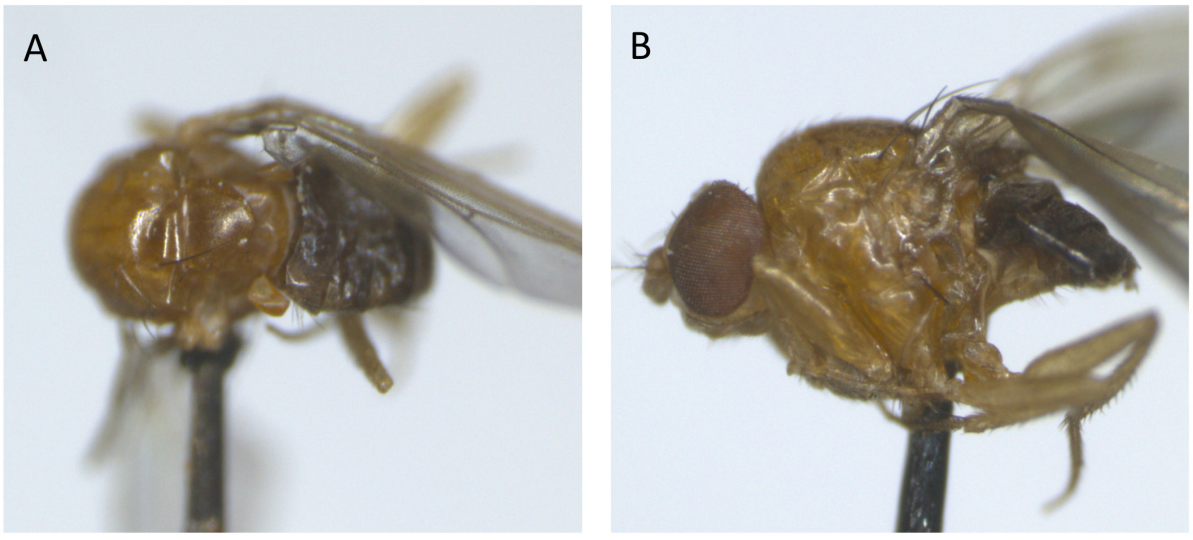

Fig. 7. Photomicrographs of *D. ifestia* Tsacas, 1984. **A**. Holotype (P. Vanschuytbroeck and J. Kelenbosch leg.; MNHN). **B**. Paratype (P. Vanschuytbroeck and J. Kelenbosch leg.; MNHN). Scale bar = 1 mm.

'*D. vulcana* species complex' new complex
Figs 1–2, 3C, 8A–D, 9A–F

Diagnosis

Male abdominal tergites yellow, T2–T4 with broad brown or black stripes, T5 and T6 with a thin black stripe expanding on the middle and fainting towards the margin (Fig. 8A); cercal ventral lobe (secondary clasper) partially separated from cerci with two or three very large curved black medial teeth on the internal margin; surstylus with a lateral row of strong, short prensisetae on the outer and inner margins (no medial prensisetae), the innermost one significantly long; hypandrial median process tapering (Fig. 3C); aedeagus hirsute with cuticular scales; inner paraphyses as long as aedeagus, with finely serrated margins (Fig. 9A–D). Female abdominal tergites darker than male's (Fig. 8B).

Remarks

Chassagnard *et al.* (1997) mentioned a species, named '*Drosophila* (*Sophophora*) sp. C', belonging to the '*D. montium* species group' in Malawi with males having diffuse brown T2–T4 and pale T5 and T6. This species either belongs to one of the two species of this complex or represents a new species belonging to the complex. Elucidating its identity needs further investigation.

Taxon content

D. vulcana Graber, 1957.
D. seguyiana sp. nov.

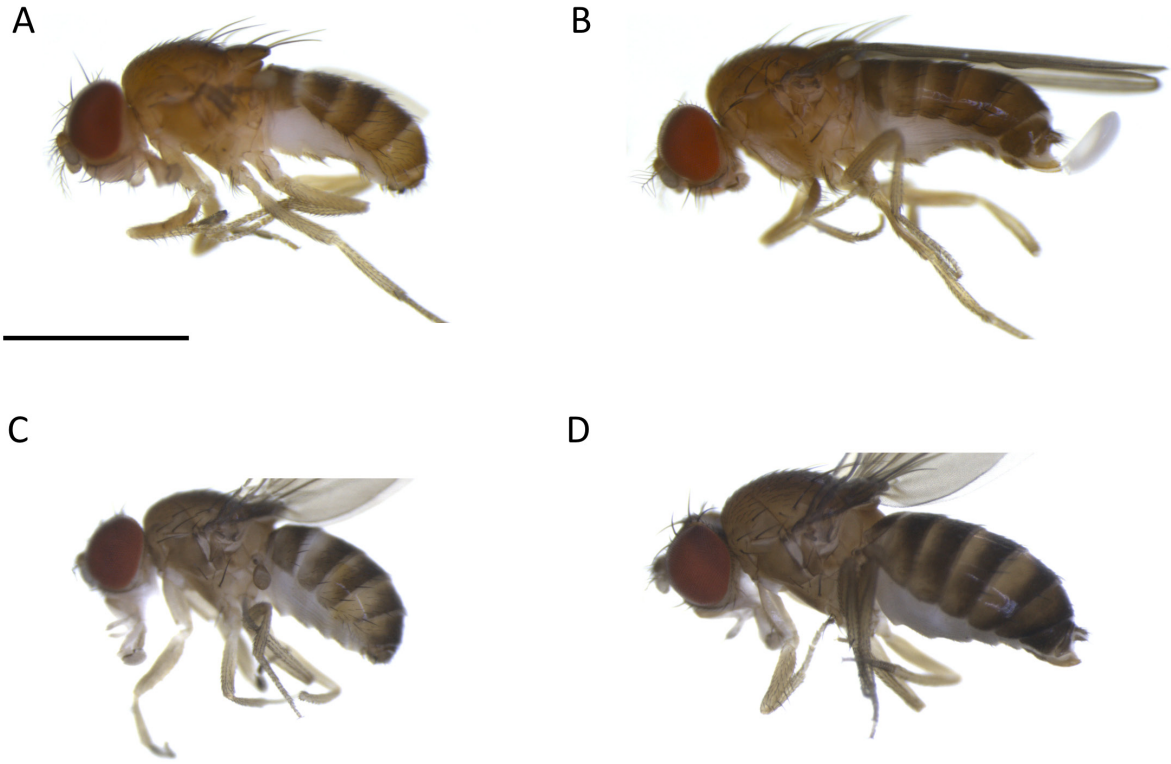

Fig. 8. Adult habitus. **A**–**B**. *D. vulcana* Graber, 1957 (MNHN). **A**. ♂. **B**. ♀. **C**–**D**. *D. mylenae* David & Yassin sp. nov. (MNHN). **C**. ♂. **D**. ♀. Scale bar = 1 mm.

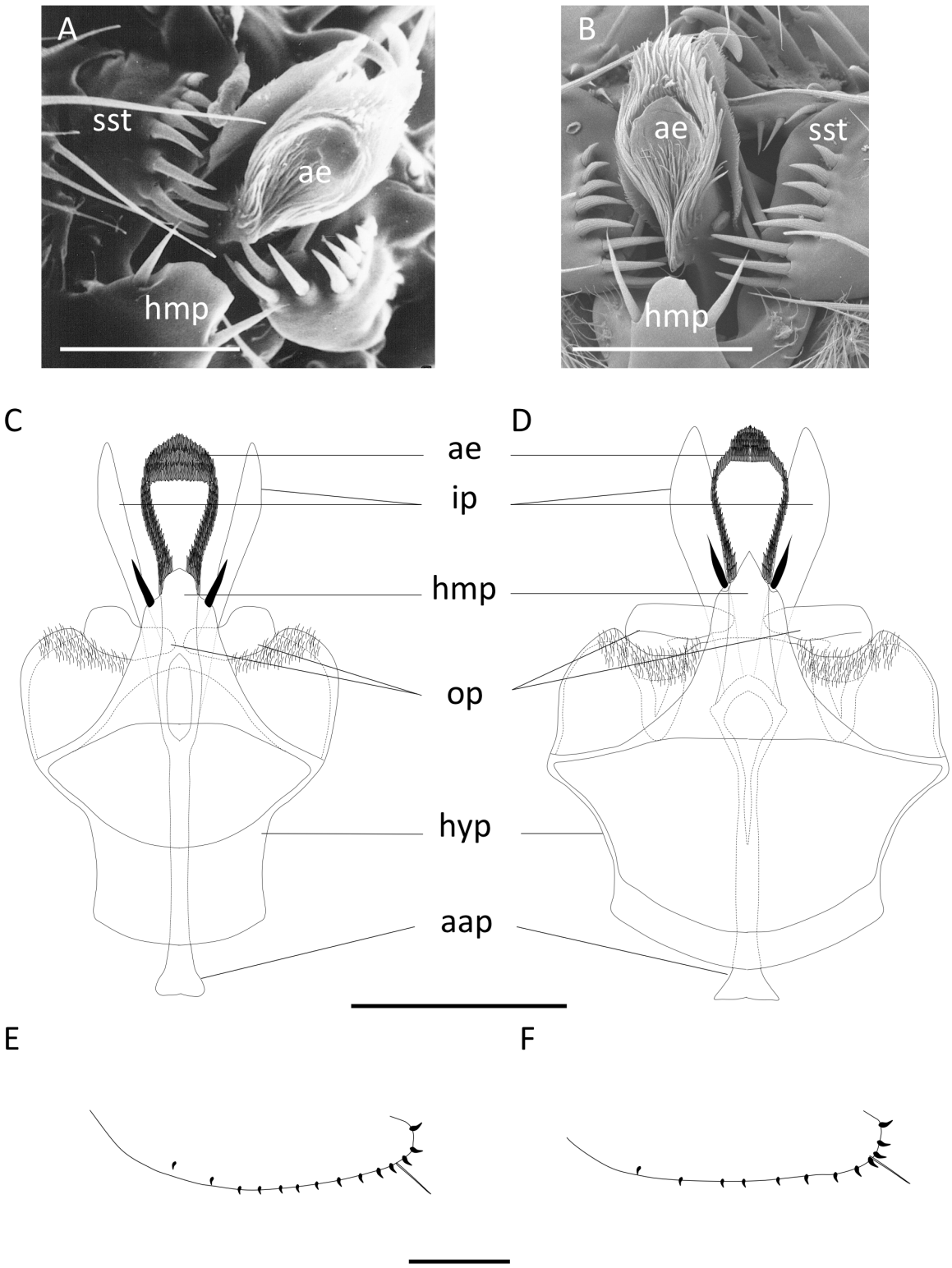

Fig. 9. Terminalia of the '*D. vulcana* species complex'. **A**, **C**, **E**. *D. vulcana* Graber, 1957 (MNHN). **A**, **C**. ♂. **E**. ♀. **B**, **D**, **F**. *D. mylenae* David & Yassin sp. nov. (MNHN). **B**, **D**. ♂. **F**. ♀. Scale bars: A–B = 50 µm; D–F = 100 µm.

Drosophila* (*Sophophora*) *vulcana Graber, 1957
Figs 1–2, 8A–B, 9A, C, E

Drosophila (*Sophophora*) *vulcana* Graber, 1957: 309.

Diagnosis

Male abdominal tergites T5 with a contiguous diffuse dark brown stripe, T6 light with a very faint dark stripe (Fig. 8A); hypandrium narrow with an elongated anterior phragma; outer paraphyses posterior margin curved; aedeagus pilosity tapering at tip (Fig. 9A, C); female abdominal tergites T2–T4 without a diffuse pale region on the antero-distal margins, T5 with diffusely dark stripe (Fig. 8B); oviscapt fourth posterior peg-like outer ovisensillum on the same axis with the third and fifth ovisensilla, with anterior ovisensilla short and thick (Fig. 9E).

Type material

Holotype

DEMOCRATIC REPUBLIC OF THE CONGO • ♂; Kivu Province, Mount Bugulumiza; 1954; ZMUZ.

Description

As in Graber (1957) for the type material and Bock & Wheeler (1972) for a strain from Mount Selinda (Zimbabwe).

Distribution

Democratic Republic of the Congo (type), Kenya, Tanzania and Zimbabwe.

Remarks

The type material of Graber (1957) consisted of six males and six females from Kivu Province (Democratic Republic of the Congo). Tsacas & Chassagnard (1992) examined this material and found five males and six females (probably the male used by Graber for dissection and genitalia illustration was lost). Of the five remaining males, only one belonged to a '*D. montium* species group' that Tsacas (1984) considered *D. ifestia*. Of the six females, five belonged to the '*D. montium* species group'. Two females sojourned in alcohol and lost coloration, whereas the remaining three females were pinned and had dark wings, pleurae and legs in agreement with the original description of Graber (1957). For abdominal coloration, this description indicates "uniform schwarzbraune glänzende Tergite" (uniform black-brown shining tergite). Bock & Wheeler (1972) described a strain from Mount Selinda (Zimbabwe) attributed to *D. vulcana* by S. Paterson. They showed, however, the presence of two types of male genitalia in this strain. Tsacas & Chassagnard (1992) reanalyzed this strain and found that only one of the two types existed at the time of their examination. They concluded that the original strain consisted of two distinct species with one having subsequently gone extinct. They also suggested that male genitalia of the type of Séguy (1938) for *D. seguyi* corresponded to the genitalia of the surviving species in this strain. We dissected males from the strain of Mount Selinda and found that the illustration of Tsacas & Chassagnard (1992) of the holotype of *D. seguyi* lacks the partial fusion of the cercal ventral lobe (secondary clasper), characteristic of the strain of Mount Selinda. Moreover, the pale male abdominal pigmentation of the strain of Bock & Wheeler (1972) (Fig. 4G) clearly contrasts with the description of Séguy (1938) of *D. seguyi*: "tergites largement bordés de brun noir, dernier segment d'un noir luisant" [tergites with large brownish black stripe, last segment shiny black]. On the contrary, abdominal pigmentation of the strain of Mount Selinda corresponded to the abovementioned original description of Graber (1957). Therefore, we concur with Bock & Wheeler (1972) for considering the strain of Mount Selinda to belong to *D. vulcana*. Okada *et al.* (1988) recorded this species from Tanzania, and Takada *et al.* (1990)

indicated its presence in Kenya, suggesting its widespread distribution in East Africa. Females do not exhibit a sex-limited color dimorphism.

Drosophila* (*Sophophora*) *mylenae David & Yassin sp. nov.
urn:lsid:zoobank.org:act:B64DA5B1-4F29-456B-B75E-0E84DFB3DA98
Figs 1–2, 8C–D, 9B, D, F

Diagnosis

Male abdominal tergites T5 and T6 with a distinct dark brown stripe expanding in the middle and fainting towards the margins (Fig. 8B); hypandrium broad with a short anterior phragma; outer paraphases posterior margin not curved; aedeagus pilosity broad at tip (Fig. 9B, D); female abdominal tergites T2–T4 with a diffuse pale region on the antero-distal margins, T5 with distinct dark stripe (Fig. 8D); oviscapt fourth posterior peg-like outer ovisensillum on the same axis with the third and fifth ovisensilla, with anterior ovisensilla short and thick (Fig. 9F).

Etimology

A species dedicated to Mylène Dauvergne, co-collector of the type strain.

Type material

Holotype

MADAGASCAR • ♂; Nosy Be; 13°20′ S, 48°15′ E; Jul. 2008 (ex-laboratory strain Feb. 2017); J.R. David and M. Dauvergne leg.; MNHN.

Paratypes

MADAGASCAR • 9 ♂♂, 10 ♀♀; same collection data as for holotype; MNHN.

Other material

MADAGASCAR • 5 ♂♂, 5 ♀♀; same collection data as for holotype; ZUAC.

Description

Male

Head (Fig. 8C). Frons pale brown, frontal length 0.35 mm; frontal index = 1.00, frontal tapering ratio = 1.29. Frontal triangle concolorous; ocellar triangle slightly darker, about 43% of frontal length. Orbital plates about 86% of frontal length. Orbital setae black, distance of or3 to or1 = 50% of or3 to vtm, or1 / or3 ratio = 1.33, or2/or1 ratio = 0.38, postocellar setae = 29%, ocellar setae = 50%, vibrissal index = 1.00 . Face white. Carina prominent, narrow. Cheek index about 12.00. Eye red, eye index = 1.15. Antennae whitish. Arista with four dorsal, three ventral branches, plus terminal fork. Proboscis brown.

Thorax (Fig. 8C). Length 1.13 mm. Scutum mid brown, shining, darker before scutellum, six rows of acrostichal setulae. H index = 1.17. Transverse distance of dorsocentral setae 200% of longitudinal distance; dc index = 0.61. Scutellum dark; scut index = 0.80. Pleura slightly darker, shining. Legs white-yellow, sex combs on protarsomeres 1 and 2, with about 18 and 13 peg-like setae, respectively. Wing dark, length 1.54 mm, length to width ratio = 2.08. Indices: C = 1.93, ac = 3.06, hb = 0.62, 4C = 1.72, 4v = 3.03, 5x = 0.56, M = 1.13, prox. x = 0.72. Haltere brown.

Abdomen (Fig. 8C). Yellow, tergites T2–T4 with a diffuse brown posterior stripes, tergites T5 and T6 pale with small posterior stripes.

Terminalia (Figs 3C, 9B, D). Epandrium pale brown, with 6 setae, the lower most being particularly long; ventral lobe with 5–7 bristles. Cercus pale brown; cercal ventral lobe yellow, partially separated from cercus, with a series of three strong, curved spines on the inner margin, and smaller spines along the ventral, outer and dorsal margins, larger dorsally. Surstylus with a regular row of five short, stout peg-like prensisetae, and a ventromedial cluster of prensisetae, the innermost pointing dorsally. Hypandrium yellow, slightly longer than broad, with a pointed medial posterior extension bearing two short, divergent thick bristles; posterior margin microtrichose with long fine hairs. Outer paraphyses large, ovoid, transverse, bearing three minute setulae. Inner paraphyses almost as long as aedeagus, swollen medially, tapering and incurved medioposteriorly. Aedeagus hirsute, broad at tip, subapically narrowed. Aedeagal apodeme yellow, broadened laterally.

Female

Habitus (Fig. 8D). Similar to male, but with no sex combs on protarsi and with abdominal tergites brown.

Terminalia (Fig. 9F). Valve of oviscapt mediodorsally mostly membranous, posteriorly rounded, ventrally slightly concave, with no discal and twelve marginal, peg-like, pointed-tipped, short and thick ovisensilla on the outer surface and one long, straight, subterminal and three tiny (microscopic) trichoid-like ovisensilla on the inner surface.

Distribution

Madagascar (endemic).

Remarks

Drosophila mylenae sp. nov. resembles *D. vulcana* in the shape of the male periphallic structures (compare Fig. 3C with figure 1 in Rafael 1984) and the female ovipositor (Fig. 9E–F) as well as in the abdominal pigmentation pattern of females being darker than males (Fig. 8), which is rare among drosophilids. However, they differ in the degree of abdominal pigmentation for both sexes and in multiple phallic structures (Figs 8–9A–D). The two species show a very low mitogenomic divergence of 0.5% (Figs 1–2). However, on the nuclear gene *Amyrel* they are quite distinct (Fig. 2), with an overall nuclear genome-wide divergence of 2.35% (Yassin, in prep.).

Drosophila mylenae sp. nov. was only collected in the littoral forest on Nosy Be. It is absent from material collected from the inland, humid forests of Madagascar, i.e., Antananarivo, Mandraka, Andasibe and Ranomafana. It is also absent from Mayotte or other islands of the Western Indian Ocean. Females do not exhibit a sex-limited color dimorphism.

Discussion

Prior to our study, the twenty-three Afrotropical species of the '*D. montium* species group' were classified under four complexes: '*D. bakoue* species complex' (7 spp.), '*D. bocqueti* species complex' (3 spp.), '*D. nikananu* species complex' (4 spp.) and '*D. megapyga* species complex' Lachaise & Chassagnard, 2001 (3 spp.), with six species not classified under any complex (Yassin 2018). Our results increased the number of Afrotropical species to twenty-five and the number of complexes to six by splitting the '*D. bakoue* species complex' into three complexes: '*D. seguyi* species complex' (4 spp.), '*D. tsacasi* species complex' (2 spp.) and '*D. vulcana* species complex' (2 spp.). Seven African species are not included in any complex since the status of *D. bakoue* is still unclear.

In drosophilid taxonomy, species complexes usually refer to morphologically similar species with partial reproductive isolation. Therefore, these complexes have a significant importance in studying the genetic

basis of speciation. Rafael (1984) tested reproductive isolation between four species (*D. bakoue* from Cameroon, *D. malagassya*, *D. tsacasi* and *D. vulcana*). None of these species are considered here to belong to the same complex, and only reciprocal crosses between *D. bakoue* from Cameroon (which may not be related to the true *D. bakoue*, see above) and *D. malagassya* produced sterile F1 males and fertile F1 females. Kopp (2016) crossed a strain of *D. bakoue* from the island of São Tomé and *D. nikananu* Burla 1954 ('*D. nikananu* species complex') each with *D. tsacasi* ('*D. tsacasi* species complex'), *D. bocqueti*, *D. burlai* and *D. chauvacae* ('*D. bocqueti* species complex') and *D. diplacantha* (unclassified) and no cross produced sterile F1 males and fertile F1 females. On the other hand, David *et al.* (2014) showed that sterile F1 males and fertile F1 females were produced when crosses were undertaken between species within the '*D. bocqueti* species complex' and the '*D. seguyi* species complex'. Further genetic analyses should be done within the new complexes defined here.

Although crossability provides strong support for delimiting species, its measure under laboratory conditions is sometimes problematic and, in many cases, may be practically unfeasible. For example, it has long been thought that *D. teissieri* Tsacas, 1971 and *D. yakuba* Burla, 1954 could not cross in the laboratory in spite of molecular evidence for mitochondrial DNA introgression in natural populations, until the recent discovery of a geographically restricted "hybrid zone" (Cooper *et al.* 2018). The estimation of genetic divergence between mitochondrial and nuclear genomes could, however, provide a proxy of the degree of reproductive isolation in nature. For example, we detect very low mitochondrial divergence in species of the '*D. seguyi* species complex' that are nuclearly divergent (Fig. 2) and partially reproductively isolated (David *et al.* 2014). Similarly, low mitogenomic divergence was found between *D. vulcana* and *D. mylenae* sp. nov. in spite of a high degree of nuclear genome divergence and a level of morphological distinction that is comparable to other bona fide *Drosophila* sister species in the '*D. montium* species group' such as species of the '*D. seguyi* species complex' (this study) or the '*D. auraria* species complex' (Watada *et al.* 2011). In an analysis of nearly seventy species of *Drosophila*, we have previously shown that mitochondrial DNA fails to distinguish closely-related species in 23% of the cases, most likely due cytological introgression events (Yassin *et al.* 2010). Further application of genome-wide analyses in systematics will definitively improve our ability to delimit closely-related species in the future.

Our study also doubled the number of identified species of the '*D. montium* species group' from the Western Indian Ocean islands. Lachaise *et al.* (1996) reported three species of the '*D. montium* species group' from these islands: *D.* cf. *bocqueti* in Comoros and Madagascar, *D. chauvacae* in Comoros, *D. kikkawai* Burla 1954 in Madagascar, Réunion and Mauritius, and *D. malagassya* in Madagascar. Yassin *et al.* (2012) did not find any species of the '*D. montium* species group' in any of the four Scattered Islands surrounding Madagascar (Europa, Juan de Nova, Glorioso and Tromelin). However, in the island of Mayotte, David *et al.* (2014) reported two species, *D. chauvacae* and *D. malagassya*. We found that these two species were *D. bocqueti* and *D. seguyi*, respectively. We reported two additional species from Madagascar, *D. mylenae* sp. nov. and *D. chocolata* sp. nov., which along with *D. malagassya*, increase the number of endemic Malagasy species to three. We also reported the presence in Madagascar of *D. curta*, a species that was only known from Malawi. Future research should consider other islands of the Comoros archipelago and other localities in Madagascar in order to draw a more complete picture of the evolution of the species of the '*D. montium* species group' in this region.

Acknowledgements

We are much grateful to Jean David (CNRS) for having maintained and provided the strains analyzed here as well as to an anonymous reviewer for their insightful comments on the manuscript. We also thank Sacha Berna and the Atelier d'Iconographie Scientifique (UMS2700 MNHN CNRS) for helping in the genitalia drawings, and Géraldine Toutirais from the Plateau technique de Microscopie Electronique du Muséum national d'Histoire naturelle for her help in taking the photomicrographs. Permissions for

collection in Madagascar were kindly obtained from the Malagasy Ministère de l'Environnement, de l'Ecologie et des Forêts (MEEF).

References

Bächli G., Vilela C.R., Andersson Escher S. & Saura A. 2004. *The Drosophilidae (Diptera) of Fennoscandia and Denmark*. Brill Publishers, Leiden.

Bock I.R. & Wheeler M.R. 1972. The *Drosophila melanogaster* species group. *The University of Texas Publication* 7213: 1–102.

Chassagnard M.-T., Tsacas L. & Lachaise D. 1997. Drosophilidae (Diptera) of Malawi. *Annals of the Natal Museum* 38: 61–131.

Chen C.-C., Watada M., Miyake H., Katoh T.K., Sun Z., Li Y.-F., Ritchie M.G. &. Wen S.-Y. 2013. Courtship patterns in the *Drosophila montium* species subgroup: repeated loss of precopulatory courtship? *Zoological Science* 30: 1056–1062. https://doi.org/10.2108/zsj.30.1056

Cooper B.S., Sedghifar A., Nash W.T., Comeault A.A. & Matute D.R. 2018. A maladaptive combination of traits contributes to the maintenance of a *Drosophila* hybrid zone. *Current Biology* 28: 2940–2947. https://doi.org/10.1371/journal.pbio.2000234

Da Lage J.-L., Kergoat G.J., Maczkowiak F., Silvain J.-F., Cariou M.-L. & Lachaise D. 2007. A phylogeny of Drosophilidae using the *Amyrel* gene: questioning the *Drosophila melanogaster* species group boundaries. *Journal of Zoological Systematics and Evolutionary Research* 45: 47–63. https://doi.org/10.1111/j.1439-0469.2006.00389.x

David J.R., Yassin A., Gidaszewski N. & Debat V. 2014. Drosophilids (Diptera) from Mayotte island: an annotated list of species collected in 2013 and comments on the colonisation of Indian Ocean Islands. *Annales de la Société entomologique de France* 50: 336–342. https://doi.org/10.1080/00379271.2014.938548

Graber H. 1957. Afrikanische Drosophiliden als Blütenbesucher. *Zoologische Jahrbucher* 85: 305–316.

Kopp A. 2016. Strong reproductive isolation among African species of the *Drosophila montium* subgroup. *Drosophila Information Service* 99: 33–35.

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

Lachaise D. 1971. Répartition du complexe *D. seguyi* (Dipt. Drosophilidae) au contact "savane-galerie", dans une savane préforestière de Côte-d'Ivoire. *Comptes Rendus des Séances de l'Académie des Sciences* 237: 1623–1624.

Lachaise D. 1979. *Spéciation, Coévolution et Adaptation des Populations de Drosophilidés en Afrique Tropicale.* PhD Thesis, *University of Paris VI, France*.

Lachaise D., Harry M. & Solignac M. 1996. Affinités biogéographiques des Drosophilidae de Madagascar et des îles de l'Océan Indien. *In*: Lourenco W.R. (ed.) *Biogéographie de Madagascar*: 467–478. ORSTOM, Paris.

Li Y.-F., Wen S.-Y., Kawai K., Gao J.-J., Hu Y.-G., Segawa R. & Toda M.J. 2012. DNA barcoding and molecular phylogeny of *Drosophila lini* and its sibling species. *International Journal of Evolutionary Biology* 2012: 1–9. https://doi.org/10.1155/2012/329434

O'Grady P. & DeSalle R. 2008. Out of Hawaii: the origin and biogeography of the genus *Scaptomyza* (Diptera: Drosophilidae). *Biology Letters* 4: 195–199. https://doi.org/10.1098/rsbl.2007.0575

Ohnishi S. & Watanabe T.K. 1984. Systematics of the *Drosophila montium* species subgroup: a biochemical approach. *Zoological Science* 1: 801–807.

Okada T., Asada N. & Kawanaka K. 1988. A result of drosophilid survey in Tanzania. *African Study Monographs* 8: 159–163.

Prigent S.R., Suwalski A. & Veuille M. 2017. Connecting systematic and ecological studies using DNA barcoding in a population survey of Drosophilidae (Diptera) from Mt Oku (Cameroon). *European Journal of Taxonomy* 287: 1–20. https://doi.org/10.5852/ejt.2017.287

Rafael V. 1984. Relations interspécifiques dans le nouveau complexe africain de *Drosophila bakoue* du groupe *melanogaster*, sous-groupe *montium* (Diptera, Drosophilidae). *Bulletin de la Société zoologique de France* 109: 179–189.

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Séguy E. 1938. Mission scientifique de l'Omo. Diptera. I. Nematocera et Brachycera. *Mémoires du Muséum national d'Histoire naturelle, Paris* 8: 319–380.

Takada H., Woodruff R.C. & Thompson J.N. 1990. Collections of Drosophilidae (Diptera) in Kenya, with description of a new species of Dettopsomyia. *Entomological News* 101: 246–255.

Toda MJ. 2018. *DrosWLD a database on taxonomy of Drosophilidae*. Available from: https://bioinfo.museum.hokudai.ac.jp/db/index.php [accessed 24 May 2019]

Tsacas L. 1979. Contribution des données africaines à la compréhension de la biogéographie et de l'évolution du sous-genre *Drosophila* (*Sophophora*) Sturtevant (Diptera, Drosophilidae). *Comptes Rendus Séances de la Société de Biogéographie* 480: 29–51.

Tsacas L. 1984. Nouvelles données sur la biogéographie et l'évolution du groupe *Drosophila melanogaster* en Afrique. Description de six nouvelles espèces (Diptera, Drosophilidae). *Annales de la Société entomologique de France* 20: 419–438.

Tsacas L. & Chassagnard M.-T. 1992. L'identité de *Drosophila* (*Sophophora*) *vulcana* Graber (Diptera, Drosophilidae). *Bulletin de la Société entomologique de France* 96: 427–432.

Tsacas L. & Lachaise D. 1974. Quatre nouvelles espèces de la Côte-d'Ivoire du genre *Drosophila*, groupe *melanogaster*, et discussion de l'origine du sous-groupe *melanogaster* (Diptera: Drosophilidae). *Annales de l'Université d'Abidjan* 7: 193–211.

Tsacas L. & Rafael V. 1982. *Drosophila malagassya* nouvelle espèce malgache du groupe *melanogaster*, sous-groupe *montium* (Diptera, Drosophilidae). *Revue Française d'Entomologie* 4: 86–90.

Turissini D.A., McGirr J.A., Patel S.S., David J.R. & Matute D.R. 2018. The rate of evolution of postmating-prezygotic reproductive isolation in *Drosophila*. *Molecular Biology and Evolution* 35: 312–334. https://doi.org/10.1093/molbev/msx271

Watada M., Matsumoto M., Kondo M. & Kimura M. T. 2011. Taxonomic study of the *Drosophila auraria* species complex (Diptera: Drosophilidae) with description of a new species. *Entomological Science* 14: 392–398. https://doi.org/10.1111/j.1479-8298.2011.00461.x

Yassin A. 2013. Phylogenetic classification of the Drosophilidae Rondani (Diptera): the role of morphology in the postgenomic era. *Systematic Entomology* 38: 349–364. https://doi.org/10.1111/j.1365-3113.2012.00665.x

Yassin A. 2018. Phylogenetic biogeography and classification of the *Drosophila montium* species group (Diptera: Drosophilidae). *Annales de la Société entomologique de France* 54: 167–175. https://doi.org/10.1080/00379271.2018.1447853

Yassin A. & Orgogozo V. 2013. Coevolution between male and female genitalia in the *Drosophila melanogaster* species subgroup. *PLoS One* 8: e57158. https://doi.org/10.1371/journal.pone.0057158

Yassin A., Markow T.A., Narechania A., O'Grady P.M. & DeSalle R. 2010. The genus *Drosophila* as a model for testing tree- and character-based methods of species identification using DNA barcoding. *Molecular Phylogenetics and Evolution* 57: 509–517. https://doi.org/10.1016/j.ympev.2010.08.020

Yassin A., Gidaszewski N., Albert B., Hivert J., David J.R., Orgogozo V. & Debat V. 2012. The Drosophilidae (Diptera) of the Scattered Islands, with the description of a novel association with *Leptadenia madagascariensis* Decne. (Apocynaceae). *Fly* 6: 298–302. https://doi.org/10.4161/fly.21583

Yassin A., Delaney E.K., Reddiex A.J., Seher T.D., Bastide H., Appleton N.C., Lack J.B., David J.R., Chenoweth S.F., Pool J.E. & Kopp A. 2016. The *pdm3* locus is a hotspot for recurrent evolution of female-limited color dimorphism in *Drosophila*. *Current Biology* 26: 2412–2422. https://doi.org/10.1016/j.cub.2016.07.016

Zhang Z., Inomata N., Cariou M.-L., Lage J.-L. & Yamazaki T. 2003. Phylogeny and the evolution of the Amylase multigenes in the *Drosophila montium* species subgroup. *Journal of Molecular Evolution* 56: 121–130. https://doi.org/10.1007/s00239-002-2384-3

Manuscript received: 8 October 2018
Manuscript accepted: 9 April 2019
Published on: 18 June 2019
Topic editor: Gavin Broad
Desk editor: Alejandro Quintanar