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Field collections reveal that São Tomé is the Afrotropical island with the highest diversity of drosophilid flies (Diptera: Drosophilidae)

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Summary. The Drosophilid fauna has been less investigated in the Atlantic Afrotropical islands than in the Indian Ocean. Located about 250 km from the continent, the volcanic island of São Tomé has been colonized mostly by natural means, probably by the wind, since the emergence of the island about 15 million years ago, and presumably also by anthropogenic transportation of invasive and domestic species. To date, 37 different Drosophilid species have been mentioned from São Tomé. The present work extends this list to 80 species. The genera *Zygothrica, Phorticella* and *Hypselothyrea* are newly recorded from the island. Among these 80 species, only 12 are putatively introduced by human activities, suggesting the preponderance of natural arrivals. Compared to other islands, São Tomé harbours a high diversity of drosophilids. At least 14 species are supposed to be endemic. Future molecular comparisons between the island flies and their continental relatives will probably help to identify other endemic species. The high diversity observed in São Tomé is certainly due to the large size of the island, and to the presence of vast natural altitudinal forests offering a variety of possible habitats. Further collections are likely to lead to an increase of the species list. From now, São Tomé island appears as an excellent laboratory for studying the ecology and evolution of the *Drosophila* model.

Résumé. Des collectes de terrain révèlent que São Tomé est l'île afrotropicale avec la plus grande diversité de drosophiles (Diptères : Drosophilidae). La faune de Drosophilidae a été moins étudiée dans les îles afrotropicales de l'Atlantique que dans l'océan Indien. Située à environ 250 km du continent, l'île volcanique de São Tomé a été colonisée principalement de façon naturelle, probablement à l'aide du vent, depuis l'émergence de l'île il y a environ 15 millions d'années, et par le transport supposé d'espèces domestiques et invasives par l'activité humaine. Jusqu'à présent, 37 espèces de Drosophilidae étaient mentionnées à São Tomé. Le présent travail accroît cette liste à 80 espèces. Les genres *Zygothrica, Phorticella* et *Hypselothyrea* sont nouvellement cités de l'île. Parmi ces 80 espèces, seulement 12 pourraient avoir été introduites par les activités humaines, révélant la prépondérance des colonisations naturelles. Comparée à d'autres îles, São Tomé abrite une plus grande diversité de drosophiles. Au moins 14 espèces sont supposées être endémiques. Il est probable que d'autres espèces endémiques seront identifiées lorsque les études moléculaires permettront de comparer les individus de São Tomé avec les espèces apparentées du continent africain. La diversité observée à São Tomé est certainement due à la grande taille de l'île et à la présence d'une vaste forêt d'altitude offrant une grande variété d'habitats. De futures collectes permettront d'accroître la liste d'espèces. L'île de São Tomé apparaît comme un excellent territoire pour l'étude de l'écologie et de l'évolution du modèle drosophile.

Keywords: *Drosophila*; *Zaprionus*; altitudinal distribution; seasonal variation; endemism

Volcanic, oceanic islands are an exciting field for evolutionary studies, with a frequent observation of new endemic species (MacArthur & Wilson 1967; Whittaker et al. 2008; Borregaard et al. 2017). In the Drosophilidae family, the most fascinating example concerns the Hawaiian flies, with a single radiation having produced almost 1000 different species (Hardy & Kaneshiro 1981; Magnacca et al. 2008). In the south-eastern part of the African continent, the Indian ocean islands have been quite extensively investigated, with the observation of many endemic species, often related to Madagascar fauna (Lemeunier et al. 1997; Cariou et al. 2009; David et al. 2014). On the Western side of Africa, the island of São Tomé is located in the Guinea Gulf, about 250 km off the continent. The climate of São Tomé is tropical and humid, with only a 2.5°C difference between the warmest month (March) and the coldest one (July) (climate-data.org). Open habitats associated with human presence

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dominate at the sea level and at low elevation, while mist natural forests are found at higher altitudes. This island is particularly famous among drosophilists due to the presence of the endemic species *Drosophila santomea* Lachaise & Harry, 2000, a close relative of *D. melanogaster* Meigen, 1830, discovered in 1998 and described in 2000 (Lachaise et al. 2000).

The first and most extensive survey of the drosophilid fauna of São Tomé is that from Rocha Pité (1993) who collected more than 36,000 individuals. Most of her collection sites were distributed at low altitudes in agricultural habitats. Among the 44 taxa listed, only 27 were clearly identified at the species level. No new taxon was described, leaving many interesting species unidentified. Later, only 10 species were added (Chassagnard & Lachaise 2000; Lachaise et al. 2000; Lachaise & Chassagnard 2002; Yassin 2008; Yassin & David 2010; Rego et al. 2016). In consequence the extant list of São Tomé species in the TaxoDros database (Bächli 2019) mentions 37 valid species names, among which only three are endemic [*D. santomea* (Lachaise et al. 2000) and two *Zaprionus* species, *Z. tsacasi* Yassin, 2008 (Yassin 2008) and *Z. santomensis* Yassin & David, 2010 (Yassin & David 2010)], all living in the altitudinal forests.

We carried out two field trips to São Tomé in 2015 and 2016. Although they were originally designed to collect *D. santomea* and its sister species *D. yakuba* Burla, 1954, it appeared interesting to make a broader survey and collect flies in diverse habitats, mostly at altitudes higher than 1000 m above the sea level (m asl). More than 25,000 collected flies were counted and identified. Several of them were able to produce laboratory strains, and the total number of recognized species corresponds to more than 70 different taxa. Our results point to the presence of several new species (to be described in a further study) and to many interesting faunistic, ecological, and biogeographical data. Here, we provide the list of the collected taxa and we also present detailed comments on a few interesting taxa as well as some general comments on the island colonization and fly ecology.

Material and methods

Dates and sampling sites

The first expedition (M.L. and J.R.D.) took place on 9–17 February 2015. The second one involved six persons (A.A., M.L., A.M.-V., O.N., V.C.-O. and J.R.D.) and took place on 16–23 September 2016. In both cases, we chose as base camp the facilities of the Bom Sucesso Botanical Garden, on the north-east part of the island, located at 1150 m asl and close to the Obô Natural Park (Parque Natural Obô de São Tomé). Bom Sucesso is connected to the city of São Tomé (at sea level, 0°20′06.6″N 6°43′43.7″E), by a steep road of about 25 km, allowing fly collections at different altitudinal ranges. Most collections were done in Bom Sucesso (0°17′19.8″N 6°36′44.2″E), in the Obô Natural Park and along the road. Collection sites on the East Coast correspond to city areas between 0 and 500 m asl, cultivated areas between 500 and 1200 m asl, and mist forest at higher altitudes. During the second expedition, some field collections were also made on the West Coast of the island, which is characterized by a drier climate and steep slopes. We were able to collect there, between Neves and Santa Catarina (0°15′59.1″N 6°28′28.7″E) at low altitude and near Manuel Morais (0°17′57.73″N 6°33′23.57″E) at 500–700 m asl.

Collection methods and species identification

We mostly used the classical method for *Drosophila* collections, of plastic bottles baited with fermenting bananas (*Musa paradisiaca*), and occasionally along the road from Bom Successo to Monté Café with other common fruits in São Tomé such as jackfruit (*Artocarpus heterophyllus*), breadfruit (*A. altilis*), mango (*Mangifera indica*) and passion fruit (*Passiflora edulis*). At each location, about 2–3 bottle traps were attached to tree branches under the shade and left for 3–4 days. Flies in bottles were collected daily with an aspirator and transferred in empty vials. We also collected by sweeping with a net over some possible natural resources, such as fermenting fruits, fungi, flowers and decaying plant materials or by using an aspirator. In the field laboratory flies were slightly anaesthetized with triethylamine vapours (T0886; Merck, Kenilworth, NJ, USA) and examined under a stereo-microscope. Females of some species were isolated in small culture vials with instant *Drosophila* medium (Formula 4–24, Carolina Biological Supply Company, Burlington, NC, USA) to establish isofemale lines, which were used to perform crosses in order to confirm sexual isolation or production of sterile hybrids. The other specimens were counted and preserved in ethanol except some too numerous specimens of well-identified invasive species that were discarded. Preserved specimens were brought to the laboratory and checked under higher magnification in order to better distinguish the species according to external morphology. Identification was based on comparison with published data, mainly from publications of Burla, Chassagnard, Lachaise and Tsacas (Burla 1954; Tsacas & Lachaise 1981; Tsacas 1984, 1990, 2003; Chassagnard 1988; Tsacas et al. 1988; Tsacas & Chassagnard 1990, 1994, 1999, 2000; Chassagnard & Tsacas 1993; Chassagnard et al. 1997; Chassagnard & Lachaise 2000) and with specimens held by the Muséum national d'Histoire naturelle (Paris, MNHN). Most of the species can be identified by the external morphology, sometimes according to the external periphallic organs and in limited cases dissection of the genitalia has been necessary. At present the specimens are kept in 70% ethanol in a cold room in the dark at MNHN. Some of them are going to be used in molecular genetics studies according to research interests of the different authors. Others are going to be deposited in the main collection of MNHN.

Results

List and abundance of collected species

The results are summarized in Table 1 and some species are illustrated in Figures 1 and 2, following the same order as in the Table 1. For the sake of clarity, the data are expressed according to altitude and year of collection. Results obtained on the east side of the island (São Tomé city and Bom Sucesso) and the west coast are also distinguished. The list of species is organized in alphabetical order according to classical taxonomy, starting with the

Table 1. List of the species collected in São Tomé with number of specimens according to sex, year, locality and altitude; the total number of specimens (N), the total number of females (Nf) and males (Nm), and the total number of specimens collected in 2015 (N2015) and 2016 (N2016) are indicated for each species; *species frequently transported by man; **species potentially endemic.

					February 2015								September 2016								
						East side								East side						West side	
Taxonomy	Species	N	Nf	Nm	N2015	0–500m	500-1000m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	N2016	0–500m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	0–500m	500–1000m
Subfamily DROSOPHILINAE																					
Genus *Chymomyza*																					
	C. bambara Burla, 1954	3	2	1	0								3	–	–	–	–	–	–	–	3
	C. mafu Burla, 1954	13	4	9	0								13	–	–	–	–	–	–	–	13
Genus *Dettopsomyia*																					
	D. nigrovittata (Malloch, 1924)	19	11	8	0								19	–	7	11	–	–	–	–	1
Genus *Drosophila*																					
Subgenus *Dorsilopha*																					
	D. busckii Coquillett, 1901*	18	6	8	0								18	4	7	7	–	–	–	–	–
Subgenus *Drosophila*																					
acrostigma complex	*Drosophila* n. sp. 1**	9	6	3	7	–	1	4	1	1	–	–	2	–	1	1	–	–	–	–	–
« *dyaramankana* group »	*D. dyaramankana* Burla, 1954	2	1	1	0								2	–	1	–	–	–	–	–	1
immigrans group	*D. nasuta* Lamb, 1914*	1802	626	998	212	49	22	107	28	2	–	1	1590	92	801	554	8	29	21	7	51
loiciana complex	*D. pruinosa* Duda, 1940	385	141	211	12	–	–	12	–	–	–	–	373	33	304	26	–	2	–	–	6
	Drosophila n. sp. 2**	2	1	1	1	–	–	–	–	–	1	–	1	–	–	–	–	1	–	–	–
repleta group	*D. repleta* Wollaston, 1858*	5	1	2	2	–	–	2	–	–	–	–	3	1	2	–	–	–	–	–	–
virilis group	*D. virilis* Sturtevant, 1916*	2	1	0	1	–	–	1	–	–	–	–	1	1	–	–	–	–	–	–	–
Subgenus *Sophophora*																					
ananassae group	*D. ananassae* Doleschall, 1858*	135	12	24	0								135	123	6	6	–	–	–	–	–
	D. malerkotliana Parshad & Paika, 1965*	1826	663	336	778	288	234	228	2	2	–	–	1048	112	80	29	1	2	–	90	733
fima group	*D. alladian* Burla, 1954	43	24	19	1	–	–	–	–	1	–	–	42	–	24	5	–	–	–	–	13
	D. fima Burla, 1954	84	26	42	21	–	16	2	–	2	–	–	63	–	26	19	–	–	–	3	15
	D. kulango Burla, 1954	3	2	1	3	–	–	–	–	3	–	–	0								
	Drosophila n. sp. 3**	23	8	15	2	–	–	1	–	1	–	–	21	–	14	2	–	–	–	–	5
melanogaster group	*D. melanogaster* Meigen, 1830*	262	57	87	24	8	–	16	–	–	–	–	238	116	115	7	–	–	–	–	–

(*continued*)

Table 1. (*Continued*).

					February 2015								September 2016								
						East side								East side						West side	
Taxonomy	Species	N	Nf	Nm	N2015	0–500m	500-1000m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	N2016	0–500m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	0–500m	500–1000m
	D. santomea Lachaise & Harry, 2000**	545	125	419	256	–	1	31	96	43	33	–	289	–	214	63	–	7	–	1	2
	D. simulans Sturtevant, 1919*	80	35	33	15	–	–	14	–	–	–	–	65	1	41	21	–	–	–	–	1
	D. yakuba Burla, 1954	2263	1120	995	138	10	69	49	7	–	–	–	2125	29	878	492	1	1	–	30	688
montium group	*D. bakoue* Tsacas & Lachaise, 1974	69	3	56	34	–	–	18	2	10	2	1	35	–	25	4	–	6	–	–	–
	D. aff. *bocqueti* n. sp.**	2486	388	2098	687	–	2	4	63	328	119	67	1799	1	781	555	81	315	20	–	13
	D. aff. *chauvacae* n. sp.**	792	246	472	129	–	31	81	4	13	–	–	663	20	425	189	–	4	–	1	22
	D. aff. *diplacantha* n. sp.**	1	0	1	0								1	–	1	–	–	–	–	–	–
	D. aff. *greeni* n. sp.**	882	279	592	5	–	–	3	2	–	–	–	877	15	439	108	–	–	–	–	312
	D. nikananu Burla, 1954	827	288	485	143	–	26	61	31	1	1	–	684	31	396	147	2	12	–	1	92
	Drosophila sp. 4	5	2	3	0								5	–	1	–	–	4	–	–	–
	Drosophila sp. 5	1	0	1	0								1	–	1	–	–	–	–	–	–
Unplaced species																					
	Drosophila sp. 6	123	54	69	10	–	–	7	3	–	–	–	113	–	67	45	–	–	–	–	1
	Drosophila sp. 7	55	17	38	3	–	–	2	–	–	1	–	52	–	43	9	–	–	–	–	–
	Drosophila sp. 8	1	1	0	1	–	–	1	–	–	–	–	0								
Genus *Hirtodrosophila*																					
	H. suma (Burla, 1954)	1	0	1	1	–	–	1	–	–	–	–	0								
	H. aff. *suma* n. sp.**	8	6	2	2	–	–	2	–	–	–	–	6	–	6	–	–	–	–	–	–
	Hirtodrosophila sp. 1	95	68	27	64	–	–	42	15	–	–	7	31	–	28	–	–	2	–	–	1
	Hirtodrosophila sp. 2	31	5	26	0								31	–	4	–	–	27	–	–	–
	Hirtodrosophila sp. 3	19	7	12	0								19	–	18	–	–	1	–	–	–
	Hirtodrosophila sp. 4	145	79	66	0								145	–	145	–	–	–	–	–	–
	Hirtodrosophila sp. 5	3	1	2	0								3	–	–	–	–	3	–	–	–
Genus *Hypselothyrea*																					
	Hypselothyrea n. sp.**	2	0	2	0								2	–	2	–	–	–	–	–	–
Genus *Microdrosophila*																					
	M. korogo Burla, 1954	7	2	5	7	–	–	7	–	–	–	–	0								
Genus *Mycodrosophila*																					
	M. aff. *fracticosta*	16	2	14	0								16	–	16	–	–	–	–	–	–
	M. aff. *kabakolo* n. sp.**	9	5	4	0								9	–	9	–	–	–	–	–	–

(*continued*)

Table 1. (*Continued*).

Taxonomy	Species	N	Nf	Nm	February 2015								September 2016								
						East side								East side						West side	
					N2015	0–500m	500-1000m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	N2016	0–500m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	0–500m	500–1000m
	M. matilei Chassagnard & Lachaise, 2000	5	1	4	0								5	–	5	–	–	–	–	–	–
	M. aff. *nigerrima*	4	1	3	2	–	–	–	–	–	–	2	2	–	2	–	–	–	–	–	–
	M. aff. *suluma*	1	0	1	0								1	–	1	–	–	–	–	–	–
Genus *Phorticella*																					
	Phorticella sp.	1	0	1	1	–	–	1	–	–	–	–	0								
Genus *Scaptodrosophila*																					
	S. caliginosa (Lamb, 1914)	76	44	32	37	–	–	37	–	–	–	–	39	–	39	–	–	–	–	–	–
	S. latifasciaeformis (Duda, 1940)*	2463	917	989	386	80	175	123	4	2	1	–	2077	68	1398	195	1	3	–	350	62
	S. mokonfim (Burla, 1954)	7	3	4	3	–	–	3	–	–	–	–	4	–	1	–	–	–	–	–	3
	S. uebe (Burla, 1954)	6	1	5	2	–	–	2	–	–	–	–	4	–	1	–	–	1	–	–	2
	Scaptodrosophila sp. 1	23	11	12	13	–	–	13	–	–	–	–	10	–	10	–	–	–	–	–	–
	Scaptodrosophila sp. 2	569	146	423	360	–	–	55	–	46	121	133	209	11	71	17	–	–	–	11	99
Genus *Scaptomyza*																					
	Scaptomyza sp. 1	1	1	0	0								1	–	1	–	–	–	–	–	–
	Scaptomyza sp. 2	3	2	1	1	–	–	1	–	–	–	–	2	–	1	1	–	–	–	–	–
Genus *Zaprionus*																					
armatus group	*Z. campestris* Chassagnard, 1988	1	1	0	1	–	–	1	–	–	–	–	0								
inermis group	*Z.* aff. *arduus*	9	3	6	7	–	5	2	–	–	–	–	2	–	–	–	–	–	–	–	2
	Z. aff. *badyi*	1	0	1	0								1	–	1	–	–	–	–	–	–
	Z. ghesquierei Collart, 1937	210	112	98	1	–	–	1	–	–	–	–	209	–	206	3	–	–	–	–	–
	Z. inermis Collart, 1937	2237	634	1554	558	–	49	339	8	2	–	–	1679	–	1161	505	–	8	–	–	2
	Z. tsacasi Yassin, 2008**	1069	415	654	35	–	–	–	5	24	1	5	1034	–	868	84	12	35	5	–	11
	Z. tuberculatus Malloch, 1932*	2496	1024	996	593	13	60	443	1	9	1	1	1903	440	780	192	–	5	–	16	467
neglectus group	*Z. neglectus* Collart, 1937	46	8	38	16	–	–	15	–	–	–	–	30	–	30	–	–	–	–	–	–
vittiger group	*Z.* aff. *camerounensis* n. sp.**	72	33	39	42	–	–	–	12	12	10	8	30	–	9	8	–	13	–	–	–
	Z. davidi Chassagnard & Tsacas, 1993	50	24	26	14	–	–	8	–	1	1	–	36	–	26	7	–	1	–	1	–

(*continued*)

Table 1. (*Continued*).

Taxonomy	Species	N	Nf	Nm	February 2015								September 2016									
						East side								East side						West side		
					N2015	0–500m	500–1000m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	N2016	0–500m	1000–1200m	1200–1300m	1300–1400m	1400–1500m	1500–1600m	0–500m	500–1000m
	Z. indianus Gupta, 1970*	1775	631	675	344	33	64	187	4	3	–	2	1431	459	780	100	–	–	–	6	84
	Z. megalorchis Chassagnard & Tsacas, 1993	51	18	33	9	–	–	8	–	–	–	–	42	–	32	4	–	–	–	–	5
	Z. aff. *proximus*	523	240	226	244	–	35	39	16	60	69	2	279	2	62	77	44	66	16	–	1
	Z. santomensis Yassin & David, 2010**	314	116	198	226	–	–	–	12	51	61	99	88	–	31	37	1	12	–	–	–
Genus *Zygothrica*																					
	Zygothrica sp.	5	1	4	1	–	–	1	–	–	–	–	4	–	–	–	–	4	–	–	–
Subfamily STEGANINAE																					
Genus *Cacoxenus*																					
	Cacoxenus sp.	1	0	1	0								1	–	1	–	–	–	–	–	–
Genus *Leucophenga*																					
	Leucophenga sp. 1	1	1	0	0								1	–	1	–	–	–	–	–	–
	Leucophenga sp. 2	1	0	1	0								1	–	–	–	–	–	–	–	1
	Leucophenga sp. 3	1	0	1	0								1	–	–	–	–	–	–	–	1
	SUM	25124	8713	13215	5455	481	790	1975	316	617	422	328	19669	1559	10446	3530	151	564	62	517	2713

Drosophilinae subfamily. For some species, the total number of individuals is different to the sum of males and females because some flies counted in the field and not preserved were not sexed. Similarly, the sum of the specimens at the different altitudes may be different to the number of collected flies in the year because the labelling of some samples did not allow us to determine the exact site of collection, and therefore the altitude.

The species list comprises 74 taxa, distributed into 14 genera, two in the Steganinae subfamily and 12 in the Drosophilinae. The most diverse genera are *Drosophila* (29 spp.), *Zaprionus* (14 spp.), *Hirtodrosophila* (7 spp.), *Scaptodrosophila* (6 spp.) and *Mycodrosophila* (5 spp.). Three genera newly reported in São Tomé, represented each by a single species, are especially interesting. They are *Zygothrica* (Figure 2F), a common genus of South America which is known in Africa by only two species, *Phorticella* (Figure 2B), a genus particularly known from Asia, which has never been reported from the African continent and *Hypselothyrea* (Figure 1G), represented by only two known species in the Afrotropical region and the species of São Tomé being a third one. Nine species are particularly abundant, with >1000 specimens while 33 species are represented by 10 individuals or less in the collection.

Several species reported by Rocha Pité (1993) were not collected during this study. They are *Drosophila hirtipes* Lamb, 1914 (cited as *D. iri* Burla, 1954) and several *Leucophenga* species, but most of them were represented by only few specimens. Thus, about 80 taxa are known from the island, but several need to be identified to the species level. Among them, only 12 species including *D. hirtipes* are known to be frequently transported by anthropogenic activities (indicated by an asterisk in Table 1). This suggests that colonization of São Tomé by Drosophilidae has been done mainly by natural means, as it has been proposed for other volcanic islands, like Galapagos, by aerial transport (actively by flight and/or passively by wind), by marine transport (on rafts of vegetation), and by transport via other animals (phoresy) (Peck 2006).

Detailed comments on some taxa

The *Drosophila melanogaster* species subgroup. In São Tomé, the *D. melanogaster* species subgroup is represented by four species, including the two cosmopolitan species, *D. melanogaster* and *D. simulans* Sturtevant, 1919. The large number of *D. melanogaster* individuals found at the sea level contrasts with the scarcity of *D. simulans* flies, which seem restricted to altitudinal places. Of course, seasonal variations of abundance may exist.

The endemic *D. santomea* seems to be localized in submontane rain forest and evergreen cloud forest up to

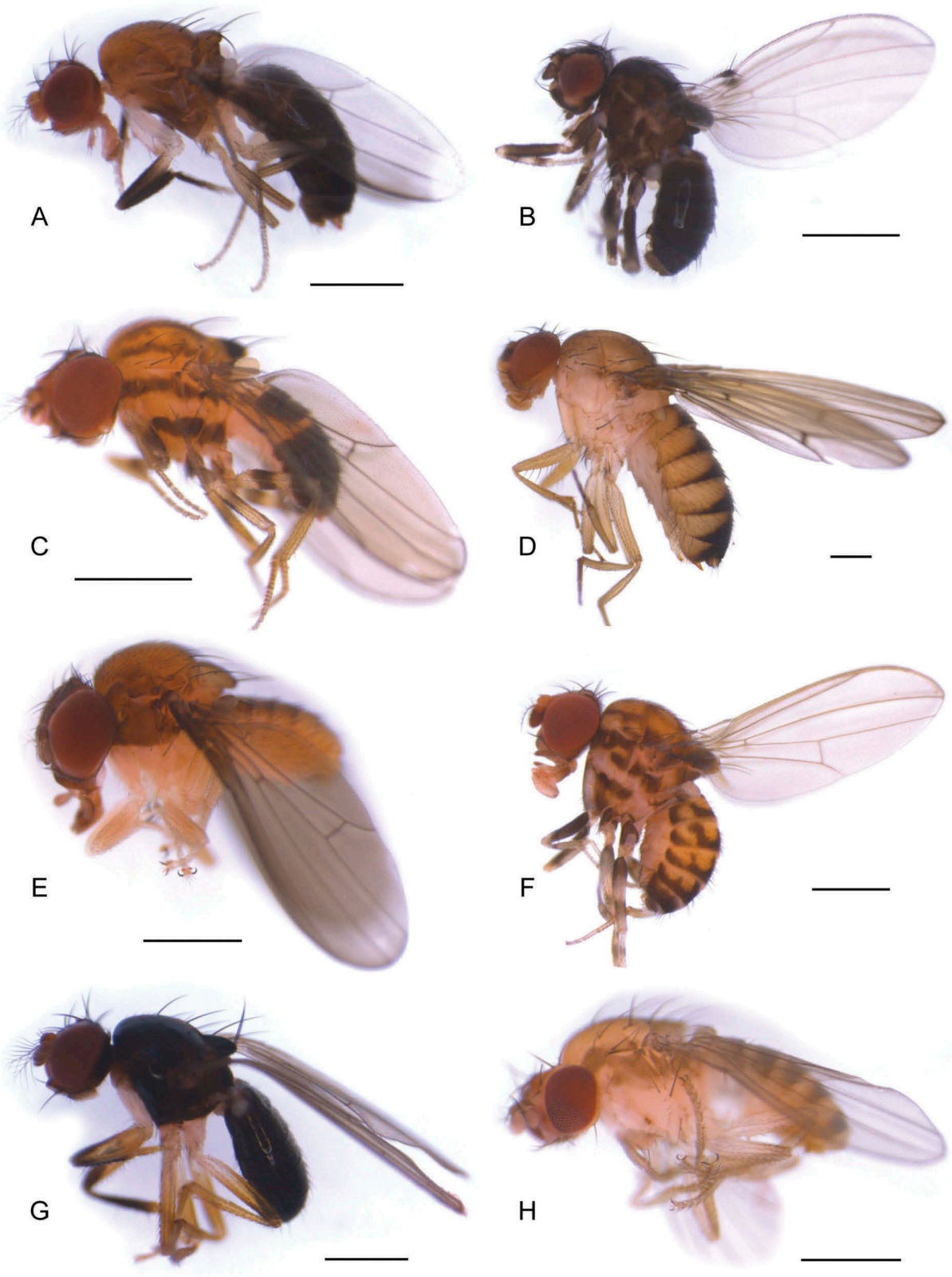

Figure 1. Morphological diversity of Drosophilidae among São Tomé species. Physical colours may be modified as specimens are in ethanol. Scale bar is 0.5 mm. **A**, *Chymomyza bambara*; **B**, *Dettopsomyia nigrovittata*; **C**, *Drosophila dyaramankana*; **D**, *Drosophila* n. sp. 2 (*loiciana* complex); **E**, *Hirtodrosophila* aff. *suma* n. sp.; **F**, *Hirtodrosophila* sp. 4; **G**, *Hypselothyrea* n. sp.; **H**, *Microdrosophila korogo.*

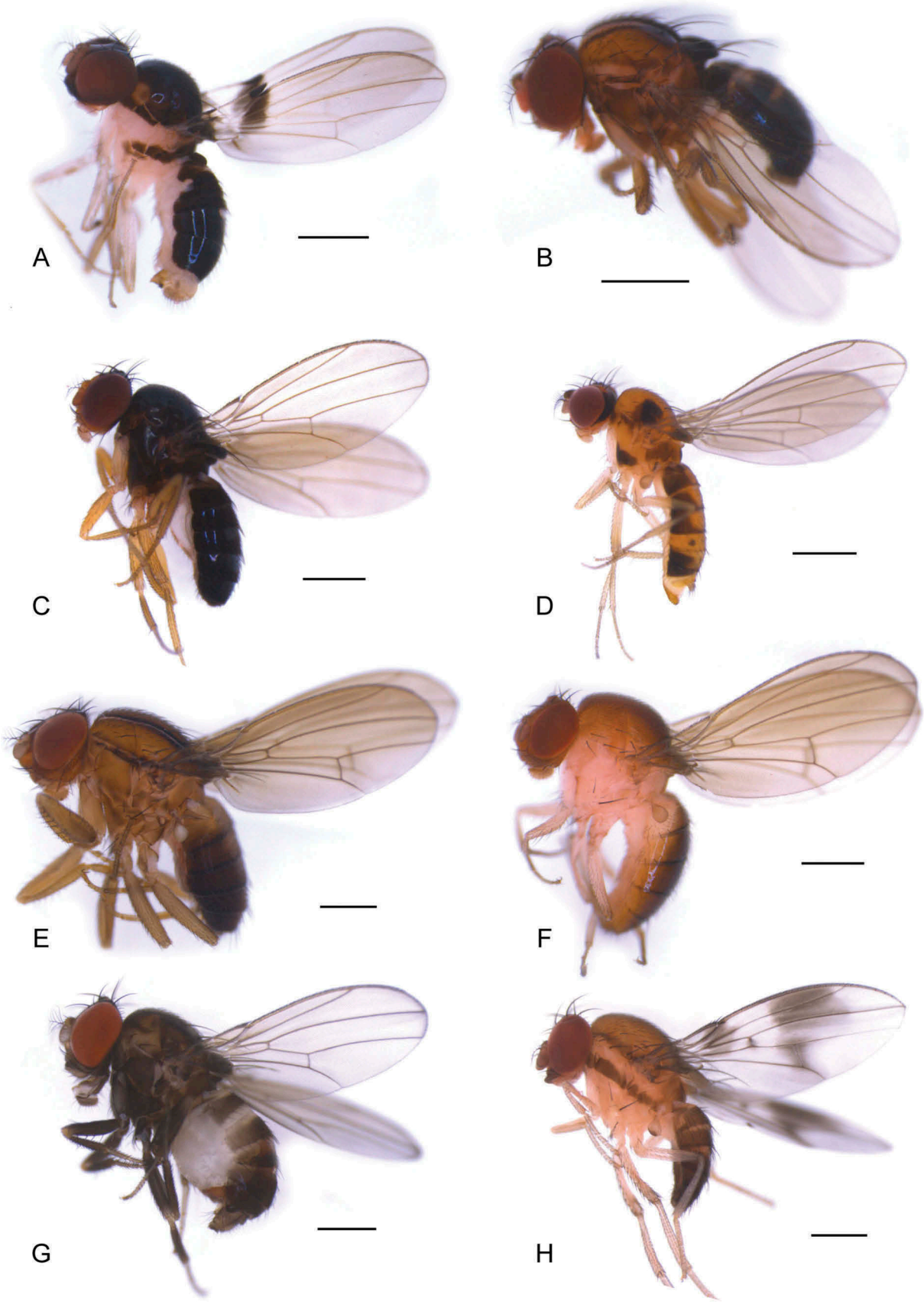

Figure 2. Morphological diversity of Drosophilidae among São Tomé species. Physical colours may be modified as specimens are in ethanol. Scale bar is 0.5 mm. **A**, *Mycodrosophila* aff. *kabakolo* n. sp.; **B**, *Phorticella* sp.; **C**, *Scaptodrosophila uebe*; **D**, *Scaptomyza* sp. 1; **E**, *Zaprionus* aff. *camerounensis* n. sp.; **F**, *Zygothrica* sp.; **G**, *Cacoxenus* sp.; **H**, *Leucophenga* sp. 1.

1450 m asl. Its sibling *D. yakuba* was collected from 0 to 1300 m asl, in agreement with previous observations (Matute 2015). In the laboratory, the two species can hybridize easily (Lachaise et al. 2000). During the 2016 expedition, 16 *D. santomea* females were isolated for establishing isofemale lines. One of them gave two kinds of progeny (light and dark), suggesting that this female was a hybrid that previously mated with a *D. yakuba* male. Male hybrids have been previously detected in São Tomé based on morphological traits and genetic analysis (Llopart et al. 2005; Turissini & Matute 2017), so that hybridization, and likely genomic introgressions appear to occur in São Tomé. On the African continent, another species belonging to the *yakuba* complex, *D. teissieri* Tsacas, 1971, is widespread and fairly abundant (Lachaise et al. 1988). Its absence from São Tomé is quite surprising if we consider the large number of successful colonizations of the island by drosophilids.

The *Drosophila montium* species group. The *D. montium* species group is the largest species group in the *Sophophora* subgenus, with about 100 described species, all from the Old World (Da Lage et al. 2007; Yassin 2018). In tropical Africa, more than 20 species are known, all belonging to the same clade, with a unique ancestral lineage which arrived on the continent around 10 million years ago (Yassin 2018). Rocha Pité (1993) mentioned four known species of this group in São Tomé, namely *D. bocqueti* Tsacas & Lachaise, 1974, *D. chauvacae* Tsacas, 1984, *D. greeni* Bock & Wheeler, 1972 and *D. nikananu* Burla, 1954, and at least three additional unnamed species. We found a similar number of species, with the addition of *D. bakoue* Tsacas & Lachaise, 1974 (observed only at high altitude). Apart from *D. nikananu*, the identity of the other species is ambiguous and would require DNA sequence analysis.

In the *bocqueti* complex we could distinguish two types of males according to the abdominal pigmentation. Males with the black abdominal tip restricted to the sixth tergite resemble *D. bocqueti* while males with the apical black area extended to the fifth tergite are more similar to *D. chauvacae*. In traps, the first type of male was mostly associated with females with entirely light-yellow abdomen (light, L) while the second type was associated with darker females (dark, D). For convenience, females were connected to males according to this criterion (Table 1). However, this approach is likely to be biased as several species of the *D. montium* species group are known to harbour both L and D females (Yassin et al. 2016b).

We have collected many such females in altitudinal forests, especially at Bom Sucesso (1150 m asl). In 2015, 20 isofemale lines were successfully established. All the collected females had a black abdomen (D). Surprisingly, two third of these lines produced a dimorphic progeny, i.e. a mixture of D and L females. This polymorphism is restricted to females. Subsequent crosses showed that the L allele is dominant, so that the L phenotype corresponds to genotypes LD or LL, while the D females are recessive DD. The conclusion is that all collected females were homozygous DD, while the males in nature should harbour the two alleles L and D. In 2016, we also collected females but several of them had a light abdomen, i.e. of genotype LD or LL. So, the relative abundance of each genotype observed in 2015 was not confirmed the following year, revealing a possible seasonal variability of allelic frequencies. *D. chauvacae* was described from Grande Comore in the Indian Ocean (Tsacas 1984), and we have a strain from another island of the Comoros (Mayotte) that we consider as real *D. chauvacae* (David et al. 2014). We crossed this strain with the one from São Tomé: the cross turned out to be difficult but indeed possible, producing fertile females and 100% sterile males. We thus consider that the flies we collected in São Tomé do not correspond to *D. chauvacae* and therefore we listed this taxon as *D.* aff. *chauvacae* n. sp. (Table 1).

We made an interesting observation regarding the other member of the *bocqueti* complex. In 2015, the females collected at higher altitudes (1200 to 1500 m asl) had a yellow abdomen. Isofemale lines from these yellow flies were very difficult to cross with the *D.* aff. *chauvacae* n. sp. from Bom Sucesso, and F1 males were fully sterile. This species appears to prefer higher altitudes, but in 2016, we found several females of that species in Bom Sucesso, again revealing some temporal variations in distribution. On the continent, *D. bocqueti* is abundantly collected at low altitude. Therefore, its altitudinal distribution in São Tomé suggests that it is another related species designated as *D.* aff. *bocqueti* n. sp. (Table 1).

Drosophila greeni is an abundant species on African mainland, and it has been mentioned in São Tomé fauna (Rocha Pité 1993). In 2015, we found very few individuals similar to that species, but in 2016, it was collected quite abundantly in several places (Table 1). The overall shape and pigmentation are typical of *D. greeni*, both sexes look alike, but an examination of the male genitalia revealed several clear-cut morphological differences between the São Tomé samples and the typical form, indicating that the São Tomé population is probably a new species.

Finally, the *D. montium* species group is represented in São Tomé by another new species, which is represented by only one specimen in Table 1. In the collections of the laboratory at Gif-sur-Yvette, we found a series of pinned flies of the same species, labelled as a new species, collected in April 2005 by D. Lachaise and M. Harry in Monte Cafe,

at around 1000 m asl. The dissection of a male revealed that this species (to be described) is a close relative of *D. diplacantha* Tsacas & David, 1978. Several traits, including a dark pigmentation of the female abdomen, justify the status of new species.

Altogether, the *D. montium* species group on São Tomé comprises now six well-characterized species, four of them remaining to be described. Since all the six species have each a relative on the continent, we hypothesize that they correspond to independent colonizations of the island.

The *Drosophila fima* species group. The *D. fima* species group is an African clade specialized on figs, and comprising more than 20 species (Tsacas & Lachaise 1981; Lachaise & Tsacas 1983). In a review paper, Lachaise and Chassagnard (2002) mentioned three species from São Tomé. All three species were collected at sea level. *Drosophila kulango* Burla, 1954 was also collected at 650 m asl and *D. fima* Burla, 1954 at 1153 m asl in Bom Sucesso while *D. alladian* Burla, 1954 was reported at different altitudes up to 1440 m asl. We found the same three species in our survey, at different altitudes (Table 1) and an additional species that might be a new one. During the two expeditions, we observed only one fig tree in fructification, while the *D. fima* species group flies were widespread: the specialization is certainly not absolute.

The *Zaprionus* genus (type subgenus). This subgenus endemic to tropical Africa comprises about 50 different species (Yassin & David 2010) distributed in four groups of species. Seven *Zaprionus* species from São Tomé are mentioned in the Taxodros database (Bächli 2019). We found 14 *Zaprionus* species, including members of the four groups (Table 1). Three species are endemic of São Tomé, namely *Z. santomensis, Z. tsacasi* (Yassin 2008; Yassin & David 2010), and another one (Figure 2F) that remains to be described but which is close to *Z. camerounensis* Chassagnard & Tsacas, 1993. Almost all species live in altitudinal forest with the exception of *Z. indianus* Gupta, 1970 and *Z. tuberculatus* Malloch, 1932, which are also found at sea level. These two species are well known for their invasive capacity and may have been introduced by human activity, while most others probably came by natural means.

***Drosophila pruinosa* Duda, 1940.** This species of the *loiciana* complex, widespread on African mainland, was also reported from São Tomé (Rocha Pité 1993). Larval breeding sites are generally difficult to find; however, we made an interesting ecological observation in the Bom Sucesso Botanical Garden. We found numerous larvae on rotting avocado fruits (*Persea americana*). The larvae and pupae were kept until adult flies emerged. We obtained a total of 38 individuals identified as one individual of *Zaprionus ghesquierei* Collart, 1937, two individuals of *D.* aff. *greeni* n. sp. and 35 individuals of *D. pruinosa*. Species that were collected abundantly using banana baits did not emerge from avocado, which led us to think that *D. pruinosa* might exhibit a preference for this fatty non-sweet fruit, although it was also collected with banana. It is worth noting that avocado is not native of Africa and that the natural breeding substrate of *D. pruinosa* is therefore still unknown. A second species of the same complex, apparently a new one (Figure 1D), was also collected in São Tomé at higher altitude (Table 1).

Species collected on flowers

Adaptation to live and develop in fresh flowers has occurred many times in the drosophilid family (Brncic 1983; Tsacas et al. 1988; Fu et al. 2016). In São Tomé we found on flowers five species from three genera. The first one is *Scaptodrosophila caliginosa* (Lamb, 1914), which is a small, black species, common on mainland and islands and displaying invasive capacities (David & Tsacas 1981). Secondly, we found two *Zaprionus* species, *Z. campestris* Chassagnard, 1988 and *Z. neglectus* Collart, 1937, inside *Brugmansia* flowers (previously known as *Datura*). The flower breeding species of *Zaprionus* are impossible to grow under usual *Drosophila* laboratory conditions (Yassin & David 2010). Our observations suggest that *Z. neglectus* is not a strict flower breeding species and that it can be reared in the laboratory. Finally, we also found *Hirtodrosophila suma* (Burla, 1954) and a related species on cultivated *Brugmansia. H. suma* is an interesting species which raises many biological questions (David et al. 2011). It has a very low reproductive potential (about 1 egg per day) but seems to be able to use a large diversity of flowers, differing in their size and colour. It is found in the huge flowers of cultivated *Brugmansia* in São Tomé (this study) and in the flowers of *Ipomoea* and *Crinum* in Congo and Mauritius Island (David et al. 2011). In spite of a very low population size and specialized ecology, it is a widespread species, both on African mainland and oceanic islands. In São Tomé it seems that two related species, *H. suma* and *H.* aff. *suma* (Figure 1E), differing in their colour pattern, co-exist in the flowers. It is very likely that the species collected on flowers in São Tomé are also breeding on that substrate, because this has been observed elsewhere for the same species or related ones (Lachaise & Tsacas 1983; David et al. 2011).

Species collected on fungi

Decaying fungi are a well-known breeding site for many drosophilids (Courtney et al. 1990; Jaenike & James 1991; Toda et al. 1999). In previous records, only one identified mycophagous species was mentioned from São Tomé, *Mycodrosophila matilei* Chassagnard & Lachaise, 2000 (Chassagnard & Lachaise 2000). We directly netted or aspirated a number of mycophagous specimens from fungi growing on tree trunks. Identified species belong to

genera *Mycodrosophila, Zygothrica* and *Hirtodrosophila* (Table 1). The *Zygothrica* species found in São Tomé resemble the species of the *Zygothrica samoaensis* species group (Prigent & Toda 2006). The various mycophagous taxa are clearly distinguished by their morphology and pigmentation (Figure 1), but they have rarely been identified to the species level since very little African material is available and it has generally not been properly preserved for molecular analysis. This is a difficult task which deserves further studies on African mycophagous species and especially in molecular diagnostics.

Discussion

Continental Africa, with its huge geographic diversity, is known to harbour more than 450 described species of Drosophilidae (Brake & Bächli 2008). The Taxodros database recorded 37 species from São Tomé (Bächli 2019). After two field surveys we distinguished 74 distinct species (Table 1). Our findings are largely, but not completely, similar to previous information (Rocha Pité 1993; Bächli 2019). For example, Rocha Pité (1993) mentioned eight species of *Leucophenga*, which were well identified, while we collected only three species. The combination of our collections and TaxoDros information amounts to a total of 80 species, revealing an obvious species richness of the island. Further surveys will certainly reveal additional species. According to the TaxoDros database (Bächli 2019), among the islands surrounding the African continent, Madagascar (686 times the size of São Tomé island) has the highest species diversity of Drosophilidae with 70 species. Mauritius and La Réunion, two islands of the Indian ocean with a size more similar to São Tomé island, have less than 40 species each. With 80 species, São Tomé is therefore harbouring the highest insular diversity of Drosophilidae known for the Afrotropical region.

Natural colonization, endemism and alien species

Endemic species seem to exist in all groups which have been thoroughly analysed. The evolution of an endemic species is likely to require a relatively long evolutionary time, on the order of several hundreds of thousands of years or more (Obbard et al. 2012). The age of São Tomé is about 15 million years (Lee et al. 1994). So, all endemic species are expected to be not older than the island. Many other species are shared by the island and the continent and raise the problem of the date of introduction in São Tomé and the possibility of repeated exchanges with continental populations. Regarding the questions of the process of island colonization and speciation by reproductive isolation, we believe that the study of the fauna of São Tomé could bring some light to these issues, which have long been considered as insoluble. Molecular genomics now provides some opportunities. For example, *D. yakuba* on Mayotte island corresponds to an original subspecies adapted to a toxic fruit (Yassin 2017). Genomic studies of this population, compared to mainland ones, suggested that Mayotte was colonized 27,000 years ago, but still some exchanges existed with the mainland (Yassin et al. 2016a). Such an analysis could be applied to many species from São Tomé, provided that sufficiently large samples are available. Species introduced by human activity are becoming frequent in recent years and are usually very common due to their invasive capacity (Acurio et al. 2010; Markow et al. 2014; Kremmer et al. 2017; Werner 2018). We did not find a specific pattern of invasiveness for the 12 alien species collected in São Tomé. While we recorded a high frequency of *D. malerkotliana* Parshad & Paika, 1965, *S. latifasciaeformis* (Duda, 1940), *Z. tuberculatus* and *Z. indianus*, which are categorized as invasive pest worldwide, other alien species were found at very low frequency in collections (Table 1).

Altitudinal distribution

A general ecological observation for drosophilids is that species distribution and richness are strongly correlated to altitude (Prigent et al. 2013). Low altitude insular habitats harbour mainly invasive, domestic species, while higher altitude ones exhibit a far more diverse fauna (David & Tsacas 1975). This is correlated to the fact that altitudinal sites are generally covered with tropical, humid forest, a habitat favourable to drosophilids. Such is the case of São Tomé, and it is clear from Table 1 that most species are found in high places. The best example is certainly the *Zaprionus* genus, with only two generalist invasive species at sea level and 12 additional species, including three endemics, in higher locations.

Temporal variations

São Tomé, located under the Equator, benefits from a very stable climate. The West Coast is drier than the East Coast, but in a given place we expect a fairly stable temperature. During the two expeditions in February 2015 and September 2016 the climatic conditions were similar at the Bom Sucesso Botanical Garden, with an average daytime temperature around 20°C and abundant rainfalls every day. Fly collections, however, revealed major differences. For example, 30 species were collected only in one year (most were in very low abundance). Two other examples are provided by *D.* aff. *greeni* and *Z. ghesquierei*, that were very poorly collected in 2015, but were abundant in 2016. Variations in the altitudinal distribution and in genotypic frequencies were also observed for *D.* aff. *chauvacae* and *D.* aff. *bocqueti* (see above). Such variations are difficult to

explain, although they are often observed (Wolda 1988). We are aware of another analogous situation on Mayotte island. The two cosmopolitan species, *D. simulans* and *D. melanogaster*, have been collected regularly at the end of the year especially in November (Montchamp-Moreau, pers. comm.). During two more recent entomological collections made in January 2013 and April 2016, these two species were almost absent, although several thousands of drosophilids were collected (David et al. 2014 and unpublished results).

Larval breeding sites

Drosophilids exhibit a huge ecological diversity. Most species are saprophagous, living on rotting material and digesting yeast and bacteria (Begon 1982; Shorrocks 1982). These rotting materials can be very diverse, including a great variety of plants, flowers, fungi and fruits. The classical way for collecting *Drosophila* species is by using traps with fermenting banana as bait. Such traps can efficiently attract generalist species. In Bom Sucesso, such a trap could attract more than 100 flies just in a few hours, even when traps were set a few meters apart. This shows that the site is harbouring a huge population of diverse drosophilids. However, larval breeding sites were practically impossible to identify. Most of the attracted species, especially those belonging to the subgenera *Sophophora* and *Zaprionus*, can be reared under laboratory conditions and are considered as fruit breeders, but we found practically no rotting fruits. This is a general problem in studies of drosophilids as an evolutionary model. We have obtained a lot of knowledge on the genetic and phenotypic characteristics of natural populations, but their ecology is still poorly known and understood (Markow 2015).

Conclusions and prospects

Oceanic islands have long been a paradigm for a variety of biological sciences, ranging from Evolutionary Biology to Ecology (MacArthur & Wilson 1967; Whittaker et al. 2008; Kier et al. 2009; Borregaard et al. 2017). São Tomé is very suitable for such investigations for the following reasons.

(1) The island is about 250 km from the Coast of Gabon, so that migrations are likely to be quite regular, but not very frequent, allowing endemic speciation.
(2) A large part of the island is covered by natural rainforest favourable to migrant individuals.
(3) The Equatorial location of the island reduces the amplitude of seasonal variations, making the climate suitable for drosophilid flies all year round.
(4) Natural, preserved habitats exist on a large part of the island, allowing the persistence of numerous species.

Among many taxonomic groups, the Drosophilidae family is particularly interesting since a large amount of information has been gathered in many parts of the world. Our study has increased the number of extant species of São Tomé from 37 to 80, even if many of them are still not identified at the species level. Among these species, we estimate that about 15% are invasive species, whose introduction on the island was probably linked to human activities. Endemic species, which evolved there in isolation for several hundreds of thousands of years without a significant rate of exchange with the continent, can be identified, in a first approximation, as the species whose morphological characteristics have never been found in other sampled areas from Africa. Our study suggests that São Tomé harbours 14 endemic species, most of them being recognized as new species here (Table 1). This number is likely to be an underestimate as it does not take into account the species not yet identified and possible cryptic species (with no morphological trait distinguishing them from their sister species). Crosses with the continental populations and DNA sequence data would be helpful to examine further the rate of endemism in São Tomé.

A general question is, for each species, what is the amount of genomic divergence between continental and island populations? Do they correspond to geographic races/subspecies? It is clear that the drosophilid model is most appropriate for answering such questions, because of the large number of worldwide investigations already engaged in genomic studies of *Drosophila* (DrosEU, Dros-RTEC). The second point of interest concerns the ecology of natural populations, especially under humid tropical climate. We have pointed out repeatedly how the natural breeding sites are difficult to identify. This would require persistent studies all year round on the island. Finally, during our collections, we have noticed a very acute interest of the Sao Tomeans for the diversity of flies and of other taxonomic groups living on their land. Future taxonomic prospects on this island should include a citizen science programme.

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