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Long-legged flies as bio-indicators in site quality assessment of mangroves on Pulau Ubin (Singapore) (Insecta: Diptera: Dolichopodidae)

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Abstract. The present study is part of a long-term survey of the insect fauna of mangroves in Singapore. A three-month survey was done in 2019 of nine back mangrove sites and a patch of coastal forest on the western side of Pulau Ubin (OBS). It showed that these undisturbed old mangroves are amongst the most species-diverse mangroves in Singapore, based on the diversity of long-legged flies. Microhabitat preferences of the species allow indication of changes in habitat. Malaise traps operated during a three-month sampling give an idea about dominant species but not about presence of rare species. This highlights the need for longer in-depth surveys. Pulau Ubin is home to a large number of unique species, never been recorded previously in Singapore. In addition, 46% of the long-legged fly species have not yet been formally named, underlining the taxonomic impediment.

Key words. bio-indicators, mangrove, Southeast Asia, new species

INTRODUCTION

The small island of Pulau Ubin, located a few kilometres off the northeast coast of Singapore, has undergone tremendous floral and faunal changes over the last two centuries. There used to be intensive quarrying, farming, and fish farming activity on the island, with mangroves transformed into fishponds, but in the last 50 years, all industrial activity was halted and the island was left to undergo a natural restoration process, with only soft recreation activities such as hiking and cycling currently allowed (National Parks Board, 2021).

The history of the vegetation of Singapore is sketched out by Corlett (1997) and Sim et al. (1992), and their studies suggest that all the land vegetation on Pulau Ubin is secondary forest. A part of this secondary forest is comprised of the unique Adinandra belukar community, which is a type of anthropogenic heath forest on a very acidic and poor soil, dominated by the tree *Adinandra dumosa* (Theaceae). Other types of secondary forest have developed on abandoned rubber plantations and farmland. However, large mangrove forests are still present on Pulau Ubin. Some mangroves are quite pristine, while others consist mostly of secondary growth, being the remains of former fishponds.

In the present survey conducted on Pulau Ubin, we selected long-legged flies or dolichopodid flies (Diptera, Dolichopodidae) as the study target, as previous studies have shown that they prove to be excellent indicators for evaluating mangrove quality (Grootaert et al., 2019; Yeo et al., 2021). Most species in the mangroves are exclusively found in this marine habitat. The larvae live in the soil or in decaying wood and feed on other insect larvae such as sand fly larvae (Grichanov & Brooks, 2017). The adults are predators as well, and each genus of this very diverse fly family represents a particular guild, having a specific microhabitat and niche. The diversity of these niches and microhabitats provides a good reflection of the maturity of the mangrove. In addition, these flies are very diverse and quite abundant, allowing statistical analysis of the data. Most species can also be found year-round. Sampling with standardised techniques such as Malaise traps is quite easy, and specimens conserved in pure 70% ethanol can easily be barcoded (Wong et al., 2014; Meier et al., 2016; Srivathsan et al., 2018, 2019; Wang et al., 2018).

A systematic survey of Pulau Ubin with Malaise traps was started in 2005. First, only the mangroves at Chek Jawa were surveyed and this resulted in the description of several new species of dolichopodid flies (Zhang et al., 2007, 2008) with Chek Jawa as type locality: *Paraclius digitatus* Zhang, Yang & Grootaert, 2007, *Hercostomus lanceolatus* Zhang, Yang & Grootaert, 2008, *Hercostomus limosus* Zhang, Yang & Grootaert, 2008 and *Hercostomus meieri* Zhang, Yang & Grootaert, 2008. Several more species of related Dolichopodidae families have also the back mangroves at Chek Jawa as type locality: two species of the dance fly genus *Empis* (*Coptophlebia*) (Daugeron & Grootaert, 2005) and 13 species of Hybotidae

(Shamshev & Grootaert, 2007; Grootaert & Shamshev, 2012, 2015). In 2012, a two-year survey was performed in Chek Jawa (Grootaert & Shamshev, 2012), but other parts of Pulau Ubin were only surveyed in 2016, 2018, and 2019, resulting in a better coverage of the diversity of the island (Fig 1).

The 2016 survey conducted in the mangroves of the western part of Pulau Ubin, revealed that several unknown species were present in that area, generally known as Outward Bound Singapore (OBS) due to the presence of an Outward Bound campground there. Hence, three mangrove basins in the OBS area were selected for a more in-depth survey in 2019:

- Basin 1. Sungei Tock Kew site, a large creek system with mudflats;
- Basin 2. Sungei Tereis, a little southeast of Sungei Tock Kew with thin mud layer areas on sandy soil;
- Basin 3. Sungei Sempit, a small mangrove in the northwest of Pulau Ubin with a mix of sandy soils and small muddy patches.

In addition, a small patch of rare coastal forest next to Sungei Sempit (Basin 3) at the base of Bukit Sempit was sampled to investigate possible interactions with the mangrove.

In the present paper, we give a preliminary assessment of the mangroves in the OBS area based on the long-legged flies.

MATERIAL & METHODS

Standardised sampling with Malaise traps started in 2005, with two traps operating for two months in the back mangrove of Chek Jawa (PU1, PU2). In 2012, a two-year survey was done from April 2012 to March 2014 (MIP: Mangrove Insect Project). Once again, the back mangrove of Chek Jawa, already famous for its remarkable marine fauna, was sampled, but this time with four Malaise traps (PU1, PU2, PU3, and PU4). The samples were retrieved every two weeks. The study showed that the activity and diversity of dolichopodid flies was largest during the transition of the dry to the rainy season from March to June. High activity of flies was also noted from September to December, but this was more variable over the years. Hence, to reduce the time and cost of sampling efforts, the standardised sampling with Malaise traps was limited to three months in 2016, 2018, and 2019; i.e., the transition of the dry season to the rainy season from March until June. This period selection allowed a better comparison of the data between the years. In 2016, 2018, and 2019, weekly sampling in 10 stations was done since more traps in a single area provide more reliable data. Fig. 1A shows the locations of all the Malaise trap surveys done on Pulau Ubin from 2005 till 2019.

In 2019, sampling stations were installed in additional locations (Fig. 1B). Malaise traps were installed from 12 March 2019 until 25 June 2019 in three basins at the west point of Pulau Ubin, known as Outbound Singapore (OBS).

Basin 1: Sungei Tock Kew: back mangrove near dam.
Basin 2: Sungei Tereis: mangrove along the creek Sungei Tereis.
Basin 3: Sungei Sempit: mangrove at base of Bukit Sempit and terrestrial forest.
An overview with the details on vegetation and soil type is given in Table 1.

The Malaise traps were always installed in the intertidal zone of the mangrove so that the base of the Malaise trap was flooded by each tide.

The material was collected weekly in 70% ethanol. After collection, the ethanol was immediately partly replaced with fresh 70% ethanol to avoid dilution. All dolichopodid specimens in the samples were sorted to family level or samples were stored before sorting in a freezer at -18°C.

Databases and tools. The database "All Singa data dolis 2000 to 2019.xls" contains information on more than 300 species of long-legged flies (files at present stored in the collection of the first author of the present paper but the database will eventually be deposited in the archives of the National Parks Board and the Lee Kong Chian Natural History Museum, Singapore). It contains 11,869 records of long-legged flies that were studied in Singapore over the last 20 years. A record of a species is represented by the number of specimens of that species that were observed on a precise date at a precise observation point. In total, the database holds information on 54,384 specimens collected over the last 20 years (cf. Grootaert, 2018). These data have been used to compile the Red Data list of Singapore (unpubl.) that shows the rarity of any given species and allows for evaluation of the extinction risk of species as well.

Deposition of material. All material is deposited in the Lee Kong Chian Natural History Museum of the National University of Singapore (LKCNHM) and conserved in 70% ethanol. Photographs of nearly all the long-legged fly species observed in Singapore can be found on the website BOS (Biodiversity of Singapore) (https://singapore.biodiversity.online/).

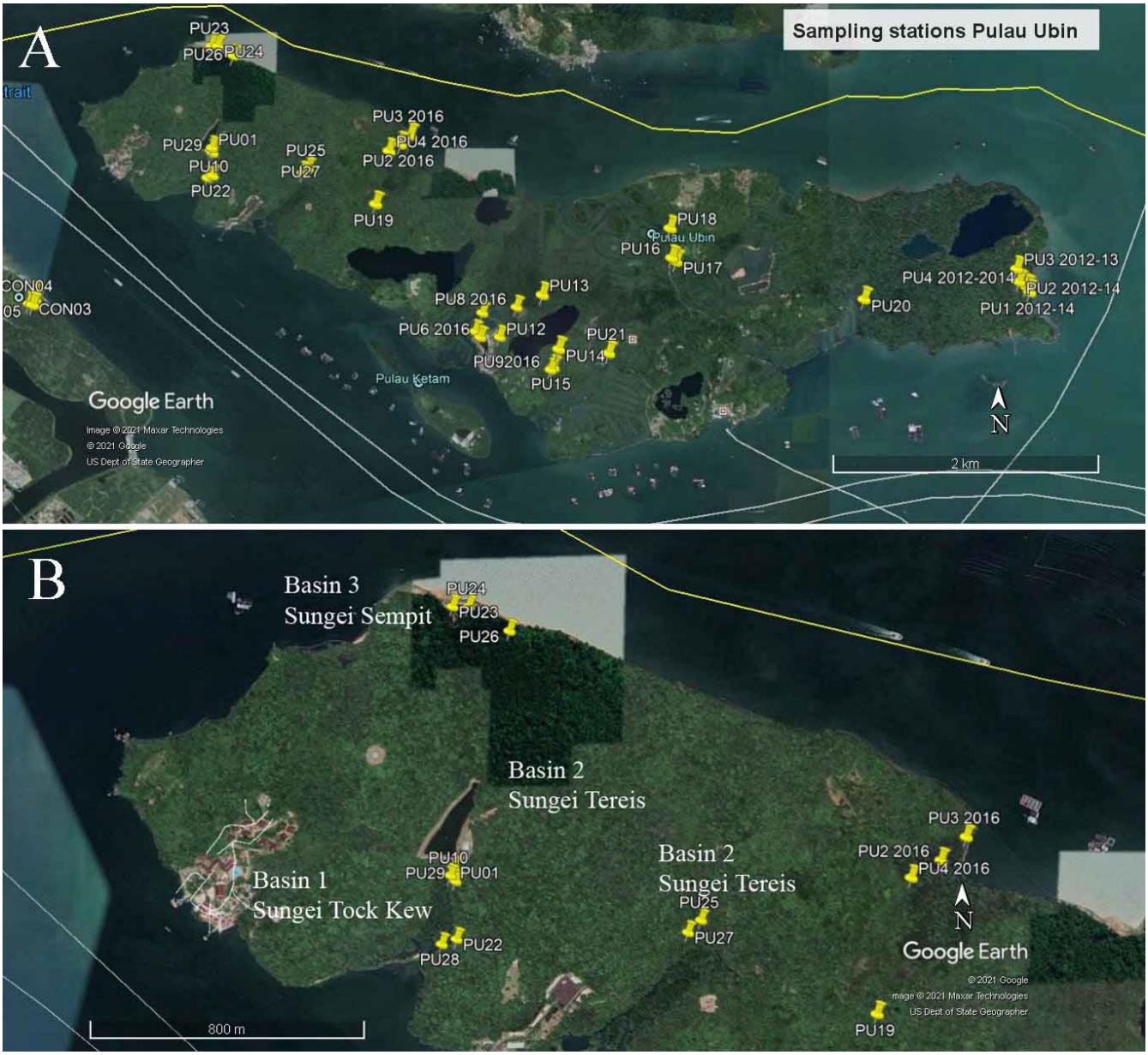


Fig. 1. Distribution of the sampling stations with Malaise traps on Pulau Ubin. A, all sites from 2005 to 2019; B, detailed position of traps in the OBS area in 2019.

Table 1. Characteristics of the sampling stations of the 2019 survey at OBS site on Pulau Ubin.

Station	Vegetation	Soil
Basin 1: Sungei Tock Kew		
PU01	pneumatophores - *Avicennia*	muddy
	kneed roots - *Bruguiera*	
	sea hibiscus, a lot of tree branches/vines	
PU10	prop roots - *Rhizophora, Bakau*	muddy, water-logged mud
PU29	prop roots - *Rhizophora, Bakau*	muddy, compacter
	mouth of creek	
PU22	sea hibiscus, many tree branches	water-logged mud
PU28	kneed roots - *Brugueria*	muddy with mud lobster mounds
Basin 2: Sungei Tereis		
PU25	kneed roots - *Brugueria*	muddy with sandy patches
PU27	kneed roots - *Brugueria*	muddy with sandy patches with big mud lobster mounds
Basin 3: Sungei Sempit		
PU23	prop roots - *Rhizophora*	muddy with sand patches
PU24	prop roots - Rhizophora, Bakau	muddy with sand patches
	mud lobster mounts	
PU26	patch of coastal forest	dry soil with leaf litter

OBSERVATIONS

Table 2 presents a list of the long-legged fly species found in the three OBS mangrove basins and the patch of coastal forest during the three-month sampling in 2019. A total number of 74 long-legged fly species were recorded. The species names marked with an asterisk * have temporary taxonomic working names. The number of specimens per species is given per station; the number of species that are exclusive in a basin is indicated as excl B1, excl B2, excl B3. The following information on each species is given:

- habitat H (B: beach; M: exclusive to mangrove; S: swamp; U: ubiquitous, known from various habitats, or blank: habitat unknown)
- microhabitat MH (LS: leaf surface, adult flies hunting for prey on the surface of leaves; SS: soil surface, adult flies foraging on the mud; TT: tree trunk, adult flies sitting and hunting for prey on tree trunks)
- number of records of the species in Singapore (RS)
- Red List status RLs (DD: data deficient is due to the paucity of old records or because the species is not well recognised; E: endangered (less than 20 records in Singapore; NE: not endangered: common species in abundant numbers in the mangroves of Singapore (more than 20 records in Singapore).

Table 2. List of species recorded in the nine mangroves and the patch of coastal forest (PU26) during the three-month sampling in 2019. H: habitat (B: beach; M: exclusive to mangrove; S: swamp; U: ubiquitous, known from various habitats, or blank: habitat unknown); microhabitat MH (LS: leaf surface, adult flies hunting for prey on the surface of leaves; SS: soil surface, adult flies foraging on the mud; TT: tree trunk, adult flies sitting and hunting for prey on tree trunks); RS: number of records of the species in Singapore; RLs: Red List status (DD: data deficient is due to the paucity of old records or that the species is not well recognised; E: endangered (less than 20 records in Singapore; NE: not endangered: common species in abundant numbers in the mangroves of Singapore (more than 20 records in Singapore).

	Basin 1. Sg Tock Kew						Basin 2. Sg Tereis			Basin 3. Sg Sempit									
	Back Mangrove						Back Mangrove			Back Mangrove			Coastal Forest						
Species	**PU01**	**PU10**	**PU29**	**PU22**	**PU28**	**Excl B1**	**PU25**	**PU27**	**Excl B2**	**PU23**	**PU24**	**excl B1**	**PU26**	**excl CF**	**B1-CF**	**Tot**	**H**	**MH**	**RS**	**RLs**
Acropsilus acroleucus *													2	x		**2**	S	SS	20	NE
Acropsilus sp	4			1						1	1		1		x	**8**	S	SS		DD
Acropsilus uncinatus *	3															**3**	S	SS	9	E
Amblypsilopus blacktrochanter*											2		1		x	**3**	M	LS	158	NE
Amblypsilopus sp											1		14		x	**15**		LS		DD
Amblypsilopus yellowantennae*													1			**1**	M	LS	21	DD
Amblypsilopus yellowcoxae*										1			2		x	**3**		LS	13	DD
Chaetogonopteron chaeturum Grootaert Meuffels	3			11							1					**15**	U	LS	96	NE
Chaetogonopteron flag*	1															**1**	S	LS	3	E
Chaetogonopteron sp	1												25		x	**26**	F	LS		DD
Chaetogonopteron spa*													1	x		**1**		LS	1	DD
Chrysosoma annulitarse Parent											8	x				**8**	M	LS	15	E
Chrysosoma cloudedwing*							1						14		x	**15**	S	LS	28	NE
Chrysotus black*	51	1		31			18	3			4		13		x	**121**		LS	241	NE
Chrysotus dot Grootaert Van de Velde	18		8	79	20		64	13		17	61		15		x	**295**	M	LS	454	NE
Chrysotus ubinensis Grootaert Van de Velde	4			6			2			1	1		2		x	**16**	M	LS	64	NE
Chrysotus prfurcatus*	17				2		3	7					15		x	**44**	M	LS	44	DD
Diaphorus blackfemora*	5	1		2	2		2			1	10					**23**	M	LS	230	NE
Diaphorus blackmidhindfemora*				1			1						6		x	**8**	M	LS	6	DD
Diaphorus blackwing*	1		1	1							1		3		x	**7**	M	LS	27	DD
Diaphorus brownhindcoxae*	1					x										**1**	F	LS	3	DD
Diaphorus mandarinus Wiedemann							1		x							**1**	F	LS	4	DD
Diaphorus noclaws*										1	10	x				**11**	M	LS	2	DD
Diaphorus prmandarinus*	1			1									9		x	**11**		LS	47	DD
Diaphorus whitering*											5					**5**	M	LS	31	DD
Eothalassius sp										2		x				**2**	B	SS	7	E
Genus unknown	3			1	1	x										**5**	M	?		DD
Hercostomus brevicornis Zhang et al.					1		7	1			9					**18**	M	SS	164	NE
Hercostomus brevidigitalis Zhang et al.	11		2	10			1			9	29					**62**	M	SS	537	NE
Hercostomus lanceolatus Zhang et al.	1		1	1	2		11			2	8					**26**	M	SS	137	NE
Hercostomus limosus Zhang et al.	1		1		3		3	1		3	3					**15**	M	SS	203	NE
Hercostomus meieri Zhang et al.				2	1		9			7	13					**32**	M	SS	263	NE
Hercostomus obtusus Zhang et al.	3		1			x										**4**	M	SS	197	NE
Hercostomus phumatus Zhang et al.	17	3		1			17	6			1					**45**	M	SS	379	NE
Hercostomus prsingaporensis*	2	1		1			1				2					**7**	M	SS	172	NE
Lichtwardtia nodulata Grootaert Tang			1										3		x	**4**	F	LS	35	NE
Mastigomyia sp							1		x							**1**	F	LS	2	DD
Medetera blackfemora*	3				1					2						**6**	F	TT	53	DD
Medetera stigma Grootaert Van de Velde										1						**1**	M	TT	1	E
Nanothinophilus hoplites Grootaert Meuffels	1						1				1					**3**	M	SS	83	NE
Neurigona angulata Meijere	1		1	10	1		1	1			1		2		x	**18**	M	TT	78	NE
Neurigona pectinata Parent				2												**2**	M	TT	16	E
Neurigona cryptica *	3			2									5		x	**10**	F	TT	3	DD
Ngirhaphium caeruleum Grootaert et al.			1													**1**	M	SS	143	NE
Ngirhaphium murphyi Evenhuis Grootaert			1	1												**2**	M	SS	21	NE
Ngirhaphium sivasothii Evenhuis Grootaert	10			2			4				1					**17**	M	SS	538	NE
Paraclius adligatoides*	1			1			1						1		1	**4**	M	SS	85	DD
Paraclius blackwing*				8			1						3		x	**12**	M	SS	13	DD
Paraclius crassatus Zhang et al.	56			2		x	2									**60**	M	SS	89	NE
Paraclius digitatus Zhang et al.	22			1	2	x	10	1		4	6					**46**	M	SS	559	NE
Paraclius serratus Zhang et al.	13				1	x	2				7					**23**	M	SS	385	NE
Paraclius singaporensis Zhang et al.				8	1		3	1		2			4		x	**19**	M	SS	72	NE
Paraclius yellowcoxae*	5	1	1										1			**8**	M	SS	8	E
Phacaspis mitis Grootaert & Meuffels	4	1	5	1	5		32	2		11	6					**67**	M	SS	521	NE
Physopyga longicauda *							2		x							**2**	M	SS	148	NE
Plagiozopelma flavipodex (Becker)													2	x		**2**	M	LS	15	NE
Plagiozopelma sp	1												4		x	**5**	F	LS	25	DD
Protomedetera uncinata Grootaert Van de Velde		1					1				1		1		x	**4**	M	LS	2	DD
Sciapodinae unknowgenus*													1	x		**1**	F	LS	2	E
Sympycnus longpp*													1	x		**1**	F	LS	34	NE
Sympycnus yellow*	4							2					1			**7**	F	LS	44	NE
Tachytrechus tessellatus Macquart											1					**1**	U	SS	8	DD
Teuchophorus limosus Grootaert	13		1	11			3						1		x	**29**	M	SS	140	NE
Teuchophorus prkrabiensis*	11		1	1	1		5	3					3		x	**25**	M	SS	16	DD
Teuchophorus tiomanensis Grootaert	19	2		4	5		7						3		x	**40**	M	SS	63	NE
Thinophilus apicatus Grootaert	1									1	1					**3**	M	SS	136	NE
Thinophilus clavatus Zhu et al.											1	x				**1**	M	SS	5	E
Thinophilus meieri Grootaert Evenhuis										2	2	x				**4**	M	SS	3	E
Thinophilus murphyi Evenhuis Grootaert											3	x				**3**	M	SS	201	NE
Thinophilus nigrilineatus Grootaert								1	x							**1**	M	SS	30	NE
Thinophilus simplex Grootaert							1			6	17	x				**24**	M	SS	636	NE
Trigonocera ubinensis Grootaert Van de Velde													1	x		**1**	M	LS	11	DD
Urodolichus singaporensis *				1									1		x	**2**	F	LS	5	DD
Urodolichus sp				2									1		x	**3**	F	LS	3	DD
Total number of specimens	**316**	**11**	**26**	**206**	**49**		**218**	**42**		**74**	**218**		**163**			**1323**				
Total number of species	**37**	**8**	**14**	**31**	**16**	**6**	**32**	**13**	**4**	**19**	**32**	**7**	**35**	**6**	**26**	**74**				
*Temporary taxonomic working name	49						34			37			35			74				

Basin 1: Sungei Tock Kew. Only the back mangrove of this basin was sampled (Fig. 1B). The basin itself is characterised by having a quite thick mud cover and the area was shaded by short trees with a rather closed canopy.

49 dolichopodid fly species were found in this basin during the three-month survey in 2019 (Table 2) which is a high number of species compared to other mangroves in Singapore. Eight species were represented by more than 20 specimens which allows robust statistical analysis, while five species are represented by doubletons and 12 species by singletons only. The very large number of singletons indicates that the sampling is not yet representative enough to get an idea of the total biodiversity of the area.

Station PU01 at the end of the back mangrove with 37 species was the most diverse site of all sites surveyed. Station PU10 had a defective trap and the trap at PU29 was placed much later to compensate for the problematic PU10 trap. PU29 had fewer species than PU01, but it also had species that were not found in the other station. The traps PU01, PU10, and PU29 sampled the back end of the mangrove at Sungei Tock Kew while station PU22 and PU28 sampled the mangrove closer to the sea.

No significant differences were found between the two areas and the typical front mangrove species belonging to the genera *Ngirhaphium* and *Phacaspis* (Fig. 2) followed the creek up to the back end. Only the robust bottom dwellers of the genus *Paraclius* (Fig. 3) were more diverse and present in large numbers in the back mangrove area (PU01) than in the area closer to the sea. The other small differences in numbers are explained by the patchiness of the occurrence of the species in that mangrove area.

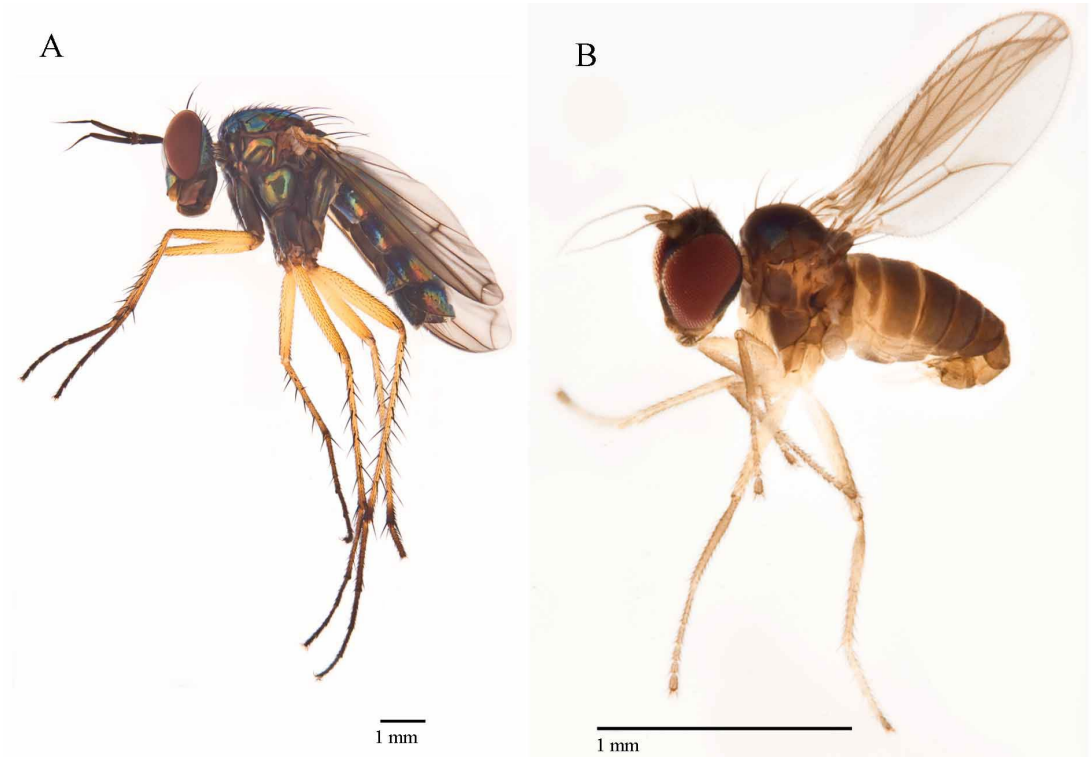


Fig. 2. The front mangrove is characterised by mud-dwelling species belonging to the large *Ngirhaphium* and the minute *Phacaspis*. A, *Ngirhaphium sivasothii* Evenhuis & Grootaert; B, *Phacaspis mitis* Grootaert & Meuffels (Photos: Jayanthi Puniamoorthy).

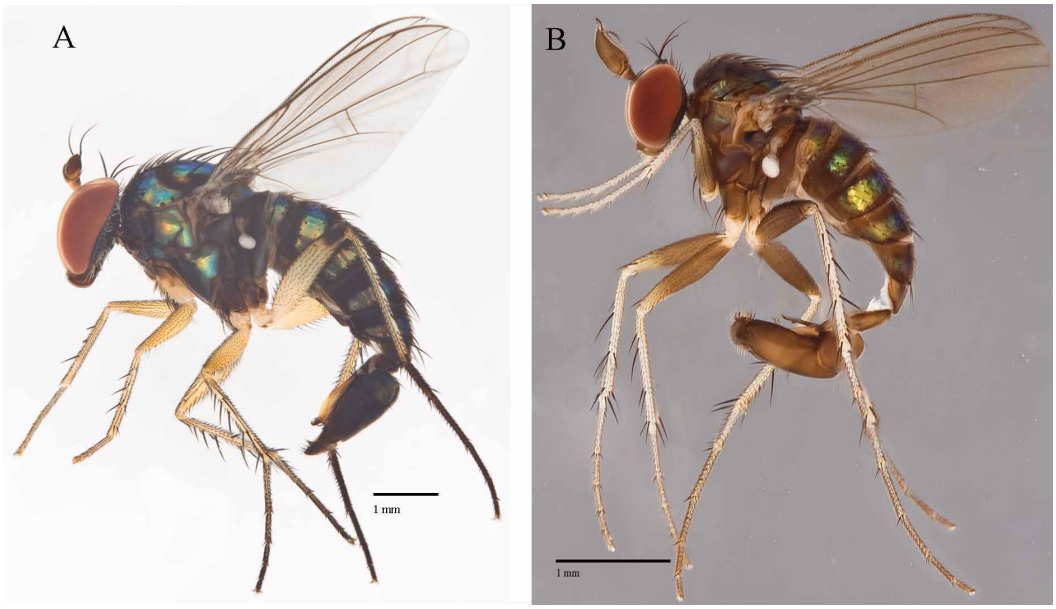


Fig. 3. Mud dwellers belonging to the genera *Paraclius* and *Hercostomus* hunt for insect larvae and other small bottom fauna in the creeks. A, *Paraclius digitatus* Zhang, Yang & Grootaert; B, *Hercostomus meieri* Zhang, Yang & Grootaert (Photos: Jayanthi Puniamoorthy).

Basin 2: Sungei Tereis. 34 species were found in Sungei Tereis (Table 2). Only four species (*Physopyga longicauda**, *Diaphorus mandarinus*, *Thinophilus nigrilineatus*, and a *Mastigomyia* sp.) were unique to this basin. Otherwise, the species diversity was very similar to Basin 1: Sungei Tock Kew.

Basin 3: Sungei Sempit, back mangrove. 37 species were recorded in the mangrove of Sungei Sempit (Table 2). Seven species were unique for this area. Most remarkable is that six *Thinophilus* species, typically found in sandy soils, were found here. Among them were the robust species *Thinophilus clavatus*, *T. meieri*, and *T. murphy* that were only observed in this mangrove (Fig. 4). In addition, an *Eothalassius* sp. typical of sandy beaches was also recorded in the mangrove and was quite abundant on the sandy beach in front of the mangrove.

It was noted during the survey visits that passing boats created strong waves that penetrated the creek. The riverbed of the mouth of the creek consisted of coarse sand while small patches of mud were only present deeper in the mangrove. The presence of large areas with sand and only small patches of mud clearly has had an impact on the species composition.

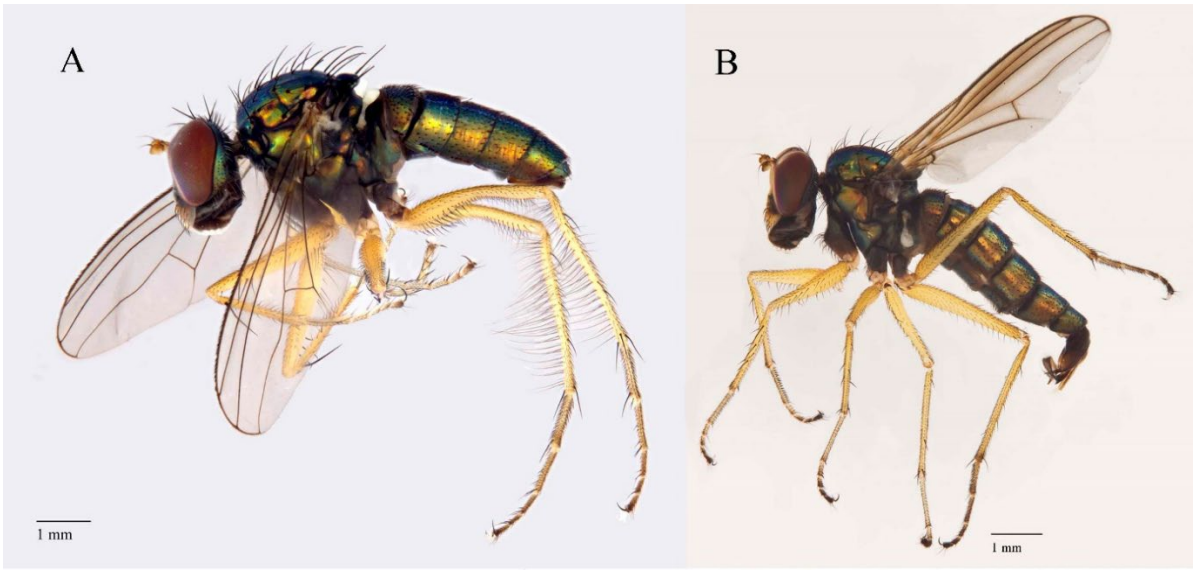


Fig. 4. The splash zone of sandy beaches is characterised by robust species of the genus *Thinophilus*: A, *Thinophilus meieri* Grootaert & Evenhuis; B, *Thinophilus murphyi* Evenhuis & Grootaert (Photos: Jayanthi Puniamoorthy).

Table 3. List of species in descending abundance, recorded in the patch of coastal forest (PU26) during the three-month sampling in 2019.

Species	26-Mar-19	02-Apr-19	10-Apr-19	16-Apr-19	24-Apr-19	08-May-19	15-May-19	22-May-19	28-May-19	03-Jun-19	11-Jun-19	19-Jun-19	25-Jun-19	Grand Total	Microhabitat
Chaetogonopteron sp		4	1	1	2	1			1		2	10	3	25	leaf surface
Chrysotus dot								15						15	leaf surface
Chrysotus prfurcatus*	1		1	1			1	4	1	1	4		1	15	leaf surface
Amblypsilopus sp	1		4	2			1	1	3	1		1		14	leaf surface
Chrysosoma cloudedwing*	1							1		2	3	4	3	14	leaf surface
Chrysotus black*		2	2					1	1		4	3		13	leaf surface
Diaphorus prmandarinus*			1	1			3				3	1		9	leaf surface
Diaphorus blackmidhindfemora*			1									5		6	leaf surface
Neurigona spd *			1			2	1				1			5	tree trunk
Plagiozopelma sp								1		2			1	4	leaf surface
Paraclius singaporensis		1							1	1		1		4	soil surface
Paraclius blackwing*								1			1	1		3	soil surface
Teuchophorus tiomanensis												3		3	soil surface
Chrysosoma sp							1						2	3	leaf surface
Teuchophorus prkrabiensis*								1	1		1			3	soil surface
Diaphorus blackwing*						1					1	1		3	leaf surface
Lichtwardtia nodulata							1	1			1			3	leaf surface
*Chrysotus furcatus**		2												2	leaf surface
Amblypsilopus yellowcoxae*		2												2	leaf surface
Diaphorus sp								1					1	2	leaf surface
Acropsilus acroleucus		2												2	soil surface
Neurigona angulata								2						2	tree trunk
Plagiozopelma flavipodex												2		2	leaf surface
Sympycnus longpp*						1								1	leaf surface
Amblypsilopus blacktrochanter*				1										1	leaf surface
Teuchophorus limosus												1		1	mud dweller
Paraclius sp											1			1	mud dweller
*Urodolichus singaporensis**				1										1	leaf surface
Paraclius yellowcoxae*						1								1	soil surface
Sympycnus yellow*				1										1	leaf surface
Urodolichus sp						1								1	leaf surface
Amblypsilopus yellowantennae*											1			1	leaf surface
Paraclius adligatoides				1										1	soil surface
Trigonocera sp							1							1	leaf surface
Protomedetera sp						1								1	tree trunk
Chaetogonopteron spa						1								1	leaf surface
Sciapodinae unknowngenus*						1								1	leaf surface
Acropsilus sp	1													1	soil surface
Number of specimens	**4**	**13**	**11**	**9**	**2**	**10**	**9**	**29**	**8**	**7**	**23**	**33**	**11**	**169**	
Number of species	**4**	**6**	**7**	**8**	**1**	**9**	**7**	**11**	**6**	**5**	**12**	**12**	**6**	**38**	

*Temporary taxonomic working name

Coastal forest. Station PU26 in the coastal forest area is located 20 m from the border of the back mangrove and 160 m from the stations PU23 and PU24 in the nearby mangrove. It is 50 m from the sandy beach.

35 species were recorded in the coastal forest (Table 2, 3). A *Chaetogonopteron* species belonging to the *chaeturum*-complex was the only species represented by more than 20 individuals. The large number of doubletons and singletons, six and 15 respectively, shows that the sampling was too poor and erratic to give a good idea of the occurrence of the species at that station.

There is a huge overlap with species normally occurring in the higher parts of the back mangrove. What was unexpected is that some true back-mangrove species such as the bottom-dwellers like the *Paraclius* species and the tiny *Teuchophorus* species were found in the coastal forest, though in small numbers. This large number of 'mangrove species' might be due to inland movement of the flies during springtides or excessive rainfall flooding the soil. Unfortunately, we do not know the extent of the flooding (which might possibly be to the point of leaving temporary pools) during the survey. Heavy rainfall was previously observed to influence movement of species. Moreover, the trap was placed perpendicular on a path through the vegetation that seemed to be a corridor where movement of insects took place. Hence, care should be taken in the interpretation of the data.

Nevertheless, the coastal forest at PU26 has a typical fauna that is dominated by leaf dwelling species (Fig. 5). They belong to the subfamilies Sympycninae (genera *Chaetogonopteron* and *Sympycnus*), to the Sciapodinae (genera *Amblypsilopus*, *Chrysosoma*, and *Plagiozopelma*) and the Diaphorinae (genera *Chrysotus*, *Diaphorus*, *and Trigonocera*) (Table 3). Although the Malaise trap was very close to the beach, no beach species were found in the coastal forest.

DISCUSSION

The three-month survey in the nine mangrove stations in the OBS area yielded 66 long-legged fly species. Fig. 6A shows that the species number is still steeply rising after the three-month sampling in these nine mangroves. The singletons curve also still continues to go up, while the doubletons seem to stagnate. When simulating sampling in an additional 50 stations, the species accumulation curve calculated according to Colwell (2013) predicts that 94 species are expected to occur in the OBS area (Fig. 6B). This maximum is already obtained when 40 simulated stations were sampled over three months and sampling in 20 stations would already obtain 90% of the total expected species.

The long-legged fly fauna of the mangroves of Pulau Ubin and especially that of Sungei Tock Kew basin (OBS) is very rich and one of the most diverse in comparison to other mangroves in Singapore. This high diversity is likely due to the presence of large mud flats that are breeding grounds for the larvae. Remarkable is the presence of a large number of *Protomedetera* species in 2016. This might be related to the presence of dead decaying wood in the mangrove.

The fauna of Sungei Sempit on the northern side of the OBS area is also very diverse but different from the southern part. Here, the mud flats are reduced to small patches while the rest is pure sand. The fauna is characterised by the presence of a number of species adapted to sandy soils instead of mudflats. From a conservation perspective, attention should be paid to ship-generated waves at Sungei Sempit leading to removal of the mud layer and to sand deposition instead. Hence, true mangrove is disappearing.

Sampling effort and diversity. The sampling effort is an important issue in a survey of mangrove fauna. Access to mangroves is difficult and most of the insects are not abundant. Taking into account costs, manpower, time, products, and impact of the sampling on the habitat and the species, the effort must be reasonable, but the data must be statistically representative.

A comparison of the survey carried out in 2016 with the one in 2019 at OBS showed that only 45% of the observed species were present during both years (Table 4). The dominant species were present in both years but a large turnover of the rare species was observed. Yearly cyclic oscillations of insect populations are a normal and common phenomenon, not only in the dominant species but also in rare species that are often not observed for years. It renders assessments based on a short sampling period difficult. However, when using the simulation models (Colwell, 2013), an estimation of the total number of species can be made and the figures obtained for the various mangrove areas can be compared.

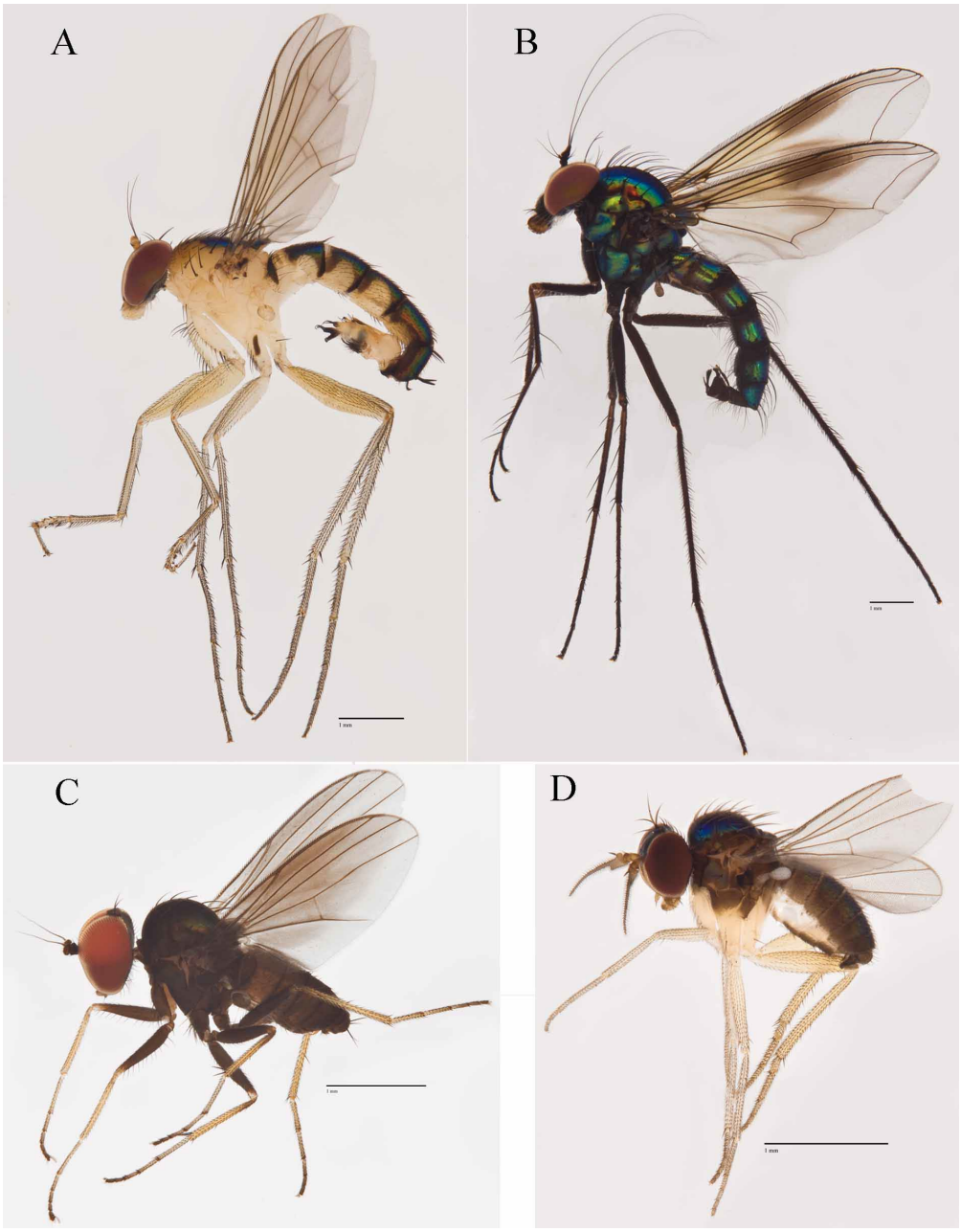
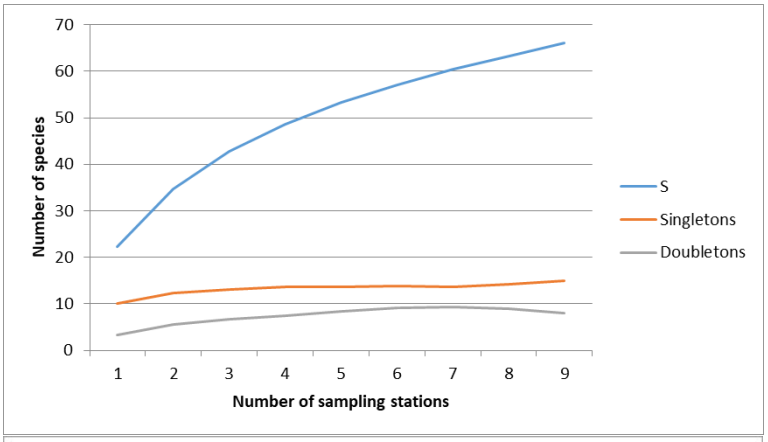


Fig. 5. Leaf dwellers are very common in the back mangrove. A, Male of an unknown genus of Sciapodinae that perhaps belongs to the genus *Amblypsilopus*, characterised by bizarre male secondary sexual characters (MSSC) such as the clipper-like fore tarsus and the strong black spines on the 6th abdominal tergite; B, *Chrysosoma* clouded wing, a new species pending description; C, *Diaphorus* sp.; D, *Teuchophorus temasek* Grootaert (photos: Jayanthi Puniamoorthy, Rene Ong, and Maimon Hussin).

We conclude that Malaise traps operated during three months in the transition of the dry to the wet season give a fair idea about the dominant species present but not about the rare species. This highlights the need for longer in-depth sampling to get a better idea on the species diversity and the presence of rare species. Furthermore, many species have a patchy distribution in a given biotope and thus there is a trade-off in the number of traps in a site and the duration of the sampling. We suggest that more sampling stations in an area will give a better idea of the presence of the 'rare' species presuming that they have different microhabitat preferences. They might not be sampled in a single station even over a long period.

In the OBS mangroves, 37 species are not endangered (they are species reported more than 20 times in Singapore), 27 are data deficient, hence their status cannot be assessed, and 10 species are considered to be endangered since they have been previously recorded less than 20 times in Singapore. Since the insect fauna of Singapore, like the rest of the



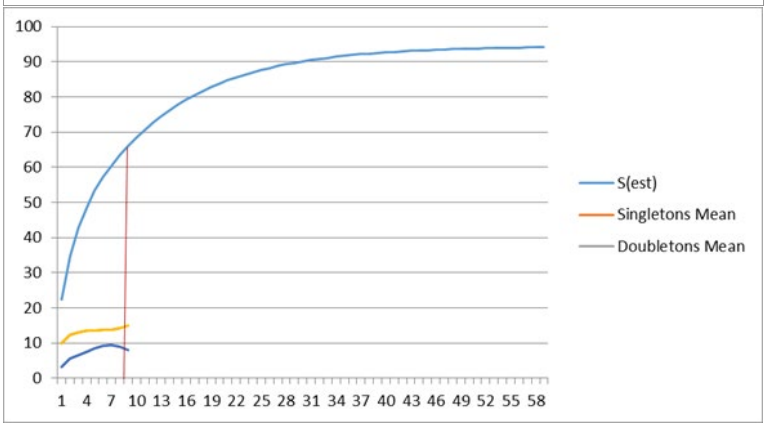


Fig. 6. Species accumulation curves for the mangroves of OBS: A, calculated for three-month sampling of nine stations; B, simulated for 50 additional sampling stations. Red line indicates the actual number of samples; S, number of species.

Oriental fauna is still poorly known, it is difficult to state the level of threat to the species using IUCN standards. However, since mangroves are endangered habitats, we might conclude that all species that live exclusively in mangroves are endangered. At present, we estimate that at least 180 long-legged fly species occur only in mangroves. They have an undeniable function in the mangrove ecosystem as predators and as food sources for other predators, thus regulating the populations of other insects and invertebrates in the mangrove.

The taxonomic impediment and the genetic barcoding. As can be seen in Table 2 and 3, no less than 34 species or 46% of the total species, have a temporary taxonomic working name (name marked with an asterisk) and thus still need formal description. In our present study, these species can be identified since a diagnosis was made for all, voucher specimens are deposited in the collection of the LKCNHM (NUS), all have been imaged (see BOS website), and moreover, almost all have been NGS barcoded.

The taxonomic impediment, even in long-legged flies that have been studied for nearly 20 years in Singapore, is still tremendous. Many Oriental species need revision since they have been described and reported from areas wide apart and even from entirely different biotopes. Access to the type material of the known species is difficult.

Table 4. Species turnover between a three-month sampling in 2016 and 2019 in the OBS stations PU01 and PU10.

	2016		2019			only in	only in	both in
	PU01	PU10	PU01	PU10	Total	2016	2019	2016/2019
Acropsilus sp			4		4		1	
*Acropsilus uncinatus**			3		3		1	
Amblypsilopus sp	7	3			10	1		
Chaetogonopteron chaeturum		2	3		5			1
Chaetogonopteron flag*			1		1		1	

Chaetogonopteron sp		**1**	**1**		**2**			**1**
Chrysosoma Si2043*	**2**				**2**	**1**		
Chrysotus black*	**19**	**10**	**51**	**1**	**81**			**1**
Chrysotus black2*	**2**				**2**	**1**		
Chrysotus dot	**236**	**61**	**18**		**315**			**1**
Chrysotus ubinensis			**4**		**4**		**1**	
*Chrysotus prfurcatus**	**20**	**24**	**17**		**61**			**1**
Chrysotus prfurcatus2*	**1**				**1**	**1**		
Diaphorus blackfemora*	**6**		**5**	**1**	**12**			**1**
Diaphorus blackwing*			**1**		**1**		**1**	
Diaphorus brownhindcoxae*			**1**		**1**		**1**	
Diaphorus prmandarinus*			**1**		**1**		**1**	
Diaphorus whitering*	**2**				**2**	**1**		
Genus unknown*			**3**		**3**		**1**	
Hercostomus brevicornis	**1**				**1**	**1**		
Hercostomus brevidigitalis	**2**	**7**	**11**		**20**			**1**
Hercostomus lanceolatus		**3**	**1**		**4**			**1**
Hercostomus limosus		**5**	**1**		**6**			**1**
Hercostomus obtusus		**1**	**3**		**4**			**1**
Hercostomus plumatus	**34**	**14**	**17**	**3**	**68**			**1**
Hercostomus prsingaporensis*	**5**	**3**	**2**	**1**	**11**			**1**
Hercostomus singaporensis	**2**				**2**	**1**		
Hercostomus truncatus	**8**				**8**	**1**		
Lichtwardtia sp	**1**				**1**	**1**		
Medetera blackfemora*	**1**		**3**		**4**			**1**
Nanothinophilus brownwing*	**2**				**2**	**1**		
Nanothinophilus hoplites	**4**		**1**		**5**			**1**
Nanothinophilus sp		**1**			**1**	**1**		
Neurigona angulata			**1**		**1**		**1**	
Neurigona spd*			**3**		**3**		**1**	
Ngirhaphium caeruleum	**2**				**2**	**1**		
Ngirhaphium sivasothii	**24**	**36**	**10**		**70**			**1**
Paraclius adligatoides*	**2**	**2**	**1**		**5**			**1**
Paraclius blackwing*	**1**				**1**	**1**		
Paraclius crassatus		**23**	**56**		**79**			**1**
Paraclius digitatus	**1**	**5**	**22**		**28**			**1**
Paraclius serratus	**5**	**3**	**13**		**21**			**1**
Paraclius yellowcoxae*			**5**	**1**	**6**		**1**	
Phacaspis mitis		**2**	**4**	**1**	**7**			**1**
Plagiozopelma sp			**1**		**1**		**1**	
Protomedetera sp	**3**				**3**	**1**		
Protomedetera uncinata	**12**				**12**	**1**		
Protomedetera squamata	**5**	**2**		**1**	**8**			**1**
Sympycnus sp		**1**			**1**	**1**		
Sympycnus yellow*			**4**		**4**		**1**	
Teuchophorus limosus	**4**		**13**		**17**			**1**

Teuchophorus prkrabiensis*	**2**	**6**	**11**		**19**			**1**
Teuchophorus tiomanensis	**45**	**9**	**19**	**2**	**75**			**1**
Thinophilus apicatus			**1**		**1**		**1**	
Thinophilus simplex		**1**			**1**		**1**	
Total number specimens	**461**	**225**	**316**	**11**	**1013**			
Total number species	**31**	**24**	**37**	**8**	**55**	**16**	**15**	**24**

By using NGS barcoding in combination with traditional morphological characters, it is possible to identify species that are difficult to recognise on the basis of morphologic characters alone. This way, female fly specimens can be identified that otherwise could not be named as they are morphologically very similar to closely related species. Eventually, the barcode data may serve in a haplotype network study that can shed some light on the provenance of the species on Pulau Ubin and the interconnectivity of the mangroves in Singapore. Such an example is *Ngirhaphium caeruleum* that has five different haplotypes in Pulau Ubin, related to a haplotype that is exclusive to Pulau Tekong and that forms a link to the cluster of 15 different haplotypes present in the mangroves of Brunei (Fig. 1 in Samoh et al., 2019).

A minority of the specimens from the present survey have been NGS barcoded, which resulted in a taxonomic paper (Grootaert & Van de Velde, 2024). This showed that barcoding provides a reliable reference for a species and is a valuable supporting tool to reduce the huge taxonomic impediment. Furthermore, barcoding is a promising tool to pre-sort the species from the samples in clusters with the same barcode and eventually ID them (Srivathsan et al., 2018, 2019; Wang et al., 2019). Ecological analyses can be done using the barcode as a reference (Yeo et al., 2021). However, Linnaean names for the species are of utmost importance since they form the link to the habitat and the niches of the species. Using numbers alone to indicate a species does not give a strong signal.

NGS COI barcodes are available for 32,800 long-legged fly specimens from Singapore and can be matched with newly barcoded specimens. In addition, a database containing barcodes from southern Thailand, Brunei, and Hong Kong is available (Yeo et al. 2021), allowing for assessment of species on a broader scale in Southeast Asia. This way, the genetic variation of the species (haplotypes) and relationship between the genetics and the geographic distribution around the South China Sea can be seen (haplotype network) as illustrated in Grootaert (2019), Samoh et al. (2018), and Yeo et al. (2021).

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