



## A survey of True flies (Insecta: Diptera) by DNA Barcoding of Malaise Trap Collection in Bangladesh

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**ABSTRACT.** True flies (Insecta: Diptera) are widely distributed and the key components in most ecosystems. The objective of this study was to identify the true flies' diversity through DNA barcoding technique (658 bp sequence from the 5'-end of cytochrome oxidase I) in Bangladesh. Specimens were collected by a Malaise trap at Chittagong University Campus between April 2014 and March 2015. In this study, we examined 36476 sequences of 38267 true flies, and resulted 105 species, 109 genera, 54 subfamilies and 59 families. Among them 79 species, 69 genera, 12 subfamilies and 23 families are new country records. All the specimen records with the Barcode Index Numbers (BINs) (the species proxies), are available on the Barcode of Life Data System (BOLD).

**Key words:** Diptera, Malaise trap, DNA barcode, Bangladesh

**Received:**

13 August, 2020

**Accepted:**

11 October, 2020

**Published:**

15 October, 2020

**Subject Editor:**

Ebrahim Gilasian

**Citation:** Mazumdar, S., Hebert, P.D.N. & Bhuiya, B.A. (2021) A survey of True flies (Insecta: Diptera) by DNA Barcoding of Malaise Trap Collection in Bangladesh. *Journal of Insect Biodiversity and Systematics*, 7 (1), 15–42.

### Introduction

Diptera (True flies) is one of the third largest insect order containing approximately 125,000 described species (ADCF, 2020). They are effective contributors in maintaining ecological balance and in considerable economic status. For instance, they play significant positive role as scavengers, parasitoids and predators of other insects, pollinators, food for predators, bioindicators of water quality, and tools for scientific research as well as effects on agriculture, animal and human health, and forestry (Courtney et al., 2017; Dhamorikar, 2017).

True flies' taxonomic works in Bangladesh has mainly focused morphological characters, by considering their medical and agriculture importance. For example, Huda (1981) worked on genus *Anopheles* from Chittagong University Campus (CUC) and its adjacent areas. Also, Huda (2000) reported species diversity of genus *Aedes* through the country. Gapud (1992) compiled true flies associated with agricultural fields. Alam et al. (2012) reported true flies of Bandarban District. Alam et al. (2009) worked on vector species of kala-azar. Pollinating true flies and their foraging behavior were assessed by Mazumdar et al. (2010, 2011). Bashar et al. (2016) worked on true flies of semi-urban areas of Dhaka city. Irish et al. (2016) reviewed articles on mosquitoes occurred in Bangladesh.

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True flies species diversity has far not been well surveyed in Bangladesh applying DNA barcoding technique. For instance, [Leblanc et al. \(2013\)](#), [Amin et al. \(2014\)](#) and [Alam et al. \(2015\)](#) identified some agricultural pests, [Rain et al. \(2019\)](#), [Siddiki et al. \(2019\)](#) and [Mazumdar \(2020\)](#) studied medically and veterinary important true flies through DNA barcoding technique.

Species identification based on DNA sequences was introduced by [Hebert et al. \(2003\)](#) and this modern technique has revealed an effective and credible modern tool in taxonomy, evolutionary biology and biodiversity research ([Hebert et al., 2016](#); [Lin et al., 2018](#)). DNA barcoding of arthropods collected by Malaise traps has captured the headlines for assessing terrestrial insect diversity ([deWaard et al., 2019](#); [Mazumdar et al., 2019](#)). A research work for insect inventories result that about 75% dipterans of total specimens collections through Malaise trap in Sweden ([Karlsson et al., 2020](#)).

Therefore, the present work was carried out to disclose the partial mitochondrial COI gene sequences of the true flies' diversity available in Bangladesh.

## Material and methods

### *Specimen Collection, Processing, Identification and Specimen Deposition*

Specimens were collected by a Townes-style Malaise trap (BioQuip Inc. USA) installed in perceived flight paths at Chittagong University Campus (Lat. 22.46359° N; Long. 91.7808° E) in Bangladesh by following the Standard Operating Protocol for the Global Malaise Trap Program ([www.dnabarcoding.ca](http://www.dnabarcoding.ca)). Between March 2014 and February 2015, the samples were harvested weekly in a 500 mL plastic Nalgene bottle that was filled with 375 mL of 95% ethanol and placed in 500 mL of fresh ethanol before storage at -20°C until analysis. Collected insects were analyzed by following standard barcoding protocols (<http://ccdb.ca/resources.php>), at the Canadian Centre for DNA Barcoding (CCDB) within the Centre for Biodiversity Genomics (CBG), University of Guelph, Canada. Collection data, voucher information and taxonomy for each specimen are available in the Barcode of Life Data Systems ([http://v3.boldsystems.org/index.php/TaxBrowser\\_Taxonpage?taxid=127](http://v3.boldsystems.org/index.php/TaxBrowser_Taxonpage?taxid=127)). All the specimens analyzed in this study have been curated at the CBG, University of Guelph, Guelph, Ontario, Canada.

### *Molecular Analysis and Data Analysis*

A small portion of each specimen (usually 1-3 legs) was removed and used for DNA extraction and from the whole body of smaller taxa, and vouchers were recovered after DNA extraction for imaging and curation. Tissue lysis, DNA extraction, PCR amplification, cycle sequencing and sequence analysis were performed at the CCDB following its standard protocols.

PCR amplification of COI-5' was performed with primers C\_LepFolF and C\_LepFolR ([http://www.ccdb.ca/docs/CCDB\\_PrimerSets](http://www.ccdb.ca/docs/CCDB_PrimerSets)). By following PCR conditions, 94°C (1 min), 5 cycles at 94°C (40 s), 45°C (40 s), 72°C (1 min); 35 cycles at 94°C (40 s), 51°C (40 s), 72°C (1 min) and a final extension at 72°C (5 min) and amplicons were sequenced using BigDye v3.1 (Applied Biosystems) on an ABI 3730XL. Sequences were assembled, aligned, and edited using CodonCode Aligner (CodonCode Corporation, USA) and submitted to Barcode of Life Data Systems (BOLD) ([www.boldsystems.org](http://www.boldsystems.org)). With a few exceptions, by considering sequence matches to records on BOLD, the specimens with barcodes were assigned to 59 families.

## Results

In current study, 36476 sequences were examined of 38267 true flies. Total 105 true fly species, 109 genera, 54 subfamilies and 59 families of Malaise trap collections were recorded through DNA barcoding technique. Among them 79 species, 69 genera, 12 subfamilies and 23 families were new country records for the fauna of Bangladesh (Table 1). Muscidae was the most dominant diversified family (subfamily: 3, genera: 11, species: 19) of the total count. Chironomidae showed next highest dominance (subfamily: 2, genera: 10, species: 7), followed by Agromyzidae (subfamily: 2, genera: 7, species: 7), Culicidae (subfamily: 2, genera: 5, species: 12) and Ceratopogonidae (subfamily: 2, genera: 2, species: 10). No species were confirmed in newly recorded 24 families such as Anthomyzidae, Asteiidae, Bibionidae, Carnidae, Chyromyidae, Clusiidae, Cryptochetidae, Empididae, Hippoboscidae, Keroplatidae, Lauxaniidae, Mycetophilidae, Mythicomyiidae, Neriidae, Odiiniidae, Perisclididae, Platypezidae, Platystomatidae, Psilidae, Rhinophoridae, Rhinophoridae, Scatopsidae, Simuliidae, Therevidae and Xylomyidae.

**Table 1.** Dipterans revealed by DNA barcoding of Malaise trap samples collected in Bangladesh.

Family	Subfamily	Scientific name	References
Agromyzidae	Agromyzinae	<i>Melanagromyza cleomae</i> Spencer, 1961 (Fig. 1)	Current study
		<i>Melanagromyza obtusa</i> (Malloch, 1914) (Fig. 2)	Mazumdar & Bhuiya, 2014
		<i>Ophiomyia phaseoli</i> (Tryon, 1895) (Fig. 3)	Mazumdar & Bhuiya, 2014
	Phytomyzinae	<i>Calycomyza lantanae</i> (Frick, 1956) (Fig. 4)	Current study
		<i>Chromatomyia horticola</i> (Goureau, 1851) (Fig. 5)	Current study
		<i>Liriomyza sativae</i> Blanchard, 1938 (Fig. 6)	Mazumdar & Bhuiya, 2014
		<i>Phytoliriomyza</i> sp.	Current study
		<i>Phytomyza thalictrella</i> Spencer, 1981 (Fig. 7)	Current study
Anthomyiidae	-	-	Miah et al., 1997
Anthomyzidae	-	-	Current study
Asilidae	Asilinae	<i>Zosteria</i> sp.	Current study
Asteiidae	-	-	Current study
Bibionidae	-	-	Current study
Bombyliidae	-	-	Arabyat et al., 2004
Calliphoridae	Chrysomyinae	<i>Chrysomya</i> sp.	Mazumdar et al., 2010, 2014
	Luciliinae	<i>Hemipyrellia</i> sp.	Current study
		<i>Lucilia</i> sp.	Islam et al., 2017
Canacidae	-	-	Mathis, 1992

Table 1. Continued.

Family	Subfamily	Scientific name	References
Carnidae	-	-	Current study
Cecidomyiidae	Cecidomyiinae	<i>Contarinia maculipennis</i> Felt, 1933	Current study
		<i>Feltiella acarisuga</i> (Vallot, 1827) (Fig. 8)	Current study
Celyphidae		<i>Spaniocelyphus bigoti</i> Karsch, 1884 (Fig. 9)	Current study
Ceratopogonidae	Ceratopogoninae	<i>Culicoides actoni</i> Smith, 1929 (Fig. 10)	Current study
		<i>Culicoides asiana</i> Bellis, 2015	Current study
		<i>Culicoides brevipalpis</i> Delfinado, 1961 (Fig. 11)	Current study
		<i>Culicoides innoxius</i> Sen and Das Gupta, 1959 (Fig. 12)	Current study
		<i>Culicoides orientalis</i> Macfie, 1932 (Fig. 13)	Current study
		<i>Culicoides oxystoma</i> Kieffer, 1910 (Fig. 14)	Current study
		<i>Culicoides peregrines</i> Kieffer, 1910 (Fig. 15)	Current study
		<i>Culicoides shortti</i> Smith and Swaminathan, 1932 (Fig. 16)	Current study
		<i>Culicoides similis</i> Carter, 1920 (Fig. 17)	Current study
		<i>Culicoides sumatrae</i> Macfie, 1934 (Fig. 18)	Current study
	Forcipomyiinae	<i>Forcipomyia</i> sp.	Current study
Chironomidae	Chironominae	<i>Chironomus circumdatus</i> (Kieffer, 1916) (Fig. 19)	Current study
		<i>Chironomus crassiforceps</i> (Kieffer, 1916) (Fig. 20)	Current study
		<i>Chironomus javanus</i> Kieffer, 1924 (Fig. 21)	Current study
		<i>Cladotanytarsus</i> sp.	Current study
		<i>Kiefferulus calligaster</i> (Kieffer, 1911) (Fig. 22)	Current study
		<i>Microchironomus cf. tener</i> (Kieffer, 1918) (Fig. 23)	Current study
		<i>Polypedilum cf. allansoni</i> Freeman, 1958 (Fig. 24)	Current study
		<i>Tanytarsus formosanus</i> Kieffer, 1912 (Fig. 25)	Current study
		<i>Zaoreliella</i> sp.	Current study
	Tanypodinae	<i>Denopelopia</i> sp.	Current study
		<i>Paramerina</i> sp.	Current study
		<i>Procladius</i> sp.	Current study

**Table 1.** Continued.

Family	Subfamily	Scientific name	References
Chloropidae	Chloropinae	<i>Pachylophus</i> sp.	Catling & Islam, 2013
	Oscinellinae	<i>Anatrichus</i> sp.	Catling & Islam, 2013
		<i>Cadrema</i> sp.	Current study
		<i>Oscinella</i> sp.	Current study
		<i>Neorhodesiella</i> sp.	Current study
	Rhodesiellinae	<i>Rhodesiella</i> sp.	Current study
Chyromyidae	-	-	Current study
Clusiidae	-	-	Current study
Cryptochetidae	-	-	Current study
Culicidae	Anophelinae	<i>Anopheles vagus</i> Donitz, 1902 (Fig. 26)	Huda & Majumder, 1985; Irish et al., 2016
		<i>Aedes albopictus</i> (Skuse, 1894) (Fig. 27)	Huda & Majumder, 1985; Irish et al., 2016
	Culicinae	<i>Armigeres subalbatus</i> (Coquillett, 1898) (Fig. 28)	Irish et al., 2016
		<i>Culex bitaeniorhynchus</i> Giles, 1901 (Fig. 29)	Irish et al., 2016
		<i>Culex fuscocephala</i> Theobald, 1907 (Fig. 30)	Irish et al., 2016
		<i>Culex gelidus</i> Theobald, 1901 (Fig. 31)	Irish et al., 2016
		<i>Culex infantulus</i> Edwards, 1922 (Fig. 32)	Current study
		<i>Culex pallidothorax</i> Theobald, 1905 (Fig. 33)	Irish et al., 2016
		<i>Culex pipiens</i> Linnaeus, 1758 (Fig. 34)	Ameen & Moizuddin, 1975
		<i>Culex rubithoracis</i> (Leicester, 1908) (Fig. 35)	Current study
		<i>Culex tritaeniorhynchus</i> Giles, 1901 (Fig. 36)	Huda & Majumder, 1985; Irish et al., 2016
		<i>Culex vishmii</i> Theobald, 1901 (Fig. 37)	Huda & Majumder, 1985; Irish et al., 2016
		<i>Lutzia</i> sp.	Irish et al., 2016
Dolichopodidae	Diaphorinae	<i>Chrysotus</i> sp.	Current study
	Dolichopodinae	<i>Paraclius digitatus</i> Zhang, Yang & Grootaert, 2007 (Fig. 38)	Current study
		<i>Thinophilus</i> sp.	Current study
	Medeterinae	<i>Medetera grisescens</i> de Meijere, 1916 (Fig. 39)	Current study
Drosophilidae	Drosophilinae	<i>Drosophila albomicans</i> (Duda, 1923) (Fig. 40)	Current study

Table 1. Continued.

Family	Subfamily	Scientific name	References
Drosophilidae	Drosophilinae	<i>Drosophila sulfurigaster</i> (Duda, 1923) (Fig. 41)	Current study
		<i>Mycodrosophila aqua</i> Bock, 1980 (Fig. 42)	Current study
		<i>Scaptodrosophila riverata</i> (Singh & Gupta, 1977)	Current study
		<i>Scaptomyza pallid</i> (Zetterstedt, 1847) (Fig. 43)	Current study
	Steganinae	<i>Leucophenga angusta</i> Okada, 1956	Current study
		<i>Leucophenga taiwanensis</i> Lin & Wheeler, 1972	Current study
		<i>Stegana kanmiyai</i> Okada & Sidorenko, 1992 (Fig. 44)	Current study
Empididae	-	-	Current study
Ephydriidae	Ephydrinae	<i>Brachydeutera</i> sp.	Current study
		<i>Scatella</i> sp.	Current study
	Ilytheinae	<i>Nostima</i> sp.	Current study
	Notiphilinae	<i>Hydrellia flaviceps</i> (Meigen, 1830) (Fig. 45)	Current study
		<i>Hydrellia pakistanae</i> Deonier, 1978 (Fig. 46)	Current study
	Psilopinae	<i>Polytrichophora</i> sp.	Current study
		<i>Psilopa</i> sp.	Current study
Hippoboscidae	-	-	Current study
Hybotidae	Tachydromiinae	-	Current study
Keroplastidae	-	-	Current study
Lauxaniidae	-	-	Current study
Limoniidae	Limoniinae	<i>Orimarga</i> sp.	Current study
		<i>Trentepohlia</i> sp.	Current study
Lonchaeidae	Lonchaeinae	<i>Lamprolonchaea</i> sp.	Current study
Micropezidae		-	Jui et al., 2007; Hill, 2008
Milichiidae	Madizinae	<i>Desmometopa sordida</i> (Fallén, 1820) (Fig. 47)	Current study
		<i>Desmometopa varipalpis</i> Malloch, 1927 (Fig. 48)	Current study
	Milichiinae	<i>Milichiella</i> sp.	Current study
Muscidae	Coenosiinae	<i>Coenosia attenuata</i> Stein, 1903 (Fig. 49)	Current study
		<i>Limnophora</i> sp.	Current study
		<i>Lispe assimilis</i> Wiedemann, 1824 (Fig. 50)	Current study
		<i>Lispe nicobarensis</i> Schiner, 1868 (Fig. 51)	Current study

**Table 1.** Continued.

Family	Subfamily	Scientific name	References
Muscidae	Coenosiinae	<i>Lispe sericipalpis</i> (Stein, 1904) (Fig. 52)	Current study
		<i>Pygophora respondens</i> (Walker, 1859) (Fig. 53)	Current study
	Muscinae	<i>Haematobia irritans</i> (Linnaeus, 1758) (Fig. 54)	Current study
		<i>Haematobosca sanguinoleant</i> (Austen, 1909) (Fig. 55)	Current study
		<i>Morellia</i> sp.	Current study
		<i>Musca cnofiscate</i> Speiser, 1924 (Fig. 56)	Current study
		<i>Musca convexifrons</i> Thomson, 1869 (Fig. 57)	Current study
		<i>Musca crassirostris</i> Stein, 1903 (Fig. 58)	Current study
		<i>Musca domestica</i> Linnaeus, 1758 (Fig. 59)	Mazumdar et al., 2010
		<i>Musca formosana</i> Malloch, 1910	Current study
		<i>Musca ventrosa</i> Wiedemann, 1830 (Fig. 60)	Current study
		<i>Neomyia timorensis</i> (Robineau-Desvoidy, 1830) (Fig. 61)	Current study
		<i>Stomoxys calcitrans</i> (Linnaeus, 1758) (Fig. 62)	EBO, 2015
		<i>Stomoxys indicus</i> Picard, 1908 (Fig. 63)	Current study
		<i>Stomoxys uruma</i> Shinonaga & Kano, 1966 (Fig. 64)	Current study
Phaoniinae	<i>Atherigona orientalis</i> Schiner, 1868 (Fig. 65)	Current study	
	<i>Atherigona oryzae</i> Malloch, 1925 (Fig. 66)	Catling & Islam, 2013	
Mycetophilidae	-	Current study	
Mythicomyiidae	-	Current study	
Neriidae	-	Current study	
Oдиниidae	-	Current study	
Periscelididae	-	Current study	
Phoridae	Metopininae	<i>Megaselia</i> sp.	Alam et al., 2015
	Phorinae	<i>Diplonevra</i> sp.	Current study
		<i>Dohrniphora cornuta</i> (Bigot, 1857) (Fig. 67)	Current study
Pipunculidae	Pipunculinae	<i>Eudorylas</i> sp.	Current study
		<i>Tomosvaryella</i> sp.	Current study
Platypezidae	-	Current study	

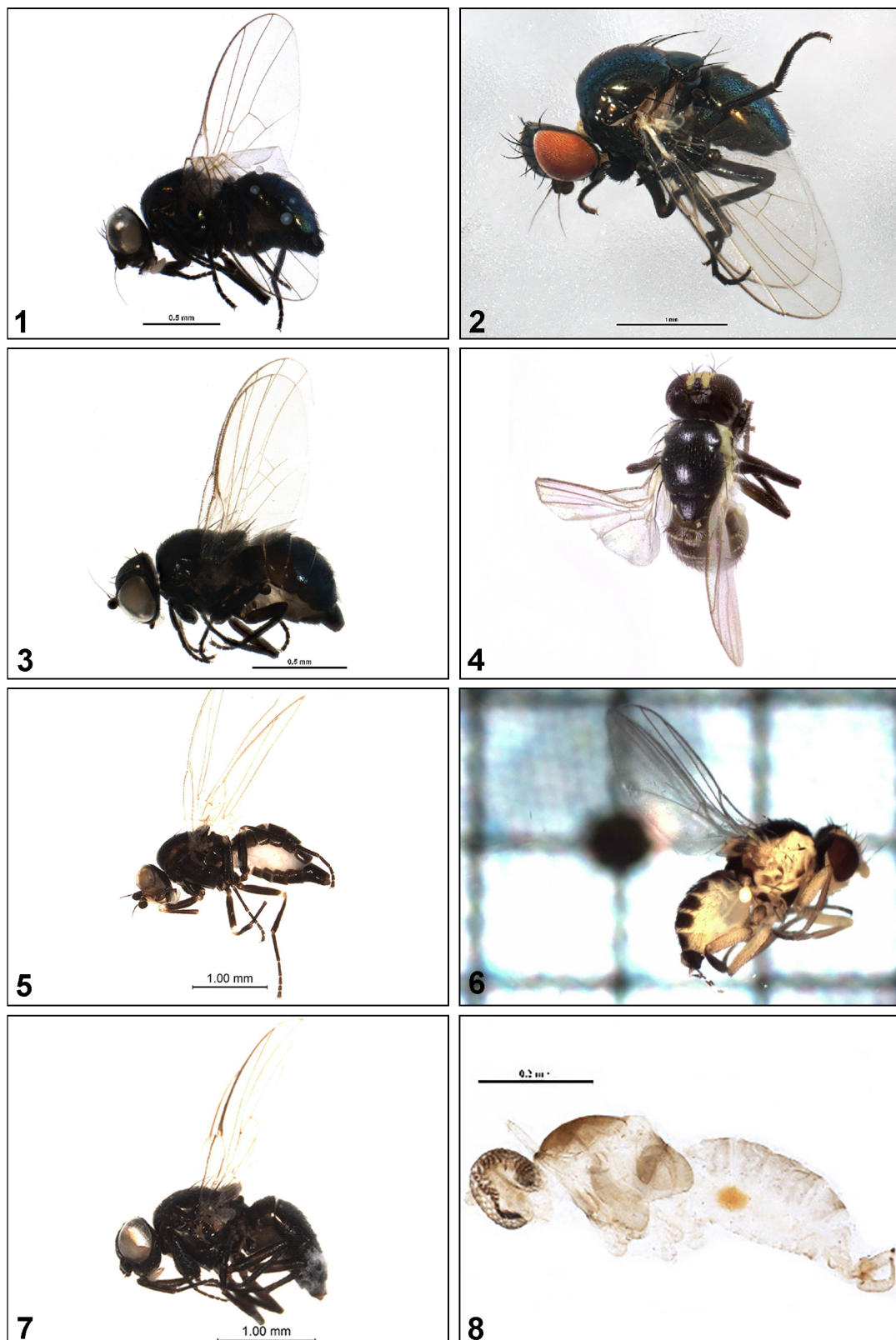
**Table 1.** Continued.

Family	Subfamily	Scientific name	References
Platystomatidae		-	Current study
Psilidae		-	Current study
Psychodidae	Psychodinae	<i>Brunettia</i> sp.	Current study
		<i>Psychoda alternate</i> Say, 1824 (Fig. 68)	Current study
Rhiniidae	Rhiniinae	<i>Stomorhina discolor</i> (Fabricius, 1794) (Fig. 69)	Sitompul et al., 2018
Rhinophoridae	-	-	Current study
Sarcophagidae	Sarcophaginae	<i>Sarcophaga albiceps</i> Meigen, 1826 (Fig. 70)	Sharma et al., 2017
		<i>Sarcophaga misera</i> Walker, 1849 (Fig. 71)	Pape, 1996; SMT, 2020
		<i>Sarcophaga princeps</i> Wiedemann, 1830 (Fig. 72)	Current study
		<i>Sarcophaga scopariiformis</i> Senior-White, 1927 (Fig. 73)	Current study
		<i>Sarcophaga taenionota</i> (Wiedemann, 1819) (Fig. 74)	Pape, 1996
Scatopsidae	-	-	Current study
Sciaridae		<i>Bradysia ocellaris</i> (Comstock, 1882) (Fig. 75)	Current study
		<i>Cosmosciara</i> sp.	Current study
		<i>Hyperlasion</i> sp.	Current study
		<i>Sciara</i> sp.	Current study
Sepsidae	Sepsinae	<i>Australosepsis frontalis</i> (Walker, 1860) (Fig. 76)	Current study
		<i>Dicranosepsis cf. sauteri</i> Ozerov, 2003	Current study
		<i>Dicranosepsis crinite</i> (Duda, 1926) (Fig. 77)	Current study
		<i>Sepsis dissimilis</i> Brunetti, 1909 (Fig. 78)	Current study
		<i>Sepsis nitens</i> Wiedemann, 1824 (Fig. 79)	Current study
	Toxopodinae	<i>Toxopoda</i> sp.	Iwasa et al., 1991
Simuliidae	-	-	Current study
Sphaeroceridae	Limosininae	<i>Coproica ferruginata</i> (Stenhammar, 1855) (Fig. 80)	Current study
		<i>Coproica hirtula</i> (Rondani, 1880) (Fig. 81)	Current study
		<i>Rachispoda</i> sp.	Current study
		<i>Spelobia bifrons</i> (Stenhammar, 1855) (Fig. 82)	Current study
		<i>Trachyopella lineafrons</i> (Spuler, 1925) (Fig. 83)	Current study

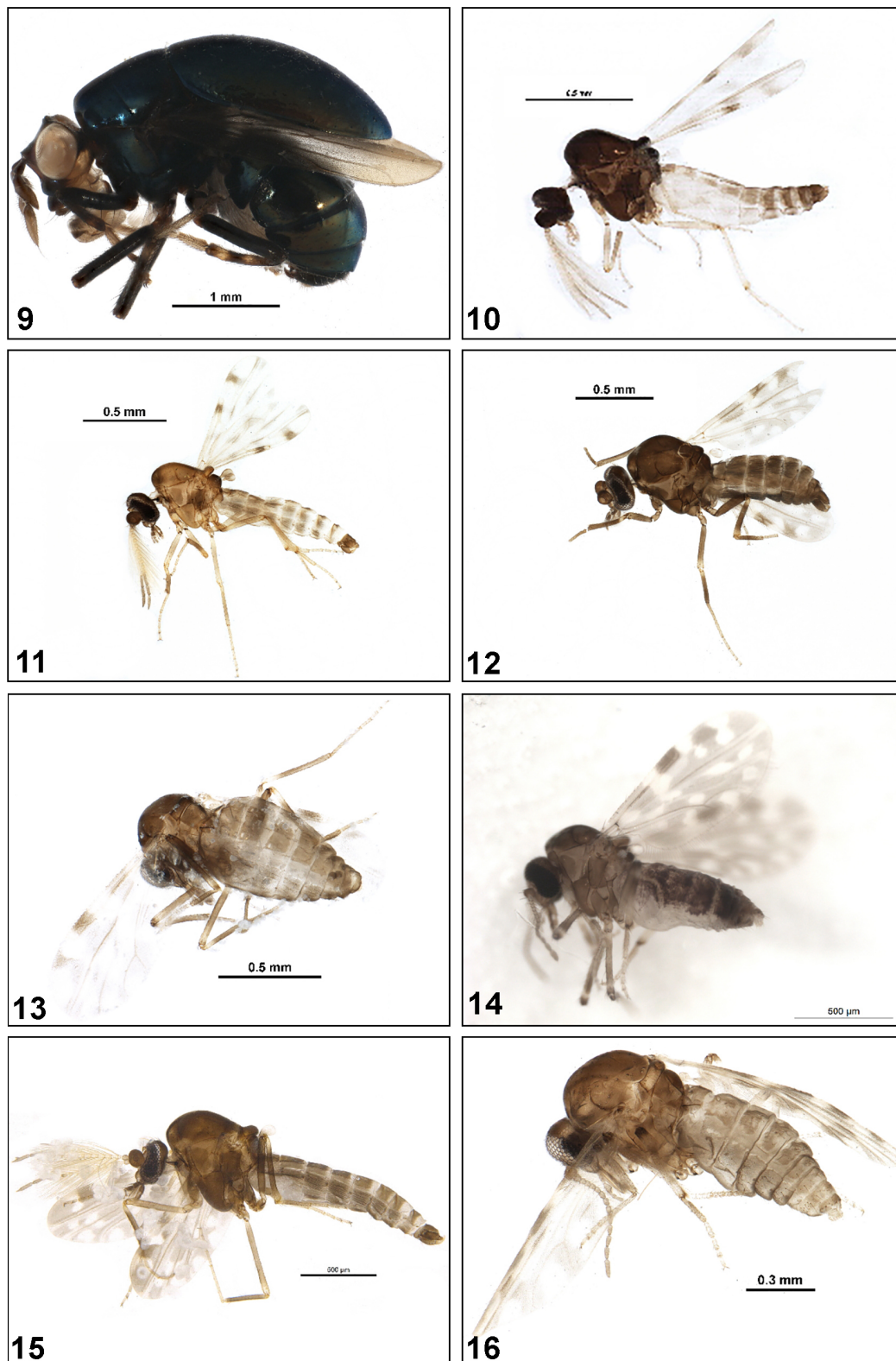


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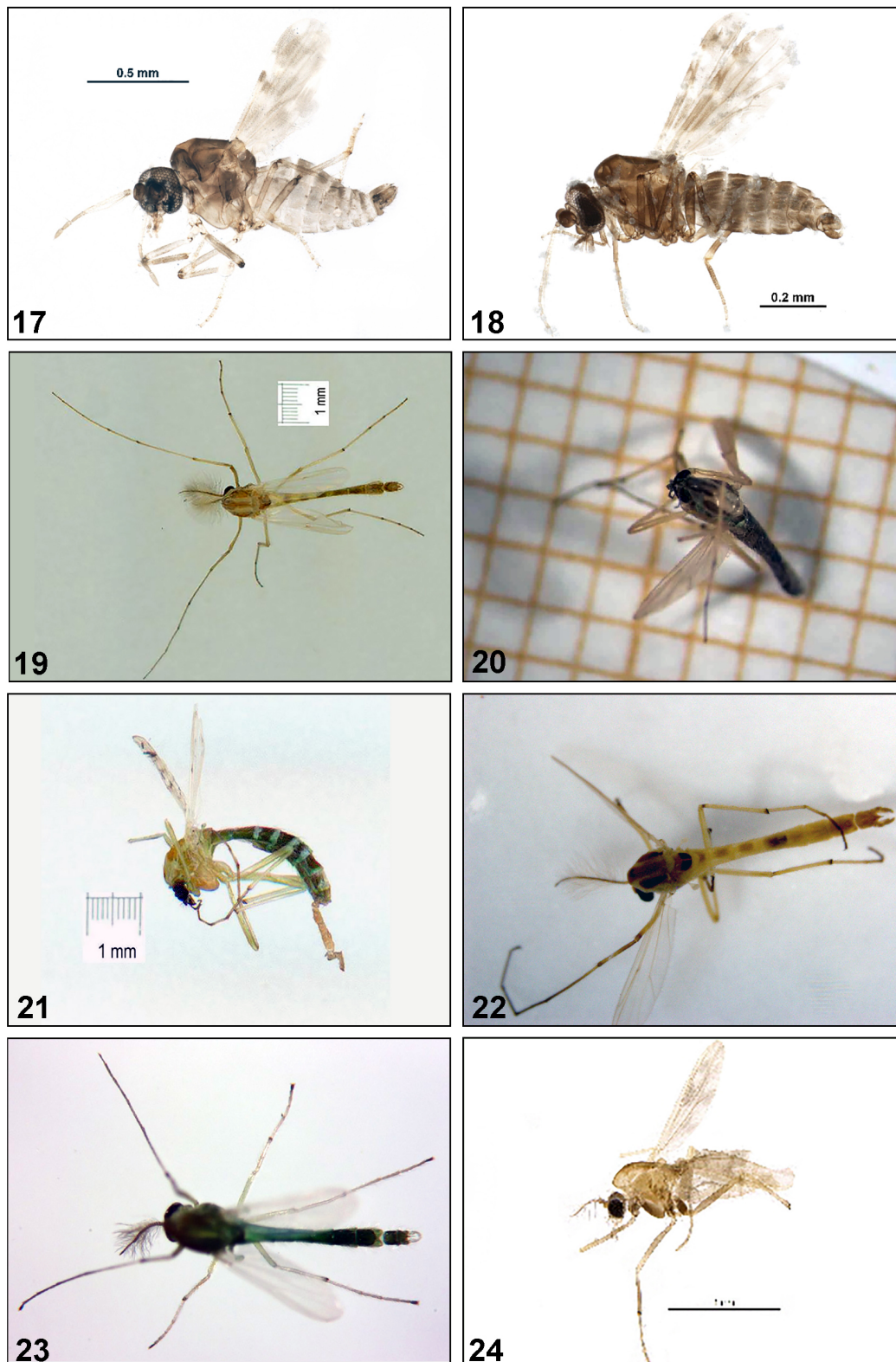
Family	Subfamily	Scientific name	References
Sphaeroceridae	Limosiniinae	<i>Trachyopella straminea</i> Rohacek & Marshall, 1986 (Fig. 84)	Current study
Stratiomyidae		-	Current study
	Clitellariinae	-	Current study
		<i>Octorthra</i> sp.	Current study
	Hermetiinae	<i>Hermetia illucens</i> (Linnaeus, 1758) (Fig. 85)	Current study
	Pachygastrinae	-	Current study
	Sarginae	<i>Microchrysa flaviventris</i> (Wiedemann, 1824) (Fig. 86)	Current study
	Stratiomyiinae	<i>Caloparyphus</i> sp.	Current study
Syrphidae	Eristalinae	<i>Syrhitta orientalis</i> Macquart, 1842 (Fig. 87)	Current study
	Syrphinae	<i>Allobaccha amphithoe</i> (Walker, 1849) (Fig. 88)	Current study
		<i>Allograpta medanensis</i> (Meijere, 1914) (Fig. 89)	Current study
		<i>Melanostoma</i> sp.	Current study
		<i>Paragus</i> sp.	Mazumdar et al., 2014
		<i>Simosyrphus scutellaris</i> (Fabricius, 1805) (Fig. 90)	Current study
		<i>Sphaerophoria</i> sp.	EBO, 2014
Tabanidae	Tabaninae	<i>Atylotus agrestis</i> (Wiedemann, 1828) (Fig. 91)	Current study
		<i>Tabanus rubidus</i> Wiedemann, 1821 (Fig. 92)	Alfred et al., 1999
		<i>Tabanus striatus</i> Fabricius, 1787 (Fig. 93)	Alfred et al., 1999; Maity et al., 2015
Tachinidae	Exoristinae	<i>Aplomya metallica</i> (Wiedemann, 1824) (Fig. 94)	Islam, 2015
		<i>Exorista xanthaspis</i> (Wiedemann, 1830) (Fig. 95)	Islam, 2015
	Phasiinae	<i>Gymnosoma</i> sp.	Current study
Tephritidae	Dacinae	<i>Bactrocera cucurbitae</i> (Coquillett, 1899) (Fig. 96)	Akhtaruzzaman et al., 1999; Leblanc et al., 2013; Rahman et al., 2019
		<i>Bactrocera diversa</i> (Coquillett, 1904) (Fig. 97)	Leblanc et al., 2013
		<i>Dacus longicornis</i> Wiedemann, 1830 (Fig. 98)	Khan, 2009; Leblanc et al., 2013
Therevidae	-	-	Current study
Ulidiidae	Ulidiinae	<i>Physiphora</i> sp. (Fig. 99)	Current study
Xylomyidae	-	-	Current study



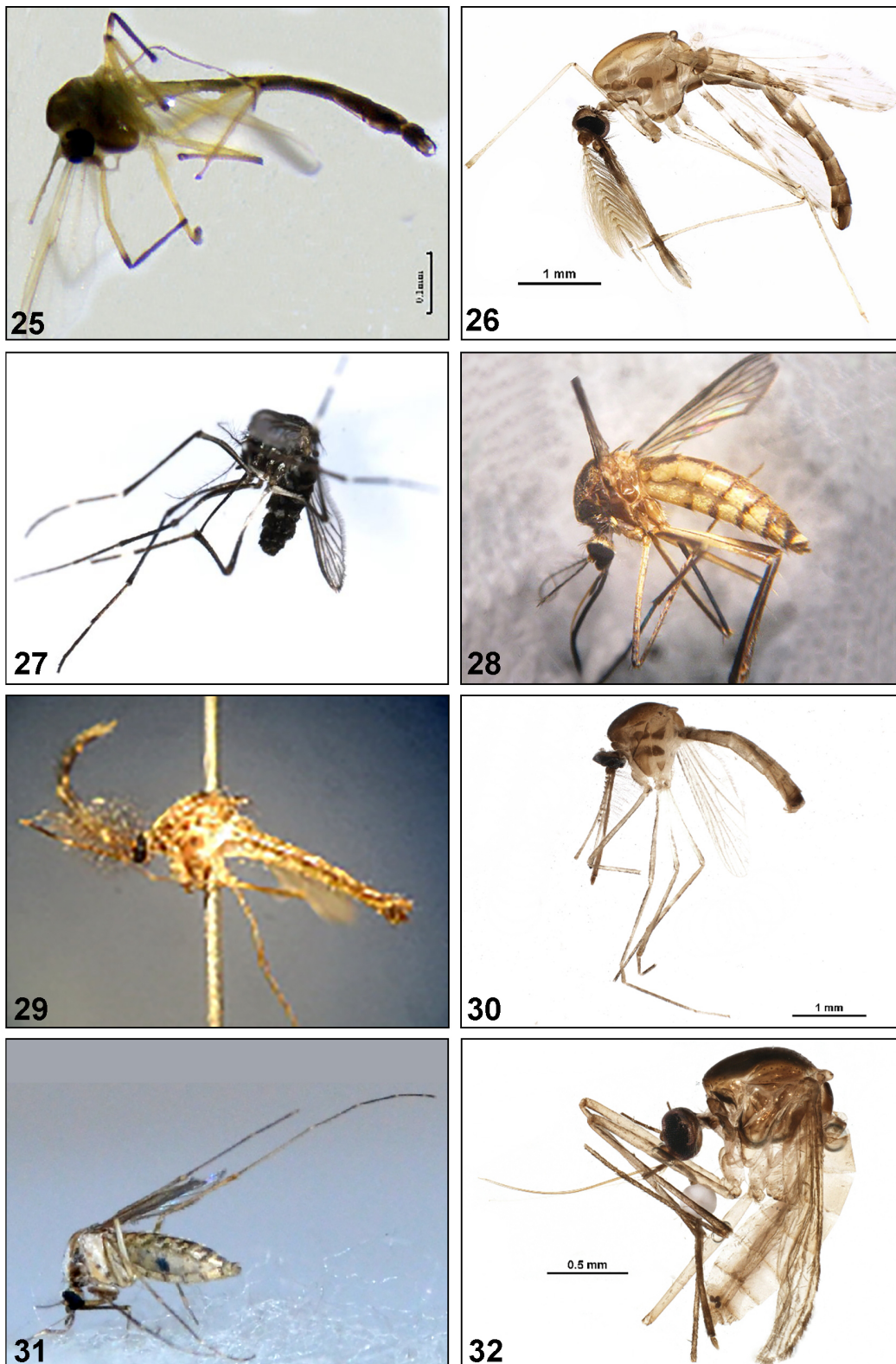
**Figures 1-8.** General habitus of True flies collected in Bangladesh. **1.** *Melanagromyza cleomae*; **2.** *Melanagromyza obtusa*; **3.** *Ophiomyia phaseoli*; **4.** *Calycomyza lantanae*; **5.** *Chromatomyia horticola*; **6.** *Liriomyza sativae*; **7.** *Phytomyza thalictrella*; **8.** *Feltiella acarisuga*.



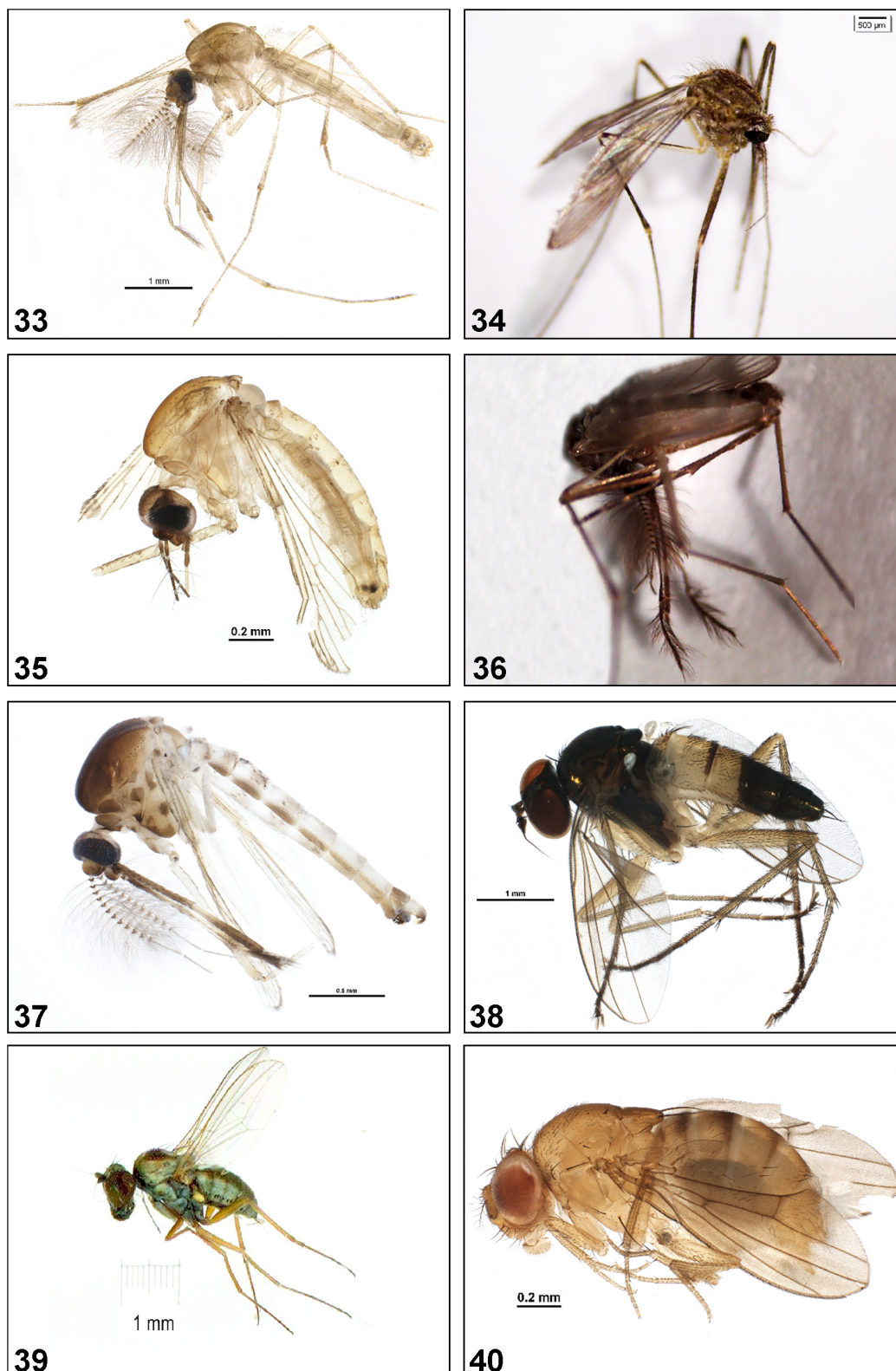
**Figures 9–16.** General habitus of True flies collected in Bangladesh. **9.** *Spaniocelyphus bigoti*; **10.** *Culicoides actoni*; **11.** *Culicoides brevipalpis*; **12.** *Culicoides innoxius*; **13.** *Culicoides orientalis*; **14.** *Culicoides oxystoma*; **15.** *Culicoides peregrines*; **16.** *Culicoides shortti*.



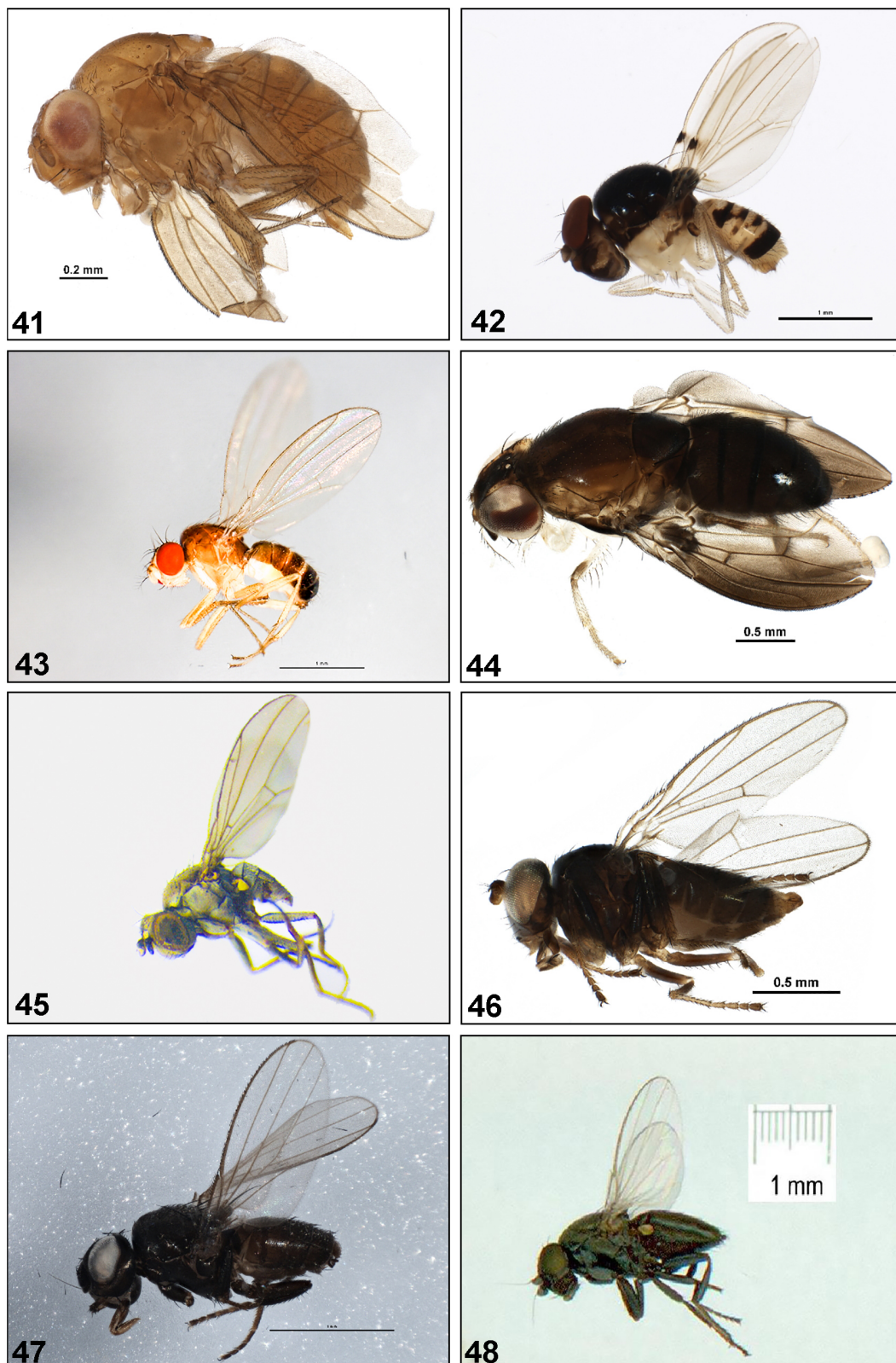
**Figures 17-24.** General habitus of True flies collected in Bangladesh. **17.** *Culicoides similis*; **18.** *Culicoides sumatrae*; **19.** *Chironomus circumdatus*; **20.** *Chironomus crassiforceps*; **21.** *Chironomus javanus*; **22.** *Kiefferulus calligaster*; **23.** *Microchironomus cf. tener*; **24.** *Polypedilum cf. allansoni*.



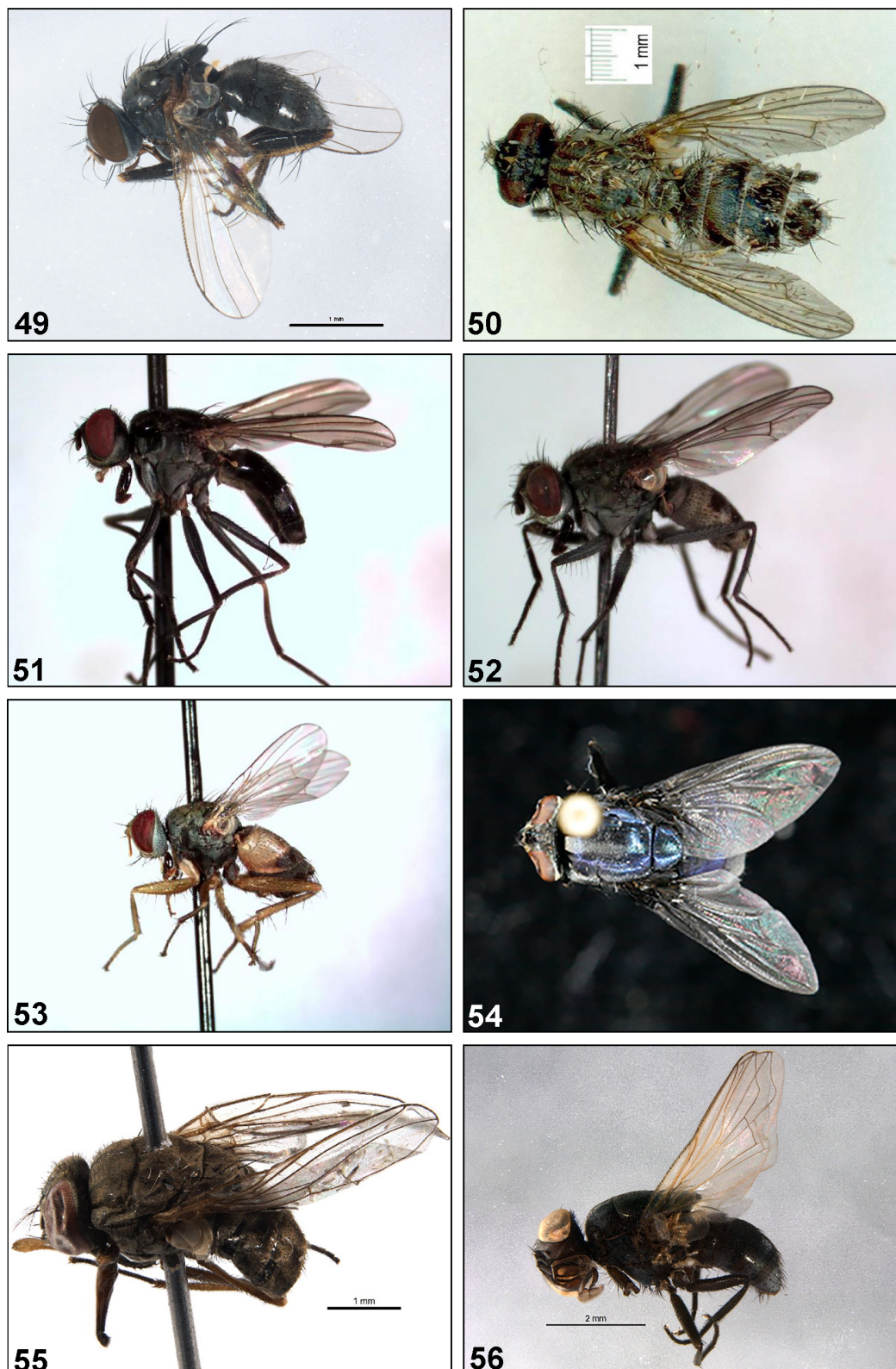
**Figures 25–32.** General habitus of True flies collected in Bangladesh. **25.** *Tanytarsus formosanus*; **26.** *Anopheles vagus*; **27.** *Aedes albopictus*; **28.** *Armigeres subalbatus*; **29.** *Culex bitaeniorhynchus*; **30.** *Culex fuscocephala*; **31.** *Culex gelidus*; **32.** *Culex infantulus*.



**Figures 33–40.** General habitus of True flies collected in Bangladesh. **33.** *Culex pallidothorax*; **34.** *Culex pipiens*; **35.** *Culex rubithoracis*; **36.** *Culex tritaeniorhynchus*; **37.** *Culex vishnui*; **38.** *Paraclius digitatus*; **39.** *Medetera grisescens*; **40.** *Drosophila albomicans*.

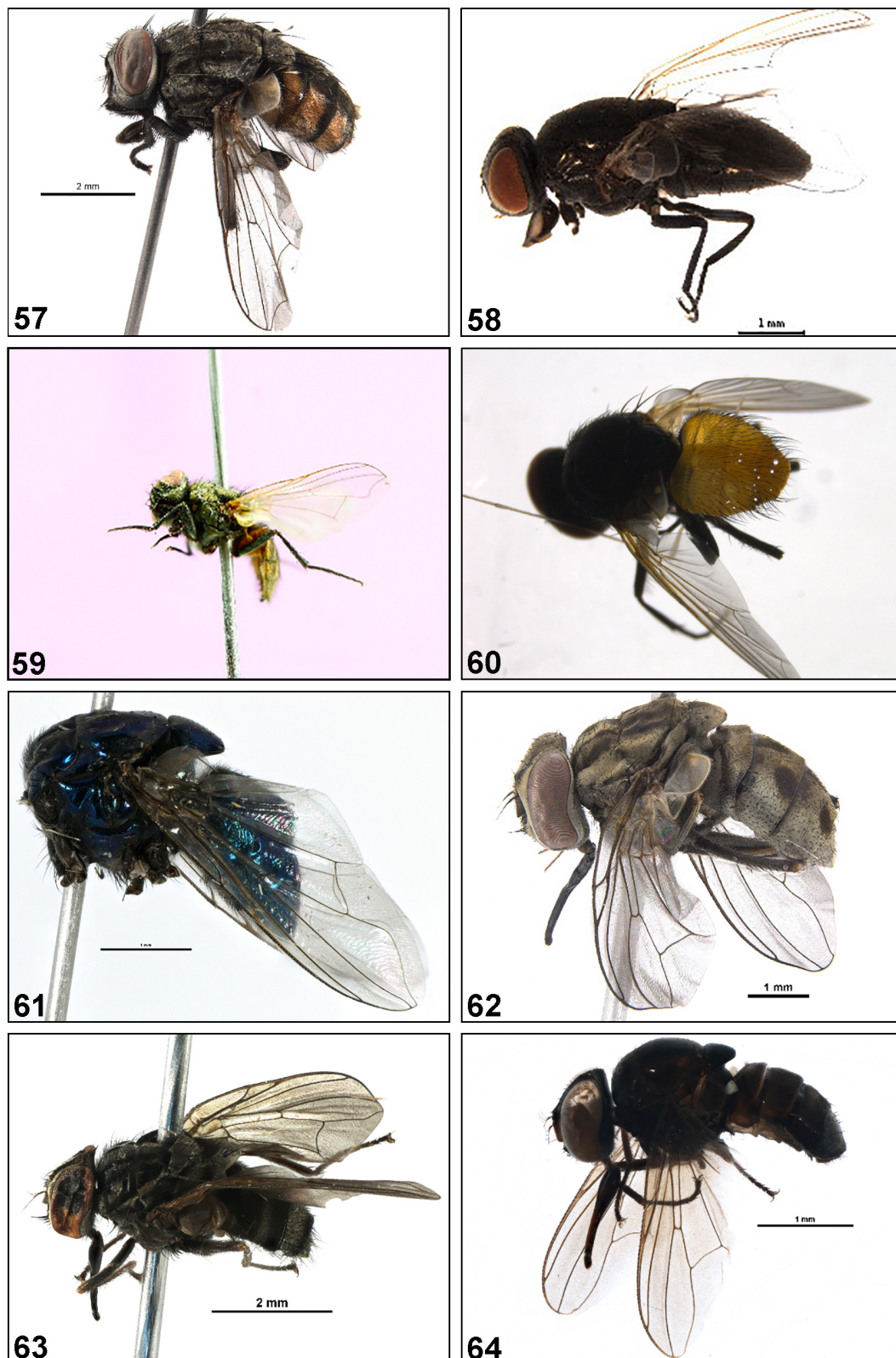


**Figures 41–48.** General habitus of True flies collected in Bangladesh. **41.** *Drosophila sulfurigaster*; **42.** *Mycodrosophila aqua*; **43.** *Scaptomyza pallid*; **44.** *Stegana kanmiyai*; **45.** *Hydrellia flaviceps*; **46.** *Hydrellia pakistanae*; **47.** *Desmometopa sordida*; **48.** *Desmometopa varipalpis*.

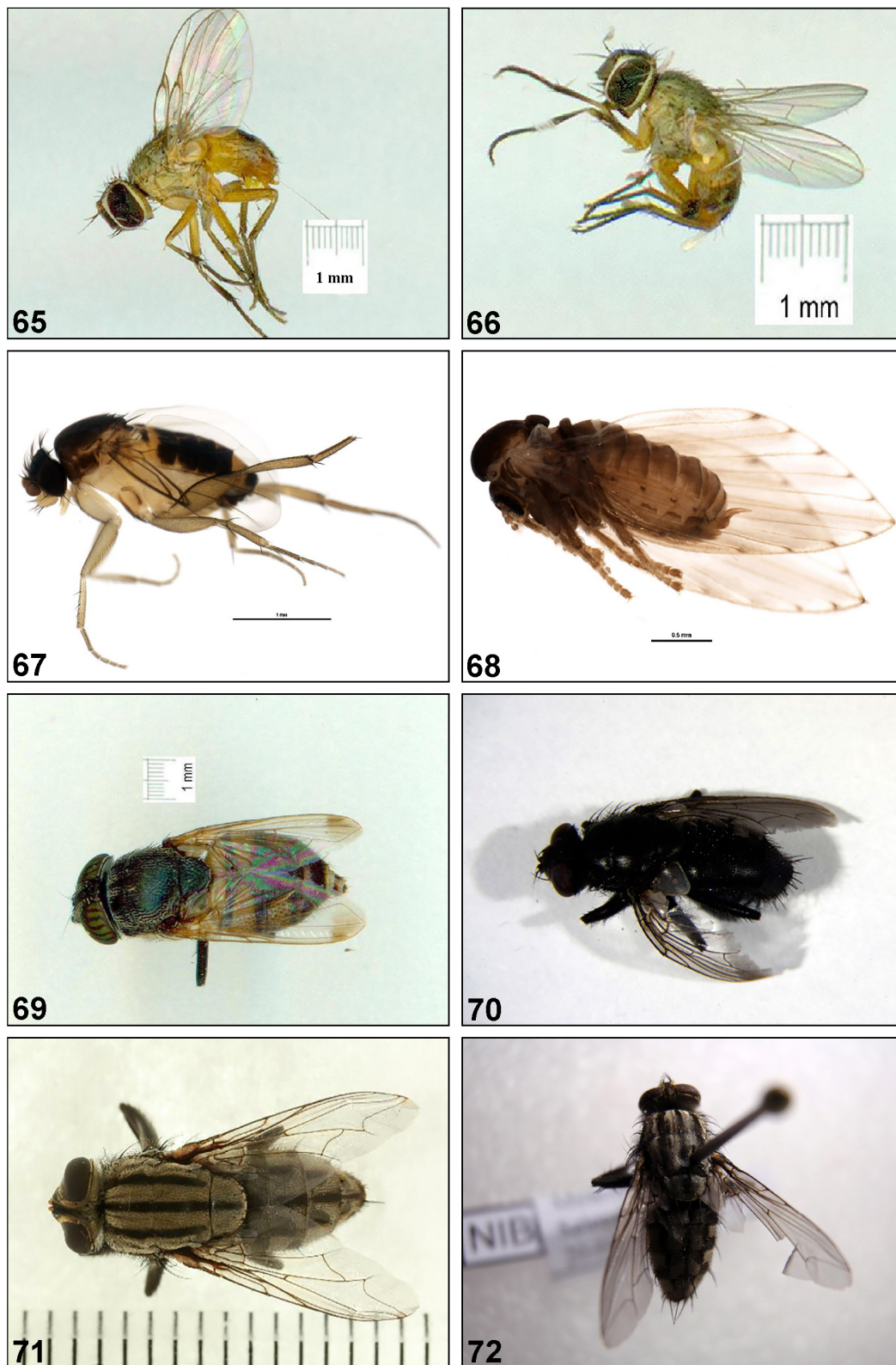


**Figures 49–56.** General habitus of True flies collected in Bangladesh. **49.** *Coenosia attenuata*; **50.** *Lispe assimilis*; **51.** *Lispe nicobarensis*; **52.** *Lispe sericipalpis*; **53.** *Pygophora respondens*; **54.** *Haematobia irritans*; **55.** *Haematobosca sanguinoleanta*; **56.** *Musca cnofiscate*.

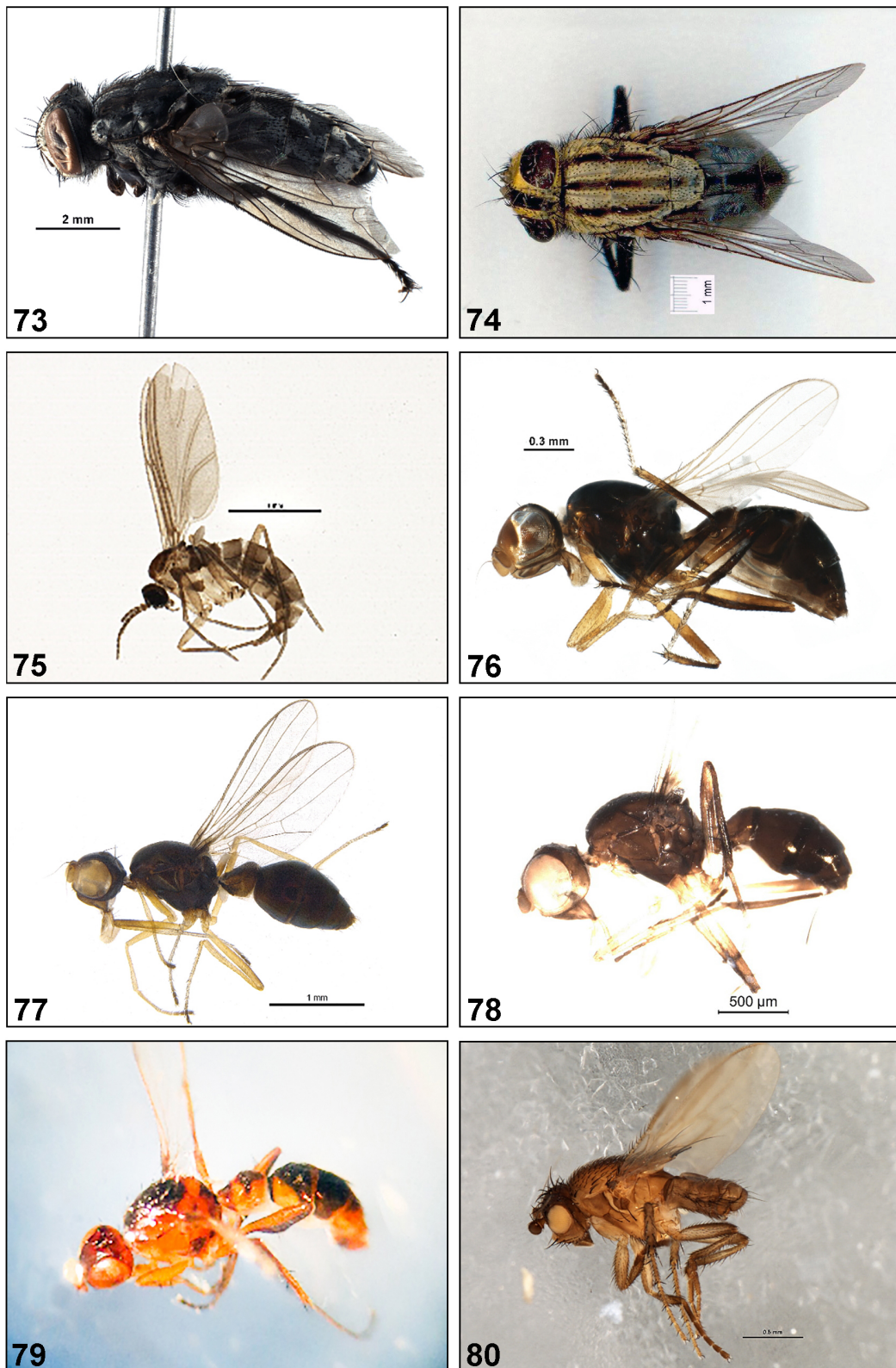




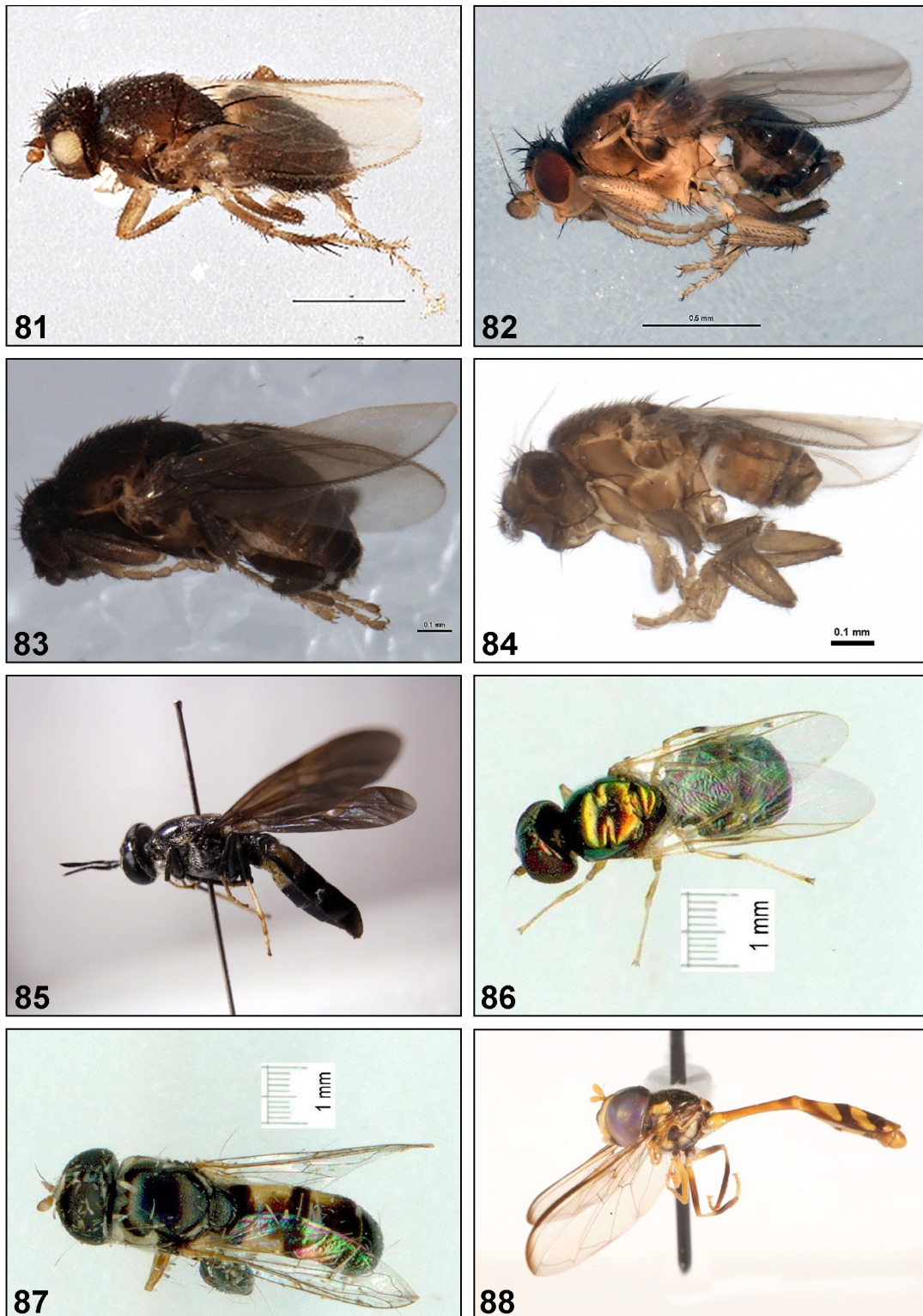
**Figures 57–64.** General habitus of True flies collected in Bangladesh. **57.** *Musca convexifrons*; **58.** *Musca crassirostris*; **59.** *Musca domestica*; **60.** *Musca ventrosa*; **61.** *Neomyia timorensis*; **62.** *Stomoxys calcitrans*; **63.** *Stomoxys indicus*; **64.** *Stomoxys uruma*.



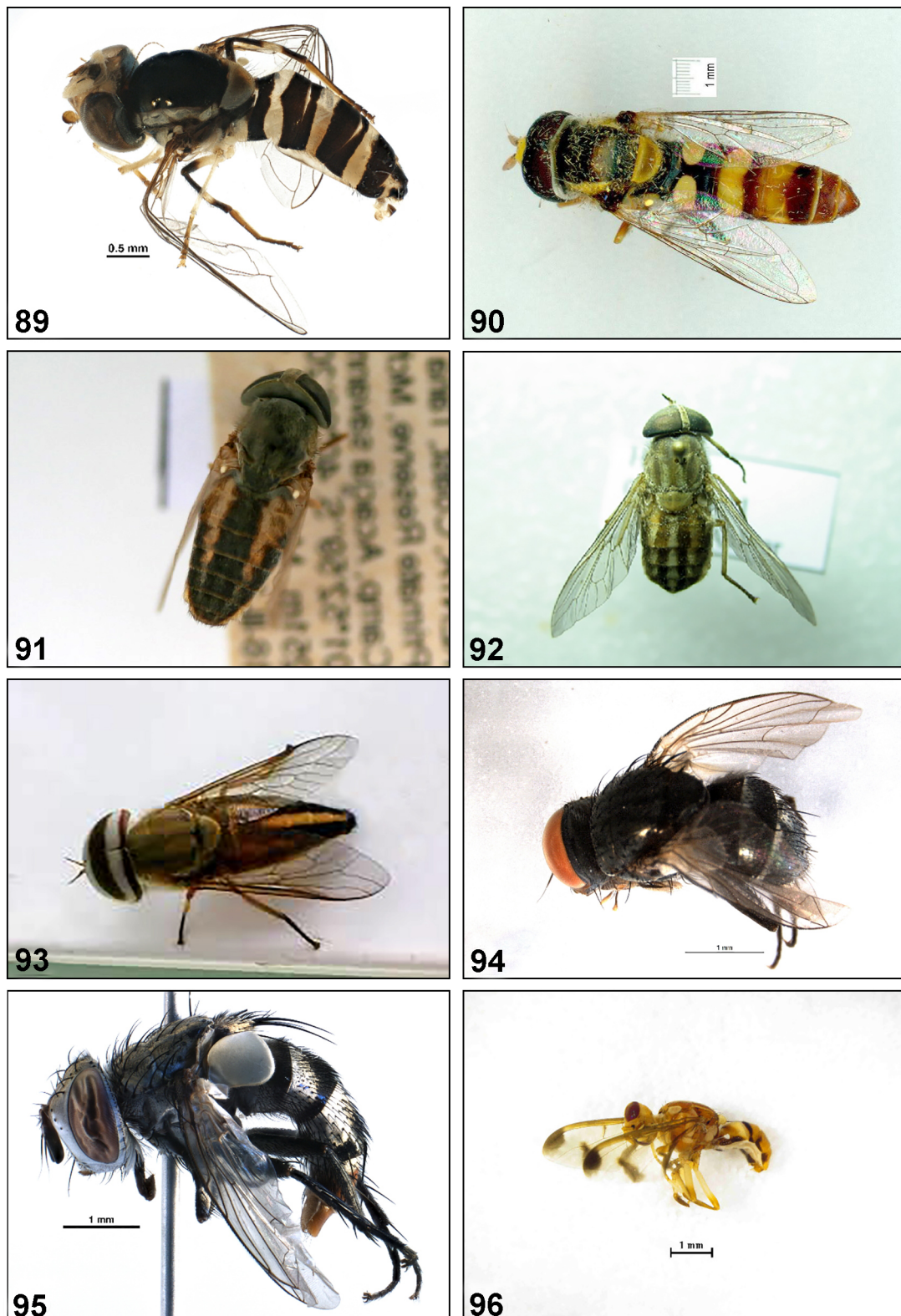
**Figures 65-72.** General habitus of True flies collected in Bangladesh. **65.** *Atherigona orientalis*; **66.** *Atherigona oryzae*; **67.** *Dohrniphora cornuta*; **68.** *Psychoda alternate*; **69.** *Stomorhina discolor*; **70.** *Sarcophaga albiceps*; **71.** *Sarcophaga misera*; **72.** *Sarcophaga princeps*.



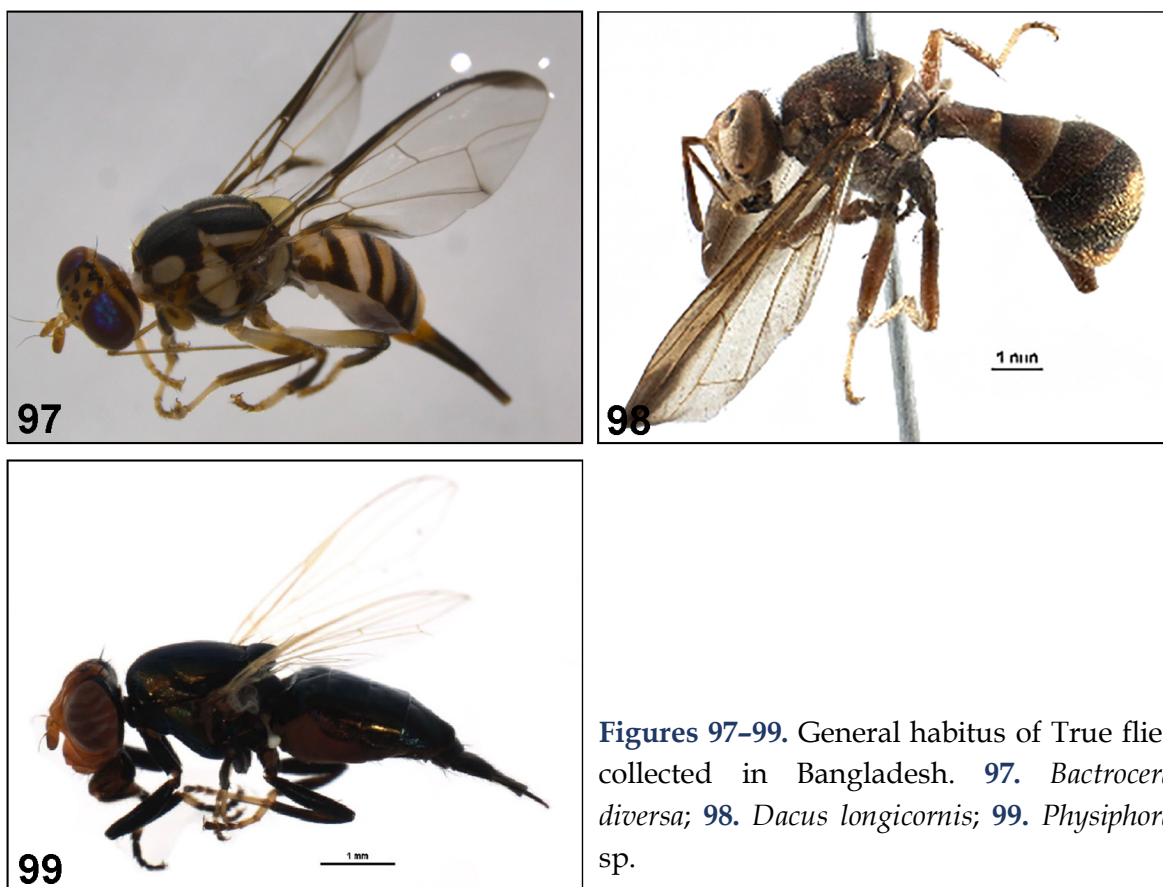
**Figures 73–80.** General habitus of True flies collected in Bangladesh. **73.** *Sarcophaga scopariiformis*; **74.** *Sarcophaga taenionota*; **75.** *Bradysia ocellaris*; **76.** *Australosepsis frontalis*; **77.** *Dicranosepsis crinite*; **78.** *Sepsis dissimilis*; **79.** *Sepsis nitens*; **80.** *Coproica ferruginata*.



**Figures 81–88.** General habitus of True flies collected in Bangladesh. **81.** *Coproica hirtula*; **82.** *Spelobia bifrons*; **83.** *Trachypella lineafrons*; **84.** *Trachypella straminea*; **85.** *Hermetia illucens*; **86.** *Microchrysa flaviventris*; **87.** *Syritta orientalis*; **88.** *Allobaccha amphithoe*.



**Figures 89–96.** General habitus of True flies collected in Bangladesh. **89.** *Allograpta medanensis*; **90.** *Simosyrphus scutellaris*; **91.** *Atylotus agrestis*; **92.** *Tabanus rubidus*; **93.** *Tabanus striatus*; **94.** *Aplomya metallica*; **95.** *Exorista xanthaspis*; **96.** *Bactrocera cucurbitae*.



**Figures 97-99.** General habitus of True flies collected in Bangladesh. 97. *Bactrocera diversa*; 98. *Dacus longicornis*; 99. *Physiphora* sp.

## Discussion

In Bangladesh, some species of three families Micropezidae (Jui et al., 2007; Hill, 2008), Canacidae (Mathis, 1992), and Anthomyiidae (Miah et al., 1997) were reported previously by different authors mentioned in parenthesis but no species were confirmed in the present study. Whilst, various authors were reported varieties of true flies that are also found in the current study. For instance, Huda (1981) noted 30 species of genus *Anopheles*. Afterwards, Huda & Majumder (1985) recorded *Anopheles vagus*, *Aedes albopictus*, *Culex tritaeniorhynchus*, *Cu. vishnui* from CUC. Also, Huda (2000) reported that out of 26 species of genus *Aedes* only *Ae. aegypti* and *Ae. albopictus* are responsible for dengue fever. In the present study *Ae. albopictus* was confirmed only.

Rahman et al. (1983) reported *Melanagromyza phaseoli* (Agromyzidae) as dipteran pest on soybean. Ali et al. (1986) evaluated abundance of *Culicoides* sp. (Ceratopogonidae) and *Chironomus* sp. (Chironomidae) at different depth range in a pond. Iwasa et al. (1991) reported a new species *Toxopoda mordax* (Sepsidae) from the country. Kabir et al. (1991) noted five species of *Dacus* sp. and their 16 host plants. Hill (2008) noted *Mimegralla coerubifrons* (Micropezidae) as a minor pest of Ginger (*Zingiber officinale*). Mazumdar et al. (2010, 2014) recorded *Allograptia* sp. *Anopheles* sp. (male), *Chrysomya megacephala*, *Musca domestica*, *Paragus serratus* as flower visiting insects. Biswas & Das (2011) reported *Asphondylia sesami* as pest of Sesame. Catling & Islam (2013) recorded *Atherigona oryzae*, *A. falcata*, *Anatrichus pygmaeus*, *Atherigona reversura*, *Pachylophus rufescens* and *Hydrellia*

*philippina* from a deepwater Rice field. Four agromyzid leafminers including *Liriomyza chinensis*, *L. sativae*, *Melanagromyza obtusa* and *Ophiomyia phaseoli* as well as their 17 plant hosts confirmed by Mazumdar & Bhuiya (2014). Asaduzzaman et al. (2015) identified *Culicoides* spp. (Ceratopogonidae) as a vector nematodes caused for Onchocercosis affect mainly cattle, and also buffaloes. Mazumdar & Bhuiya (2016) recorded Cecidomyiidae (unidentified specimens). Bashir et al. (2016) identified *Aedes aegypti*, *Ae. albopictus*, *Anopheles vagus*, *Culex gelidus*, *Cu. tritaeniorhynchus* from Dhaka city. Irish et al. (2016) recorded for 123 species of mosquitoes including *Anopheles pseudowillmori*, *Armigeres malayi* and *Mimomyia luzonensis*. Khan et al. (2017) reported six different bamboo-shoot fly species like *Felderimyia gombakensis*, *Rioxoptilona dunlopi*, *R. vaga*, *Acroceratitis distincta*, *A. ceratitina* and *Gastrozona soror* from Dhaka. Sultana et al. (2017) studied the association of five mosquito species namely *Aedes aegypti*, *Ae. albopictus*, *Armigeres subalbatus* and *Culex tritaeniorhynchus* with their breeding habitat diversity and species distribution in the parks of Dhaka city. Hossain et al. (2017) observed the abundance of *Bactrocera zonata* in mango orchards. Islam et al. (2017) reported *Lucilia cuprina*, *Chrysomya* sp., *Musca domestica* as Dipteran fly pests of dried fishes at the Sonadia offshore island, Cox's Bazar. Mazumdar (2019) reported *Medetera* sp. (Medeterinae) from Chittagong City.

By applying DNA barcoding in Bangladesh, Amin et al. (2014) confirmed leafminer pest *Liriomyza sativae* (Agromyzidae). Alam et al. (2015) identified four *Drosophila* species like *Drosophila ananassae*, *D. parabipectinata*, *D. repleta* and *D. melanogaster*. Leblanc et al. (2019) surveyed for fruit fly, and a new species, 33 new country records and discovery of the highly invasive *Bactrocera carambolae*. Based on morphological and molecular identification, Siddiki et al. (2019) reported *Aedes aegypti*, *Ae. albopictus* and *Culex pipiens* from Chittagong city. Mazumdar (2020) ensured *Ae. albopictus* from Chittagong University Campus through DNA barcoding technique.

The present endeavor was to assess the dipterans diversity by applying DNA barcoding of Malaise trap samples. The results of the current study may a taxonomic baseline study of agro-dipterans for further research.

### Acknowledgments

The authors are extremely grateful to associates at the Centre for Biodiversity Genomics, University of Guelph, for supporting with sequence analysis.

### Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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## بررسی دوبالان (Insecta: Diptera) جمع‌آوری شده توسط تله مالیز با استفاده از DNA بارکدینگ در بنگلادش

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| تاریخ دریافت: ۲۳ مرداد ۱۳۹۹ | تاریخ پذیرش: ۲۰ مهر ۱۳۹۹ | تاریخ انتشار: ۲۴ مهر ۱۳۹۹ |

**چکیده:** دوبالان (Insecta: Diptera) پراکنش زیادی داشته و یکی از اجزای اصلی اغلب اکوسیستم‌ها هستند. هدف از این مطالعه، شناسایی تنوع دوبالان از طریق بارکدگذاری DNA (توالی ۶۵۸ جفت‌باز از انتهای ۵' سیتوکروموکسیداز ۱) در بنگلادش بود. نمونه‌ها توسط تله مالیز در پردیس دانشگاه چیتاگونگ بین آوریل ۲۰۱۴ تا مارس ۲۰۱۵ جمع‌آوری شدند. در این مطالعه، ۳۶۴۷۶ توالی از ۳۸۲۶۷ نمونه از دوبالان بررسی گردید و ۱۰۵ گونه، ۱۰۹ جنس، ۵۴ زیرخانواده و ۵۹ خانواده ثبت شد. از میان آنها، ۷۹ گونه، ۶۹ جنس، ۱۲ زیرخانواده و ۲۳ خانواده برای فون دوبالان بنگلادش گزارش جدید هستند. تمام گونه‌های گزارش شده با شماره شاخص بارکد (BINs) (پروکسی‌های گونه) در سامانه Barcode of Life Data System (BOLD) موجود است.

**واژگان کلیدی:** دوبالان، تله مالیز، بارکد DNA، بنگلادش