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Parasitic Hymenoptera Recovered by DNA Barcoding of Malaise Trap Collection at the Chittagong University Campus, Bangladesh

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Abstract: In the natural ecosystems, parasitic Hymenoptera composes the most significant group of biocontrol agents. DNA barcode (658 bp sequence from the 5′-end of cytochromeoxidase I) analysis of hymenopterans collected in a Malaise trap in Chittagong university campus was performed to analyze the diversity of parasitic wasps. In the present study a total of 3,468 sequences were generated that represented 31 species, 83 genera and 22 families from seven superfamilies of Hymenoptera. Among them 25 species namely *Aphanogmus fijiensis* Ferriere, *Telenomus remus* Nixon, *Ganaspis xanthopoda* Ashmead, *Encarsia sophia* Girault & Dodd, *Copidosoma floridanum* Ashmead, *C. thebe* Walker, *Ceranisus menes* Walker, *Hemiptarsenus varicornis* Girault, *Eupelmus martellii* Masi, *Trichogramma achaeae* Nagaraja and Nagarkatti, *Trichogrammatoidea bactrae* Nagaraja, *Binodoxys acalephae* Marshall, *B. communis* Gahan, *Aspidobracon noyesi* van Achterberg, *Bracon crassicornis* Thomson, *Cardiochiles fuscipennis* Szepligeti, *Apanteles boaris* Walker, *Glyptapanteles creatonoti* Viereck, *Phaedrotoma depressa* Li & van Achterberg, *Psyttalia fletcheri* Silvestri, *Aleiodes malichi* Quicke & Butcher, *A. prillae* Quicke & Butcher, *A. sutthisani* Quicke & Butcher, *Diplazon orientalis* Cameron, *Exochus pictus* Holmgren, 58 genera, 12 subfamilies and 7 named families specifically Megaspilidae, Figitidae, Eupelmidae, Ormyridae, Perilampidae, Torymidae and Trigonalyidae are the first reports from Bangladesh. The results of this study will be resulted to determining of parasitic hymenopteran fauna in Bangladesh.

Keywords: Parasitic Hymenoptera, Malaise Trap, DNA Barcode, Bangladesh

1. Introduction

Parasitic Hymenoptera are natural control agents for insects mainly in the terrestrial habitats. About one quarter [1] of the 150,000 described species of Hymenoptera are parasites [1-2]. DNA barcoding (658 bp sequence from the 5′ end of COI) is an effective method to identify animal species [3]. DNA barcoding of arthropods collected by Malaise traps has gained popularity for assessing terrestrial insect diversity [4], this method is leading to its frequent use in insect biodiversity assessments [5]. A study coupled Malaise trap and DNA barcoding to analyze the diversity of parasitic Hymenoptera in a sub-arctic environment [6]. In Bangladesh, the study of parasitic hymenopterans has been properly studied. The study presents a preliminary list of parasitic Hymenoptera that were collected by Malaise trap at the Chittagong university campus of Bangladesh and analyzed using DNA barcoding. Though rationally many more parasitic Hymenoptera still remain undescribed, present taxonomic work will be a reference for future work in Bangladesh.

2. Materials and Methods

2.1. Specimen Collection, Processing, Identification and Specimen Deposition

A Townes-style Malaise trap (BioQuip Inc. USA) was installed 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 [7]. Insects were collected from March 2014 to 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, following standard barcoding protocols [8], at the Canadian Centre for DNA Barcoding within the Centre for Biodiversity Genomics, University of Guelph, Canada. Collection data, voucher information and taxonomy for each specimen are available in the Barcode of Life Data Systems [9]. All the specimens analyzed in this study have been curated at the Centre for Biodiversity Genomics, University of Guelph, Guelph, Ontario, Canada.

2.2. Molecular Analysis and Data Analysis

DNA extracts were prepared from a single leg from each large specimen 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 atthe Canadian Centre for DNA Barcoding following the standard protocols (CCDB). PCR amplification of COI-5′ was performed with primers C_LepFolF and C_LepFolR [10] 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) [11]. With a few exceptions, by considering sequence matches to records on BOLD, the specimens with barcodes were assigned to 22 families of 8 superfamilies.

3. Result

Table 1 presents the parasitic hymenoptera from Bangladesh analyzed in this study. The specimens represent six superfamilies *viz.* Ceraphronoidea (1 species/1 genus), Diaprioidea (no species/1 genus), Platygastroidea (1 species/2 genera), Cynipoidea (1 species/1 genus), Chalcidoidea (10 species/28 genera), Ichneumonoidea (16 species/49 genera). In Evanioidea and Trigonalyidea only family Evaniidae and Trigonalyidae, respectively were confirmed.

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

Superfamily	Family	Subfamily	Scientific name	Recorded from Bangladesh
Ceraphronoidea Haliday 1833	CeraphronidaeHaliday 1833	-	*Aphanogmus fijiensis* Ferriere 1933	New record
	Megaspilidae	-	-	New record
Evanioidea Latreille 1802	Evaniidae Latreille 1802	-	-	[12]
Diaprioidea Halliday 1833	Diapriidae Halliday 1833	Diapriinae Haliday 1833	*Trichopria* Ashmead1893	New record
PlatygastroideaHaliday 1833	Platygastridae Förster 1856	Scelioninae Förster 1856	*Telenomus remus* (Nixon 1937)	New record
		Platygastrinae Haliday 1833	*Leptacis* Förster 1856	New record
Cynipoidea Latreille 1802	Cynipidae Latreille 1802	-	-	New record
Chalcidoidea Latreille 1817	Figitidae Hartig 1840	Eucoilinae Thomson 1862	*Ganaspis xanthopoda* (Ashmead1896)	New record
	Aphelinidae Thomson 1876	Aphelininae Thomson 1876	*Aphelinus* Dalman 1820	[13-15]
			Aphytis Howard 1900	[15]
		CoccophaginaeFörster 1878	*Centrodora* Förster 1878	New record
			Coccobius Ratzeburg1852	New record
			Encarsia Sophia Girault & Dodd 1915	New record
		Eretmocerinae Shafee & Khan 1978	*Eretmocerus* Haldeman 1850	[15]
	Chalcididae Latreille 1817	-	-	[12- 15]
	Encyrtidae Walker 1837	Encyrtinae Howard 1881	*Copidosoma floridanum* Ashmead 1900	New record
			C. thebe (Walker 1838)	New record
			Syrphophagus Ashmead 1900	New record
	Eulophidae Westwood (Förster 1856)	Entedontinae Förster 1856	*Asecodes* Förster1856	New record
			Ceranisus menes (Walker1839)	New record
			Closterocerus Westwood 1833	New record
			Neochrysocharis formosa (Westwood 1833)	[16]
		Eulophinae Westwood 1829	*Hemiptarsenus varicornis* (Girault 1913)	New record
		Tetrastichinae	*Aprostocetus* Westwood 1833	New record

Superfamily	Family	Subfamily	Scientific name	Recorded from Bangladesh
		Graham 1987		
	Eupelmidae Walker 1833	Eupelminae Walker 1833	*Anastatus* Motschulsky 1859	New record
			Eupelmus martellii Masi 1941	New record
			Zaischnopsis 1904	New record
	Eurytomidae Walker 1832	-	-	[17]
	Mymaridae Haliday 1833		*Alaptus* Westwood 1839	New record
			Anagrus Haliday 1833	[18]
			Anaphes Haliday1833	[18]
			Gonatocerus Nee 1834	[19]
			Lymaenon Walker 1846	New record
			Mymar Curtis 1829	New record
			Neomymar Crawford1913	New record
			Omyomymar Schauff (1983)	New record
			Stethynium Enock1909	[18]
	Ormyridae Förster 1856	-	-	New record
	Perilampidae Latreille 1809		-	New record
	Pteromalidae Dalman 1820	-	-	[20]
	Torymidae	-	-	New record
	Trichogrammatidae Haliday1851	-	*Trichogramma achaeae* Nagaraja and Nagarkatti 1970	New record
		-	*T. chilonis* Ishii1941	[21, 22]
		-	*Trichogrammatoidea bactrae* Nagaraja1979	New record
Ichneumonoidea Latreille 1802	Braconidae Latreille 1829	Alysiinae Leach 1815	*Asobara* Förster 1862	New record
			Dinotrema Förster 1862	New record
		Aphidiinae Haliday 1833	*Binodoxys acalephae* (Marshall 1896)	New record
			B. communis (Gahan 1926)	New record
			Lipolexis oregmae (Gahan 1932)	[21]
		Brachistinae Förster 1862	-	New record
		Braconinae Nees 1811	*Aspidobracon noyesi* van Achterberg 1984	New record
			Bracon crassicornis Thomson 1892	New record
			B. hebetor Say 1836	[12, 23]
			Cratocnema Szépligeti 1914	New record
			Physaraia Shenefelt 1978	New record
			Plesiobracon Cameron (1903)	New record
			Pycnobracon Cameron 1902	New record
			Syntomernus Enderlein 1920	New record
			Tropobracon Cameron1905	[12, 23]
		CardiochilinaeAshmead 1900	*Cardiochiles fuscipennis* Szepligeti 1900	New record
		Cheloninae Förster 1862	*Chelonus* Panzer 1806	[12, 23]
		Doryctinae Förster 1862	*Rhaconotus* Ru 1854	[12, 23]
			Spathius Nees 1818	[23-24]
		Euphorinae Förster 1862	-	New record
		Exothecinae Förster 1862	*Shawania* van Achterberg 1983	New record
		Gnamptodontinae Fischer 1970	*Gnamptodon* Haliday 1833	New record
		Hormiinae Förster 1862	*Hormius* Nees 1818	New record
		Ichneutinae Förster 1862	*Paroligoneurus* Muesebeck 1931	New record
		Lysiterminae Tobias 1968	*Pentatermus* Hedqvist 1963	New record
		Microgastrinae Förster 1862	*Apanteles boaris* Walker	New record
			Choeras Mason 1981	New record
			Cotesia Cameron 1891	[12]
			Diolcogaster Ashmead 1900	New record
			Dolichogenidea Viereck 1911	New record
			Glyptapanteles Creatonoti (Viereck)	New record
			Microplitis Förster 1863	[12]
			Neoclarkinella Rema & Narendran 1996	New record
			Parapanteles Ashmead (1900)	New record

Superfamily	Family	Subfamily	Scientific name	Recorded from Bangladesh
			Xanthomicrogaster Cameron 1911	New record
		Opiinae Blanchard 1845	*Opius* Wesmael 1835	[12]
			Phaedrotoma depressa Li & van Achterberg 2013	New record
			Psyttalia fletcheri Silvestri 1916	New record
		Orgilinae Ashmead 1900	-	New record
		Pambolinae Marshall 1885	*Pambolus* Haliday 1836	New record
		Rogadinae Ashmead 1900	*Aleiodes malichi* Quicke & Butcher 2012	New record
			A. prillae Quicke & Butcher 2012	New record
			A. sutthisani Quicke & Butcher 2012	New record
			Clinocentrus Haliday 1833	New record
	IchneumonidaeLatreille 1802	AnomaloninaeViereck 191	-	[25]
		Banchinae Wesmael 1845	*Apophua* Morley 1913	New record
		BrachycyrtinaeViereck 1919	*Brachycyrtus* Kriechbaumer 1880	[25]
		CampopleginaeFörster 1869	*Xanthocampoplex* Morley 1913	[25]
		Cryptinae Kirby 1837	-	New record
		Diplazontinae Viereck 1918	*Diplazon orientalis* (Cameron 1905)	New record
		IchneumoninaeLatreille1802	-	[12]
		Mesochorinae Förster 1869	*Stictopisthus* Thomson 1886	[12, 26]
		Metopiinae Förster 1869	*Acerataspis* Uchida (Townes 1971)	New record
			Exochus pictus Holmgren 1858	New record
			Metopius Panzer1806	[26]
			Triclistus Förster 1869	New record
		Ophioninae Shuckard 1840	*Enicospilus* Stephens 1835	[25]
		Orthocentrinae Förster1869	-	New record
		Pimplinae Wesmael 1845	*Theronia* Holmgren 1859	[26]
			Xanthopimpla Saussure 1892	New record
TrigonaloideaCarmean and Kimsey (1998) Trigonalidae (=Trigonalyidea)	TrigonalyidaeCresson 1887	-	-	New record

4. Discussion

In Bangladesh, Rahman provided a list of 34 species from 17 genera of braconid parasitoids from different crop pest [27]. Gapud compiled a list of 182 species of parasitic hymenopterans [12]. Miah *et al.* prepared a preliminary list of parasitic wasps of family Ichneumonidae representing 28 genera and 57 species under 9 subfamilies [25]. Ahmad *et al*. mentioned 236 species of Hymenoptera in their book [28]. Bhuiya and Miah listed 65 species under 2 subfamilies and 33 genera belonging to Encyrtidae [29]. Mazumdar *et al.* reported 72 species in 36 genera under 14 families of Hymenoptera [1]. Recently, Huber and Islam provided identification key to 10 species in 4 genera of the family Mymaridae [18]. *Trichogramma* spp. and *Bracon hebetor* are commercially reared and implemented in controlling various vegetable pests of Bangladesh [30]. In the present study, 25 species, 58 genera, 12 subfamilies, 7 families and 1 Superfamily have been newly recorded from Bangladesh.

5. Conclusion

This piece of work is the first extensive taxonomic work to explore the natural enemies present in Bangladesh. This taxonomic work will be an excellent reference for future work. On the basis of this work and with the addition of further works suitable biocontrol agents will be find out from parasitic Hymenoptera for uses in different sectors.

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Conflict of Interest Statement

There are no conflicts of interest.

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