

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 cytochrome oxidase 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, *Ceraninus 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, *Phaerotoma depressa* Li & van Achterberg, *Psytalia 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 at the 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 Trigonalioidea only family Evanidae and Trigonalidae, 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	Ceraphronidae Haliday 1833	-	<i>Aphanogmus fijiensis</i> Ferriere 1933	New record
	Megaspilidae	-	-	New record
Evanioidea Latreille 1802	Evanidae Latreille 1802	-	-	[12]
Diaprioidea Haliday 1833	Diapriidae Haliday 1833	Diapriinae Haliday 1833	<i>Trichopria</i> Ashmead 1893	New record
Platygastroidea Haliday 1833	Platygastriidae Förster 1856	Scelioninae Förster 1856	<i>Telenomus remus</i> (Nixon 1937)	New record
		Platygastriinae Haliday 1833	<i>Leptacis</i> Förster 1856	New record
Cynipoidea Latreille 1802	Cynipidae Latreille 1802	-	-	New record
	Figitidae Hartig 1840	Eucoilinae Thomson 1862	<i>Ganaspis xanthopoda</i> (Ashmead 1896)	New record
Chalcidoidea Latreille 1817	Aphelinidae Thomson 1876	Aphelininae Thomson 1876	<i>Aphelinus</i> Dalman 1820	[13-15]
			<i>Aphytis</i> Howard 1900	[15]
		Coccophaginae Förster 1878	<i>Centrodora</i> Förster 1878	New record
			<i>Coccobius</i> Ratzeburg 1852	New record
			<i>Encarsia Sophia</i> Girault & Dodd 1915	New record
		Eretmocerinae Shafee & Khan 1978	<i>Eretmocerus</i> Haldeman 1850	[15]
	Chalcididae Latreille 1817	-	-	[12-15]
	Encyrtidae Walker 1837	Encyrtinae Howard 1881	<i>Copidosoma floridanum</i> Ashmead 1900	New record
			<i>C. thebe</i> (Walker 1838)	New record
			<i>Syrphophagus</i> Ashmead 1900	New record
	Eulophidae Westwood (Förster 1856)	Entedontinae Förster 1856	<i>Asecodes</i> Förster 1856	New record
			<i>Ceranisus menes</i> (Walker 1839)	New record
			<i>Closterocerus</i> Westwood 1833	New record
			<i>Neochrysocharis formosa</i> (Westwood 1833)	[16]
		Eulophinae Westwood 1829	<i>Hemiptarsenus varicornis</i> (Girault 1913)	New record
		Tetrastichinae	<i>Aprostocetus</i> Westwood 1833	New record

Superfamily	Family	Subfamily	Scientific name	Recorded from Bangladesh
	Eupelmidae Walker 1833	Graham 1987		
		Eupelminae	<i>Anastatus</i> Motschulsky 1859	New record
		Walker 1833	<i>Eupelmus martellii</i> Masi 1941	New record
	Eurytomidae Walker 1832	-	<i>Zaischnopsis</i> 1904	New record
			-	[17]
			-	
	Mymaridae Haliday 1833	-	<i>Alaptus</i> Westwood 1839	New record
			<i>Anagrus</i> Haliday 1833	[18]
			<i>Anaphes</i> Haliday 1833	[18]
			<i>Gonatocerus</i> Nee 1834	[19]
			<i>Lymaenon</i> Walker 1846	New record
			<i>Mymar</i> Curtis 1829	New record
			<i>Neomymar</i> Crawford 1913	New record
			<i>Omyomymar</i> Schauf (1983)	New record
			<i>Stethynium</i> Enock 1909	[18]
			-	New record
	Ormyridae Förster 1856	-	-	New record
	Perilampidae	-	-	New record
	Latreille 1809		-	
	Pteromalidae	-	-	[20]
	Dalman 1820	-	-	
	Torymidae	-	-	New record
	Trichogrammatidae	-	<i>Trichogramma achaeae</i> Nagaraja and Nagarkatti 1970	New record
	Haliday 1851	-	<i>T. chilonis</i> Ishii 1941	[21, 22]
		-	<i>Trichogrammatoidea bactrae</i> Nagaraja 1979	New record
Ichneumonoidea Latreille 1802	Braconidae Latreille 1829	Alysiinae Leach 1815	<i>Asobara</i> Förster 1862	New record
		Aphidiinae Haliday 1833	<i>Dinotrema</i> Förster 1862	New record
			<i>Binodoxys aculephae</i> (Marshall 1896)	New record
			<i>B. communis</i> (Gahan 1926)	New record
		Brachistinae Förster 1862	<i>Lipolexis oregmae</i> (Gahan 1932)	[21]
			-	New record
			<i>Aspidobracon noyesi</i> van Achterberg 1984	New record
		Braconinae Nees 1811	<i>Bracon crassicornis</i> Thomson 1892	New record
			<i>B. hebetor</i> Say 1836	[12, 23]
			<i>Cratocnema Szépligeti</i> 1914	New record
		Cardiochilinae Ashmead 1900	<i>Physaraia</i> Shenefelt 1978	New record
			<i>Plesiobracon</i> Cameron (1903)	New record
			<i>Pycnobracon</i> Cameron 1902	New record
		Cheloninae Förster 1862	<i>Syntomernus</i> Enderlein 1920	New record
			<i>Tropobracon</i> Cameron 1905	[12, 23]
			<i>Cardiochiles fuscipennis</i> Szépligeti 1900	New record
		Doryctinae Förster 1862	<i>Chelonus</i> Panzer 1806	[12, 23]
			<i>Rhaconotus</i> Ru 1854	[12, 23]
			<i>Spathius</i> Nees 1818	[23-24]
		Euphorinae Förster 1862	-	New record
			<i>Shawania</i> van Achterberg 1983	New record
			<i>Gnamptodon</i> Haliday 1833	New record
		Hormiinae Förster 1862	<i>Gnamptodon</i> Haliday 1833	New record
			<i>Hormius</i> Nees 1818	New record
			<i>Paroligoneurus</i> Muesebeck 1931	New record
		Ichneutinae Förster 1862	<i>Pentatermus</i> Hedqvist 1963	New record
			<i>Apanteles boaris</i> Walker	New record
			<i>Choeras</i> Mason 1981	New record
		Lysiterminae Tobias 1968	<i>Cotesia</i> Cameron 1891	[12]
			<i>Diolcogaster</i> Ashmead 1900	New record
			<i>Dolichogenidea</i> Viereck 1911	New record
		Microgastrinae Förster 1862	<i>Glyptapanteles</i>	New record
			<i>Creatonoti</i> (Viereck)	New record
			<i>Microplitis</i> Förster 1863	[12]
			<i>Neoclarkinella</i> Rema & Narendran 1996	New record
			<i>Parapanteles</i> Ashmead (1900)	New record

Superfamily	Family	Subfamily	Scientific name	Recorded from Bangladesh
		Opiinae Blanchard 1845	<i>Xanthomicrogaster</i> Cameron 1911	New record
			<i>Opius</i> Wesmael 1835	[12]
			<i>Phaeditoma depressa</i> Li & van Achterberg 2013	New record
			<i>Psytalia fletcheri</i> Silvestri 1916	New record
		Orgilinae Ashmead 1900	-	New record
			<i>Pambolus</i> Haliday 1836	New record
		Pambolinae Marshall 1885	<i>Aleiodes malichi</i> Quicke & Butcher 2012	New record
		Rogadinae Ashmead 1900	<i>A. prillae</i> Quicke & Butcher 2012	New record
			<i>A. sutthisani</i> Quicke & Butcher 2012	New record
			<i>Clinocentrus</i> Haliday 1833	New record
			-	[25]
	Ichneumonidae Latreille 1802	Anomaloniinae Viereck 191	-	[25]
		Banchinae Wesmael 1845	<i>Apophua</i> Morley 1913	New record
		Brachycyrtinae Viereck 1919	<i>Brachycyrtus</i> Kriechbaumer 1880	[25]
		Campopleginae Förster 1869	<i>Xanthocampoplex</i> Morley 1913	[25]
		Cryptinae Kirby 1837	-	New record
		Diplazontinae Viereck 1918	<i>Diplazon orientalis</i> (Cameron 1905)	New record
		Ichneumoninae Latreille 1802	-	[12]
		Mesochorinae Förster 1869	<i>Stictopisthus</i> Thomson 1886	[12, 26]
		Metopiinae Förster 1869	<i>Acerataspis</i> Uchida (Townes 1971)	New record
			<i>Exochus pictus</i> Holmgren 1858	New record
			<i>Metopius</i> Panzer 1806	[26]
			<i>Triclistus</i> Förster 1869	New record
			<i>Enicospilus</i> Stephens 1835	[25]
		Ophioninae Shuckard 1840	-	New record
		Orthocentrinae Förster 1869	-	[26]
		Pimplinae Wesmael 1845	<i>Theronia</i> Holmgren 1859	New record
			<i>Xanthopimpla</i> Saussure 1892	New record
Trigonaloidea Carmean and Kimsey (1998)	Trigonalidae (=Trigonalidae)			New record
Trigonalidae				
(=Trigonalidae)				

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 found 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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