

A Novel Host Association of *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae), Evidenced by Molecular Studies

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ABSTRACT

The present study from Indian Sundarbans, reports a new host-parasitoid association of *Spodoptera pecten* Guenée (Lepidoptera) and *Telenomus remus* Nixon (Hymenoptera). *T. remus* was identified integrating both morphological and molecular studies, while identification of *S. pecten* was based on molecular data (mt COI) obtained from reared larvae. The study confirms the natural occurrence of *T. remus* populations in Indian Sundarbans, with *S. pecten* as their host. Maximum likelihood trees based on the mt COI DNA gene sequences were generated for *T. remus*, and *S. pecten* to confirm the conspecificity. Further, the work also reports *Musa* sp. as a new host plant of *S. pecten*.

Keywords: host-parasitoid, India, new records, mt COI, *Spodoptera pecten*.

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INTRODUCTION

Insect parasitoids play a crucial role in regulating the populations of their hosts, offering natural control over pest species (Taekul, Valerio, Austin, Klompen & Johnson, 2014). However, there is limited understanding of how parasitoids fit into broader species interaction networks (Miller, Polaszek & Evans, 2021). Scelionidae, a prominent family of parasitic Hymenoptera, is characterized by endoparasitism of insect or spider eggs (Austin, Johnson & Dowton, 2005). Within this family, the genus *Telenomus* Haliday is particularly notable, as its members are common egg parasitoids of various insect orders, including Lepidoptera, Hemiptera (suborders Heteroptera and Auchenorrhyncha), Neuroptera, and Diptera (Taekul et al., 2014).

Telenomus remus Nixon, 1937, a well-known egg parasitoid of Lepidoptera (Cave, 2000; Pomari, Bueno, Freitas Bueno, Menezes Junior, 2012), was released as a biocontrol agent against several pest species of *Spodoptera* Guenée (Noctuidae) globally (Pomari et al., 2012). This parasitoid target at least 10 *Spodoptera* species (Table 1), many of which are serious agricultural pests causing significant economic damage (Cave, 2000; Colmenarez, Babendreier, Wurst, Freytez & Bueno, 2022). DNA barcoding proves to be a valuable tool for determining host-parasitoid relationships and for constructing ecological networks efficiently and cost-effectively (Garipey, Haye & Zhang, 2014; Miller, Polaszek & Evans, 2021; Wengrat et al., 2021). This is particularly useful in groups like Lepidoptera, where morphological identification is often restricted to specific life stages or sexes.

The present study aimed to investigate parasitoid associations with lepidopteran eggs in the Indian Sundarbans. The results revealed *T. remus* utilizing *Spodoptera pecten* (Guenée) as a host, a relationship that was previously unknown. Furthermore, *S. pecten* was found to use *Musa* sp. (banana plants) as its larval host, which had not been documented before.

Table 1. List of *Spodoptera* species parasitised by *T. remus*

Species	References
<i>S. albula</i> (Walker)	Cave 2000; Pomari et al. 2012
<i>S. cosmioides</i> (Walker)	Pomari et al. 2012
<i>S. dolichos</i> (Fabricius)	Cave 2000; Wojcik et al. 1976
<i>S. eridania</i> (Stoll)	Cave 2000; Pomari et al. 2012; Wojcik et al. 1976
<i>S. exigua</i> (Hübner)	Cave 2000; Wojcik et al. 1976
<i>S. frugiperda</i> (Smith)	Cave 2000; Kenis et al. 2019; Liao et al. 2019; Pomari et al. 2012; Wojcik et al. 1976
<i>S. latifascia</i> (Walker)	Cave 2000; Wojcik et al. 1976
<i>S. littoralis</i> (Boisduval)	Cave 2000; Gerling 1972
<i>S. litura</i> (Fabricius)	Cave 2000
<i>S. mauritia</i> (Boisduval)	Cave 2000; Gautam 1987

METHODS

Field sampling and rearing

Exploratory surveys were conducted in Indian Sundarbans, to study host-parasitoid associations. Egg masses of Lepidoptera were collected from natural and agricultural landscapes and were reared in glass vials with their mouth covered by muslin cloth. The vials were monitored daily for the emergence of parasitoids and host larvae, and the emerged specimens were preserved in 90% alcohol.

Species identification

The morphological identification of *T. remus* followed Nixon (1937) and Chou (1987) whereas the male genitalia slide was prepared after Polaszek and Kimani (1990). Molecular identification employed DNA barcoding for mitochondrial cytochrome c oxidase I COI (mt COI) gene. Genomic DNA was extracted from parasitoid specimens using the DNeasy Blood and Tissue Kit (QIAGEN, Inc.), following the kit protocol. DNA quantitation was performed in Qubit 2.0 fluorometer followed by polymerase chain reaction (PCR) amplification using primers LCO1490 and HCO2198 (Folmer, Black, Hoch, Lutz & Vrijenoeck, 1994). The PCR was carried out following Rajmohana et al. (2024) in a total volume of 25µL containing 12.5µL of 2X hot start master mix (Promega), 10µM of each forward and reverse primers, 30-40ng of template DNA and nuclease-free water up to final volume. Thermal cycling profile was as per Gariepy et al. (2014). Positive PCRs were confirmed on agarose gel by electrophoresis, followed by PCR purification. Purified products were bidirectionally sequenced by Sanger's dideoxy method on ABI 377 (Applied Biosciences) sequencer. The mt COI barcodes of the lepidopteran larvae, from cases III, IV, and V (Table 2), were generated by following the same procedure as mentioned above.

All the obtained chromatogram files were manually assessed for quality and the sequences were subjected to National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) to confirm the genetic identity. The voucher specimens of both the parasitoids and host larvae were deposited in the National Zoological Collections at the Zoological Survey of India (ZSI), Kolkata.

Digital imaging

Photomicrographs of the parasitoids were taken using a Leica DFC 500 digital camera attached to Leica M205A stereomicroscope (1X objective), and processed with LAS version 3.6 extended focus software. Images of the male genitalia slides were procured using Leica DM 1000 digital upright microscope.

Parasitism rate

The parasitism rate was calculated as per Sari, Buchori & Nurkomar (2021), using the formula $PR = (n/N) \times 100 \%$. Where, PR= parasitism rate; n =number of parasitised eggs; N = number of total eggs.

Phylogenetic analysis

For phylogenetic analyses of *Telenomus* species, the generated sequences were aligned with the mt COI dataset of Polaszek et al. (2021) (excluding *Trissolcus thyantae* Ashmead and *Hadronotus cultratus* (Masner)) in MEGA X (Kumar, Stecher, Li, Knyaz & Tamura, 2018). Additionally, mt COI sequences of *T. gregalis* Rajmohana (Rajmohana et al. 2024), *T. dendrolimi* (Matsumura) (Chen et al. 2024) and *T. dilophonotae* Cameron (Wengrat et al. 2024) were incorporated and aligned. A final maximum likelihood tree was built using 66 sequences (Table A1) in IQ-TREE multicore version 1.6.12 (Trifinopoulos, Nguyen, von Haeseler & Minh, 2016) web server. A total of 1000 ultrafast bootstrap replicates were performed under the GTR+F+I+G4 substitution model, which

was automatically selected based on the Bayesian Information Criterion with default parameters. The consensus tree was visualised in FigTree v 1.4 to treat *Trissolcus basal* (Walloston) as outgroup (Chen et al. 2024).

For phylogenetic analyses of *Spodoptera* species, a total of 2029 sequences were downloaded from GenBank belonging to the Noctuidae family. These sequences were aligned with sequences generated in the present study in MEGA X (Kumar et al., 2018). All the sequences which didn't align and created gaps were removed from the alignment. Many sequences that were too divergent were not included in the analyses (example, a few sequences of Kergoat et al., 2021). An initial tree was built with ~ 1000 mt COI sequences to check the monophyly of the *Spodoptera* spp. From the monophyletic sequences, only one sequence per species was used in the final tree construct, while the outgroup and member of other genera under Noctuidae were taken from Kergoat et al., (2021). A final Maximum likelihood tree was built using 56 sequences (Table A2) including 28 species of *Spodoptera* in IQ-TREE multicore version 1.6.12 (Trifinopoulos et al., 2016) web server for 1000 ultrafast bootstraps under GTR+F+I+G4 substitution model auto selected according to Bayesian Information Criterion with default parameters. The consensus tree was visualised in FigTree v 1.4 treating members of the genus *Catocala* Schrank as outgroup (Kergoat et al., 2021).

RESULTS

The emergence of both parasitoids and host larvae from the collected egg masses (Fig. 1a), was documented (Table 2). In cases I, II, and III, the parasitoids were observed emerging from a single egg mass over 2-12 days of rearing, suggesting that, the parasitism did not occur in a single stretch. The rate of parasitism was highest for case I (77.03%) and lowest for III (21.78%). A few host larvae also emerged in cases III, IV, and V.

The egg parasitoids were identified as *T. remus* (Fig. 1b-c) through an integrative taxonomic approach, utilizing both the morphological characters including male genitalia (Fig. 1d) and the DNA barcoding. The specimens from all four sites exhibited no morphological differences and matched the description of *T. remus* provided by Nixon (1937) and Chou (1987). Similarly, the male genitalia showed no significant variations and aligned with the description provided by Wengrat et al. (2021).

Table 2. Host- parasitoid emergence data.

Case	Date of collection of egg masses	Area surveyed	Geographical coordinates	Egg parasitoid identity	Host identity	Host plant	Parasitoid emergence	♀:♂	Parasitism rate (%)
I	27-02-2021	Madhya Gurguria, Kultali, SBR	21.97694 N 88.56722 E	<i>T. remus</i>	Unidentified (no larva emerged)	Unidentified	2nd, 3rd and 8th day of rearing	7:6	77.03
II	21-11-2021	Raidighi, Mathurapur II, SBR	21.99366 N 88.43861 E	<i>T. remus</i>	Unidentified (no larva emerged)	Unidentified	4th and 5th day of rearing	14:5	44.78
III	04-04-2022	Maipit, Kultali, SBR	21.91888 N 88.53583 E	<i>T. remus</i>	<i>S. pecten</i> (Identified from emerged larva)	Unidentified	7th and 9th day of rearing	5:12	21.78
IV	05-04-2022	Jamtala, Kultali, SBR	22.10888 N 88.56805 E	<i>T. remus</i>	<i>S. pecten</i> (Identified from emerged larva)	Banana (<i>Musa</i> sp.)	12th day of rearing	10:13	36.70
V	04-04-2022	Maipit, Kultali, SBR	21.91888 N 88.53583 E	—	<i>S. pecten</i> (Identified from emerged larva)	Unidentified	No emergence	—	—

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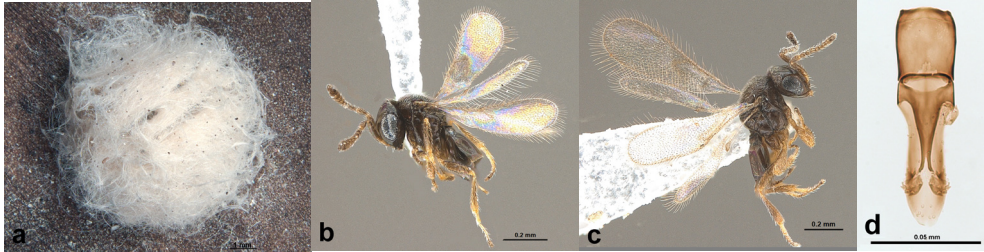


Figure 1. a) egg mass of *Spodoptera pecten*; b) *Telenomus remus* female; c) *T. remus* male; d) *T. remus* male genitalia.

The four sequences of *T. remus* (accession numbers: PP524920, PP481554, PP475445, PP537412) generated from four samples, proved identical. The NCBI BLAST results of these sequences indicated a genetic identity ranging from 99.6% to 100% with *T. remus*. The mitochondrial COI-based single-gene ML tree (Fig. 2) for species of *Telenomus* demonstrated that the samples collected from the four sites clustered together with other *T. remus* sequences from Kenya, China and Egypt under a single clade (Fig. 2).

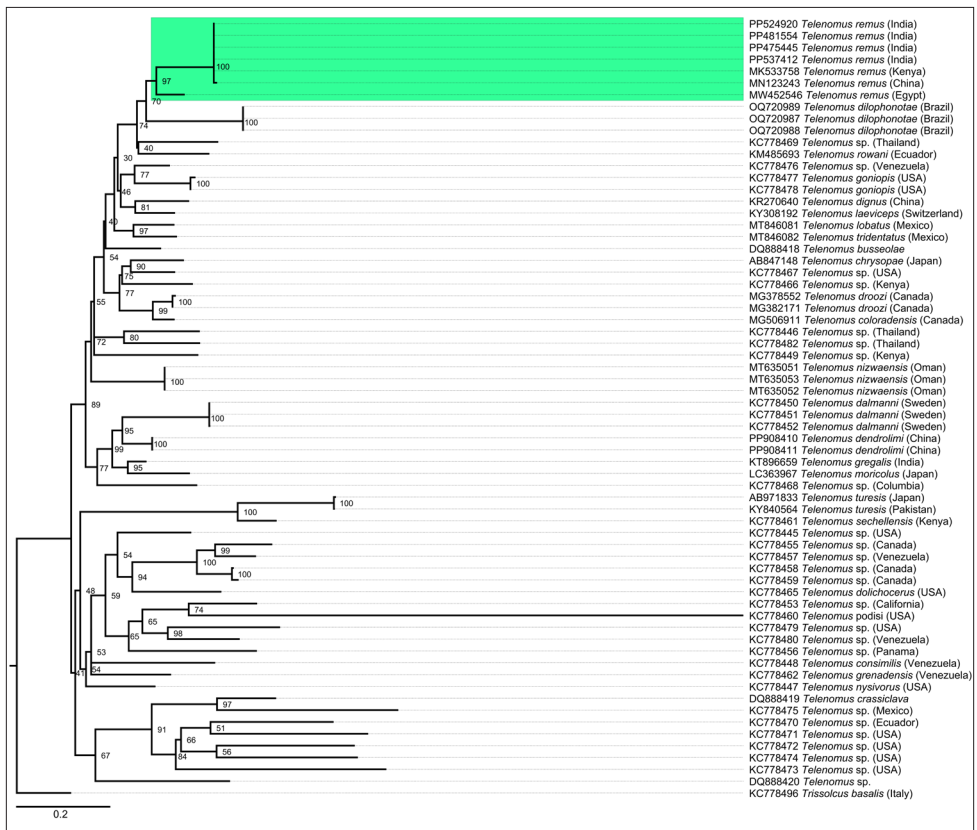


Figure 2. Maximum likelihood tree for the species of *Telenomus* based on 851 bp of mt COI DNA gene sequence. Green highlight indicates *T. remus* clade.

A total of six sequences of the lepidopteran larvae generated in this work, were analysed and compared using NCBI BLAST to verify their genetic identity. The results indicated a genetic identity ranging from 99.6 to 100% with *S. pecten* (accession numbers: PP078731, PP079035, PV037624, PV039679, PV053503, PV055052). The mitochondrial COI-based single-gene ML tree (Fig. 3) for the species of *Spodoptera* was prepared to confirm the monophyly of sequences on the tree to ascertain the BLAST identification. In the phylogenetic tree presented, all the species of *Spodoptera* showed a single monophyletic clade with poor bootstrap support among most of the subclades of *Spodoptera*. But, among the sister species pairs, bootstrap support was robust for most, however, with a few exceptions. Understanding the limitations of the mt COI gene for such phylogenetic inferences, we warrant further multigene species trees.

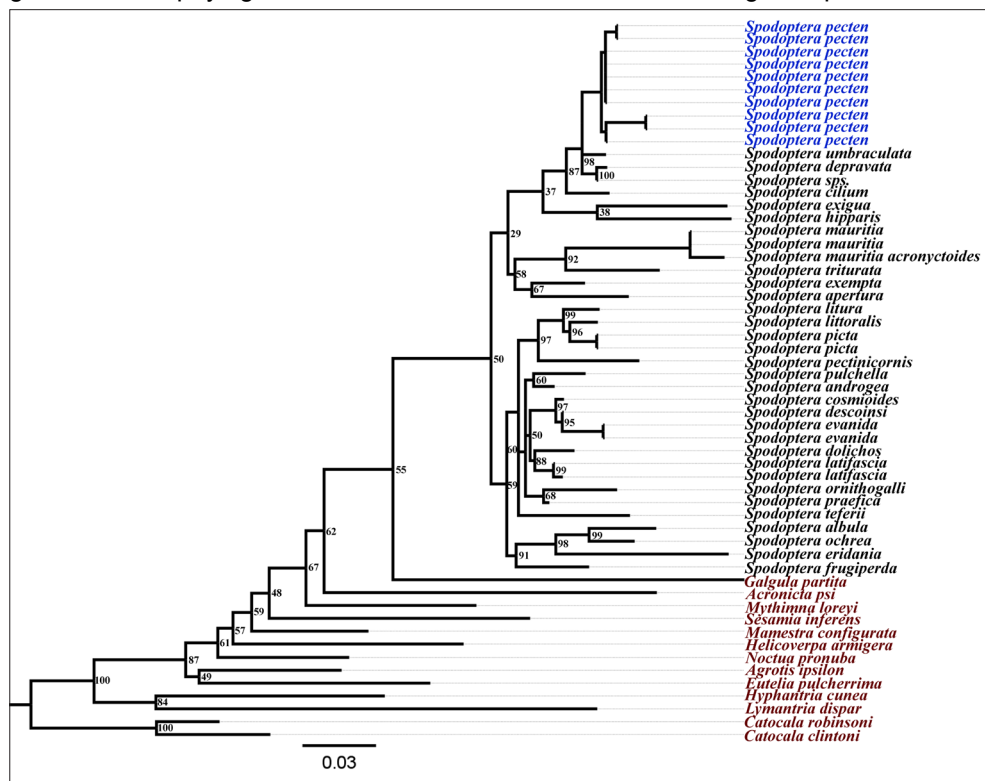


Figure 3. Maximum likelihood tree for the species of *Spodoptera* based on 603 bp of mt COI DNA gene sequence.

Sequences from the earlier studies of *S. pecten* from Pakistan (KX860418) by Ashfaq, Akhtar, Rafi, Mansoor & Hebert, 2017 and from Papua New Guinea (GU695454) by Watson et al., (unpublished data) formed a monophyletic clade with our samples of *S. pecten* sequences from West Bengal showing 0.2 % to 0.4% genetic distance within the populations of India, Pakistan and Papua New Guinea. This forms the first DNA barcode report for the species from India (although there is a report of

S. pecten sequence (MZ895792) from India by Kumar, Sharma, Paunikar & Sharma (2022) the sequence MZ895792 is not available for verification).

DISCUSSION

In addition to reporting the new host association of *S. pecten* and *T. remus*, the present study also finds *Musa* sp. as a new larval host plant of *S. pecten* (Table 2). The larvae of *S. pecten* usually feed on grasses and *Shorea curtisii* Dyer ex King (Dipterocarpaceae) seeds (Kimura 1980 & Nagoshi, Brambila & Meagher, 2011). Biological data on *S. pecten*, a minor pest in Asia (Ellis, 2004) is extremely scanty. Associations of such hosts and parasitoids are poorly understood. Non-target host species may indirectly affect the effectiveness of parasitoids as biological control agents (Miller et al., 2021). Cronin (2007) demonstrated that two hosts can influence each other's populations through shared parasitoids. The presence of alternate or non-target host species and their susceptibility to parasitism can impact pest population dynamics, hence evaluation of non-target host range turns important. The conservation biological control strategies also consider alternative hosts as a reservoir to conserve potential parasitoids (Abram, Brodeur, Urbaneja & Tena, 2019). Several host species have proven effective for the mass rearing of *T. remus* in the laboratory. *T. remus* has been reported to attack 27 species belonging to family Noctuidae, two species of Pyralidae, one species of each under Arctiidae (Cave, 2000) and Crambidae (kiruthika, Jevarani, Murugan, Nakkeera, & Balasubramai, 2022) highlighting its generalistic nature. Greater host diversity increases the chances of survival of the parasitoids in the field. New host-parasitoid association records as in the present work, gain relevance in these contexts. The recovery of *T. remus* from four different localities in Sundarbans, is indicative of the natural occurrence of well-established extant populations (Table 2). *S. pecten* as a host aid in natural survival of *T. remus*.

In the phylogenetic tree for the species of *Telenomus* (Fig. 2), MW452546 (Unpublished data) from Egypt, assigned originally as a sequence of *T. remus*, is not clustered in the monophyletic clade here. This species from Egypt possibly being a misidentification, we prefer to treat it as *Telenomus* sp. On the preliminary phylogenetic tree (Fig. 3) for the species of *Spodoptera*, among the global 31 species, 28 are represented, including *S. pecten*.

In India, the taxonomic studies of moths utilizing DNA barcodes, are limited, though such studies help to prevent taxonomic inflation and enable the identification of morphologically variant species, also uncovering the cryptic diversity (Kalawate, Pawara, Shabnam & Dinesh, 2020). In the present study, the moth species was identified as *S. pecten* from the mt COI barcode of larval samples. Keeping this as a classical example of the utility of DNA barcodes in species identification, we call for the generation of more DNA barcodes for the lesser-known insect taxa from biodiversity-rich countries like India, to facilitate building a robust DNA barcode library for future studies.

CONCLUSIONS

Extensive studies on taxonomic and bioecological aspects of both the parasitoids and their hosts are indispensable in integrated pest management programs. Sustainable pest management strategies like biocontrol, need to be promoted in regions like Indian Sundarbans, where the ecosystem is fragile and the major livelihood source of the masses is agriculture.

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APPENDICES

Table A1. The mt COI sequence details used in ML tree construction for *Telenomus remus*.

Sl.	Taxon	NCBI accession no.	Collection Locality	References
1	<i>Telenomus remus</i>	PP475445	India	This study
2	<i>Telenomus remus</i>	PP481554	India	This study
3	<i>Telenomus remus</i>	PP524920	India	This study
4	<i>Telenomus remus</i>	PP537412	India	This study
5	<i>Telenomus remus</i>	MK533758	Kenya	Kenis et al. 2019
6	<i>Telenomus remus</i>	MN123243	China	Liao et al. 2019
7	<i>Telenomus remus</i>	MW452546	Egypt	Unpublished
8	<i>Telenomus dilophonotae</i>	OQ720989	Brazil	Wengrat et al. 2024
9	<i>Telenomus dilophonotae</i>	OQ720987	Brazil	Wengrat et al. 2024
10	<i>Telenomus dilophonotae</i>	OQ720988	Brazil	Wengrat et al. 2024
11	<i>Telenomus</i> sp.	KC778469	Thailand	Taekul et al. 2014
12	<i>Telenomus rowani</i>	KM485693	Ecuador	Unpublished
13	<i>Telenomus</i> sp.	KC778476	Venezuela	Taekul et al. 2014
14	<i>Telenomus goniopis</i>	KC778477	USA	Taekul et al. 2014
15	<i>Telenomus goniopis</i>	KC778478	USA	Taekul et al. 2014
16	<i>Telenomus dignus</i>	KR270640	China	Unpublished
17	<i>Telenomus laeviceps</i>	KY308192	Switzerland	Unpublished
18	<i>Telenomus lobatus</i>	MT846081	Mexico	Ramirez-Ahuja et al. 2020
19	<i>Telenomus tridentatus</i>	MT846082	Mexico	Ramirez-Ahuja et al. 2020
20	<i>Telenomus busseolae</i>	DQ888418	-	Murphy et al. 2007
21	<i>Telenomus chrysopae</i>	AB847148	Japan	Matsuo et al. 2014
22	<i>Telenomus</i> sp.	KC778467	USA	Taekul et al. 2014
23	<i>Telenomus</i> sp.	KC778466	Kenya	Taekul et al. 2014
24	<i>Telenomus droози</i>	MG378552	Canada	Unpublished
25	<i>Telenomus droози</i>	MG382171	Canada	Unpublished
26	<i>Telenomus coloradensis</i>	MG506911	Canada	Unpublished
27	<i>Telenomus</i> sp.	KC778446	Thailand	Taekul et al. 2014
28	<i>Telenomus</i> sp.	KC778482	Thailand	Taekul et al. 2014
29	<i>Telenomus</i> sp.	KC778449	Kenya	Taekul et al. 2014
30	<i>Telenomus nizwaensis</i>	MT635051	Oman	Polaszek et al. 2021
31	<i>Telenomus nizwaensis</i>	MT635053	Oman	Polaszek et al. 2021
32	<i>Telenomus nizwaensis</i>	MT635052	Oman	Polaszek et al. 2021
33	<i>Telenomus dalmanni</i>	KC778450	Sweden	Taekul et al. 2014
34	<i>Telenomus dalmanni</i>	KC778451	Sweden	Taekul et al. 2014
35	<i>Telenomus dalmanni</i>	KC778452	Sweden	Taekul et al. 2014
36	<i>Telenomus dendrolimi</i>	PP908410	China	Chen et al. 2024
37	<i>Telenomus dendrolimi</i>	PP908411	China	Chen et al. 2024
38	<i>Telenomus gregalis</i>	KT896659	India	Rajmohana et al. 2024
39	<i>Telenomus moricolus</i>	LC363967	Japan	Matsuo et al. 2018
40	<i>Telenomus</i> sp.	KC778468	Colombia	Taekul et al. 2014
41	<i>Telenomus turesis</i>	AB971833	Japan	Mita et al. 2015
42	<i>Telenomus turesis</i>	KY840564	Pakistan	Unpublished
43	<i>Telenomus sechellensis</i>	KC778461	Kenya	Taekul et al. 2014
44	<i>Telenomus</i> sp.	KC778445	USA	Taekul et al. 2014
45	<i>Telenomus</i> sp.	KC778455	Canada	Taekul et al. 2014
46	<i>Telenomus</i> sp.	KC778457	Venezuela	Taekul et al. 2014

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Table
continued

SI.	Taxon	NCBI accession no.	Collection Locality	References
47	<i>Telenomus</i> sp.	KC778458	Canada	Taekul et al. 2014
48	<i>Telenomus</i> sp.	KC778459	Canada	Taekul et al. 2014
49	<i>Telenomus dolichocerus</i>	KC778465	USA	Taekul et al. 2014
50	<i>Telenomus</i> sp.	KC778453	California	Taekul et al. 2014
51	<i>Telenomus podisi</i>	KC778460	USA	Taekul et al. 2014
52	<i>Telenomus</i> sp.	KC778479	USA	Taekul et al. 2014
53	<i>Telenomus</i> sp.	KC778480	Venezuela	Taekul et al. 2014
54	<i>Telenomus</i> sp.	KC778456	Panama	Taekul et al. 2014
55	<i>Telenomus consimilis</i>	KC778448	Venezuela	Taekul et al. 2014
56	<i>Telenomus grenadensis</i>	KC778462	Venezuela	Taekul et al. 2014
57	<i>Telenomus nysivorus</i>	KC778447	USA	Taekul et al. 2014
58	<i>Telenomus crassiclava</i>	DQ888419	-	Murphy et al. 2007
59	<i>Telenomus</i> sp.	KC778475	Mexico	Taekul et al. 2014
60	<i>Telenomus</i> sp.	KC778470	Ecuador	Taekul et al. 2014
61	<i>Telenomus</i> sp.	KC778471	USA	Taekul et al. 2014
62	<i>Telenomus</i> sp.	KC778472	USA	Taekul et al. 2014
63	<i>Telenomus</i> sp.	KC778474	USA	Taekul et al. 2014
64	<i>Telenomus</i> sp.	KC778473	USA	Taekul et al. 2014
65	<i>Telenomus</i> sp.	DQ888420	-	Murphy et al. 2007
66	<i>Trissolcus basalis</i>	KC778496	Italy	Taekul et al. 2014

Table A2. The mt COI sequence (603 bp based) details used in ML tree construction for Noctuidae.

SI	Taxon	NCBI Accession number	Collection locality	References
1	<i>Spodoptera pecten</i>	PP078731	India	This study
2	<i>Spodoptera pecten</i>	PP078735	India	This study
3	<i>Spodoptera pecten</i>	PV037624	India	This study
4	<i>Spodoptera pecten</i>	PV039679	India	This study
5	<i>Spodoptera pecten</i>	PV053503	India	This study
6	<i>Spodoptera pecten</i>	PV053502	India	This study
7	<i>Spodoptera pecten</i>	KX860418	Pakistan	Ashfaq et al. 2017
8	<i>Spodoptera pecten</i>	GU695454	Papua New Guinea	Unpublished
9	<i>Spodoptera pecten</i>	HQ177400	Indonesia	Kergoat et al. 2012
10	<i>Spodoptera pecten</i>	HQ177399	Indonesia	Kergoat et al. 2012
11	<i>Spodoptera umbraculata</i>	HQ950507	Australia	Unpublished
12	<i>Spodoptera depravata</i>	AB733682	Japan	Watabiki et al. 2013
13	<i>Spodoptera</i> sp.	KX243390	China	Unpublished
14	<i>Spodoptera ciliium</i>	JN988598	Pakistan	Unpublished
15	<i>Spodoptera exigua</i>	AB733674	Japan	Watabiki et al. 2013
16	<i>Spodoptera hipparis</i>	JN262118	USA	Unpublished
17	<i>Spodoptera mauritia</i>	AB733409	Japan	Watabiki et al. 2013
18	<i>Spodoptera mauritia</i>	KC601856	India	Unpublished
19	<i>Spodoptera mauritia acronyctoides</i>	HQ177386	Papua New Guinea	Kergoat et al. 2012
20	<i>Spodoptera trituratora</i>	KJ634311	Zimbabwe	Van De Vossenber, & Van der Straten, 2014
21	<i>Spodoptera exempta</i>	JQ315120	Tanzania	Graham & Wilsom, 2012
22	<i>Spodoptera apertura</i>	KF388292	Australia	Hebert et al. 2013
23	<i>Spodoptera litura</i>	AB733672	Japan	Watabiki et al. 2013
24	<i>Spodoptera littoralis</i>	FN907993	United Kingdom	Unpublished

Table
continued

SI	Taxon	NCBI Accession number	Collection locality	References
25	<i>Spodoptera picta</i>	HQ950412	Australia	BOLD database
26	<i>Spodoptera picta</i>	LOQC210-05	Australia	BOLD database
27	<i>Spodoptera pectinicornis</i>	MW666002	Australia	Kergoat et al. 2021
28	<i>Spodoptera pulchella</i>	HM756075	USA	Nagoshi et al. 2011
29	<i>Spodoptera androgea</i>	KJ634282	Suriname	Van De Vossenberg, & Van der Straten, 2014
30	<i>Spodoptera cosmioides</i>	JF854736	Brazil	Unpublished
31	<i>Spodoptera descoinsi</i>	KF854163	France	Dumas et al. 2015
32	<i>Spodoptera evanida</i>	KF854176	France	Dumas et al. 2015
33	<i>Spodoptera evanida</i>	KF854175	France	Dumas et al. 2015
34	<i>Spodoptera dolichos</i>	JQ602911	Costa Rica	Unpublished
35	<i>Spodoptera latifascia</i>	BLPAA21562-20	Costa Rica	BOLD database
36	<i>Spodoptera latifascia</i>	MHMYK15102-16	Costa Rica	BOLD database
37	<i>Spodoptera ornithogalli</i>	JF855012	USA	Unpublished
38	<i>Spodoptera praefica</i>	KF492567	USA	Unpublished
39	<i>Spodoptera teferii</i>	MH817649	Ethiopia	Le et al. 2018
40	<i>Spodoptera albula</i>	KJ634281	Costa Rica	Van De Vossenberg, & Van der Straten, 2014
41	<i>Spodoptera ochrea</i>	KJ634308	Peru	Van De Vossenberg, & Van der Straten, 2014
42	<i>Spodoptera eridania</i>	KJ634290	Suriname	Van De Vossenberg, & Van der Straten, 2014
43	<i>Spodoptera frugiperda</i>	KJ634299	Peru	Van De Vossenberg, & Van der Straten, 2014
44	<i>Galgula partita</i>	JN262120	USA	Unpublished
45	<i>Acronicta psi</i>	HM875345	Finland	Unpublished
46	<i>Mythimna loreyi</i>	LC548630	Japan	Unpublished
47	<i>Sesamia inferens</i>	LC548617	Japan	Unpublished
48	<i>Mamestra configurata</i>	KJ393069	Canada	Zahiri et al. 2014
49	<i>Helicoverpa armigera</i>	LC548612	Japan	Unpublished
50	<i>Noctua pronuba</i>	KM552700	Canada	Unpublished
51	<i>Agrotis ipsilon</i>	JN284032	Germany	Unpublished
52	<i>Eutelia pulcherrima</i>	GU694338	USA	Unpublished
53	<i>Hyphantria cunea</i>	AB105318	USA	Unpublished
54	<i>Lymantria dispar</i>	KY923064	Russia	Djoumad et al. 2017
55	<i>Catocala robinsoni</i>	KJ380304	USA	Unpublished
56	<i>Catocala clintoni</i>	KJ379740	USA	Unpublished