

# A new species of *Galleria* Fabricius (Lepidoptera, Pyralidae) from Korea based on molecular and morphological characters

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## Abstract

The greater wax moth, *Galleria mellonella* Linnaeus, is well known as a pest of honey bees and for the biodegradation of wax and polyethylene by their larvae. The genus *Galleria* has long been considered monotypic and found worldwide. A taxonomic study of the genus *Galleria* is presented based on morphological and molecular characters (*COI*, *CAD*, *wg*). A new species (*Galleria similis* Roh & Song, **sp. nov.**) is recognized on the Korean peninsula. The new species is superficially similar to *G. mellonella* but they can be separated by the structures of hindwing venation and male genitalia. Habitus photographs and illustrations of diagnostic characters are provided.

## Keywords

cryptic species, Galleriinae, new species, plastic eating moth, Pyraloidea, wax worms

## Introduction

The family Pyralidae is large group of Lepidoptera, placed in the superfamily Pyraloidea consisting of 1055 genera with 5921 described species (van Nieukerken et al. 2011). A molecular phylogeny and revised classification of the Pyralidae recognized five subfamilies, Chrysauginae, Epipaschiinae, Galleriinae, Phycitinae, and Pyralinae (Regier et al. 2012).

Among the Galleriinae, the monotypic genus *Galleria* Fabricius, 1798 was established with the type species *Phalaena cereana* Blom, 1764. *Galleria mellonella* (Linnaeus) is a ubiquitous pest of honey bees, *Apis mellifera* Linnaeus and *A. cerana* Fabricius (Ellis et al. 2013; Kwadha et al. 2017). They live on honeycomb in beehives, feeding on honey, beeswax, and the skin of bee pupae (Oldroyd 1999, 2007; Martel et al. 2006; Klein et al. 2007; Kong et al. 2019). Recent studies have shown that the larvae have the ability to biodegrade polyethylene in their guts (Yang et al. 2014; Bombelli et al. 2017; Kong et al. 2019).

The genus *Galleria* is superficially similar to the genus *Achroia* Hübner, 1819 (Kwadha et al. 2017), but can be distinguished from the latter by the presence of four stemmata on the head of the larva, concaved in the termen of the forewing, and the Cu vein apparently four-branched from the hindwing (Ellis et al. 2013).

In this paper, we describe *Galleria similis* Roh & Song, sp. nov. based on morphological and molecular characters, and provide habitus photographs and illustrations of diagnostic characters for identification of the two species of the genus *Galleria*.

## Materials and methods

The material examined in this study is deposited in the Systematic Entomology Laboratory, National Institute of Agricultural Sciences (NAS), Wanju, Korea. Specimens were dissected and examined after mounting on glass slides; male genitalia in 60% Euparal and wing venation based on dried specimens. Photographs of adults and male genitalia were taken using a Dhyana 95 scientific CMOS camera (Tucsen, Fuzhou, China) attached to a Leica DM 2000 LED optical microscope (Leica, Wetzlar, Germany). Terminology for morphological characters of the adult follow Smith (1965).

Genomic DNA from four specimens of *Galleria similis* and 19 specimens of *G. mellonella* was extracted from the legs of dried specimens of adults in 100% alcohol using a MagListo 5M Genomic DNA Extraction Kit (Bioneer Corporation, Daejeon, Republic of Korea) according to the manufacturer's protocol. One mitochondrial protein coding gene, the cytochrome oxidase subunit I gene (*COI*) (Folmer et al. 1994) and two nuclear protein coding genes, Carbamoyl-phosphate synthetase 2, Aspartate transcarbamylase, and Dihydroorotase (*CAD*) and Wingless (*wg*) were sequenced (Haines and Rubinoff 2012) (Table 1). Primers and amplification strategies followed Haines and Rubinoff (2012) and are detailed in Table 2. PCR conditions for ampli-

fication followed Haines and Rubinoff (2012), and directly sequenced at Macrogen (Geumcheon-gu, Seoul, Korea). Contigs were assembled in Geneious prime (Kearse et al. 2012). Successful *COI*, *CAD* and *Wingless* sequences were uploaded to GenBank (Table 1).

The barcodes were compared to 93 DNA barcodes of the genera *Galleria* and *Achroia* downloaded from BOLD systems v4 (BIN numbers: BOLD:AAA0965, BOLD:AAL2955, BOLD:ACO9701). A neighbor-joining analysis (NJ) was performed with MEGA X (Kumar et al. 2018) using the Kimura-2-Parameter (K2P)

**Table 1.** *Galleria* species and their *COI* barcodes and nuclear protein coding gene sequences with their associated and GenBank accession numbers as used in this study. Dashes indicate missing data.

Species	Voucher No.	<i>COI</i>	<i>CAD</i>	<i>wg</i>
<i>Galleria mellonella</i>	15310	MT439336	MT447104	MT447124
	15311	MT439337	MT447105	MT447125
	15312	MT439338	MT447109	MT447126
	15313	MT439349	MT447106	MT447127
	15314	MT439350	MT447107	MT447128
	15616	MT439351	MT447110	MT447129
	15617	–	MT447108	MT447130
	21361	MT439339	–	MT447131
	21362	MT439340	MT447111	MT447132
	21363	MT439341	MT447115	MT447133
	21364	MT439342	MT447114	MT447134
	21365	–	MT447119	MT447135
	21412	MT439343	MT447116	MT447136
	21413	MT439352	–	–
	21414	MT439346	MT447112	MT447137
	21415	MT439344	MT447113	MT447138
	21416	MT439347	MT447120	MT447139
	21417	MT439345	MT447118	MT447140
	21418	MT439348	MT447117	MT447141
	<i>G. similis</i>	15315	MT447100	MT447121
21366		MT447101	MT447122	MT447143
21367		MT447102	MT447123	MT447144
21368		MT447103	–	MT447145

**Table 2.** List of primers and amplification strategies used in this study (abbreviations: s = second, min = minute).

Genes	Primers	Sequences (5' to 3')	Amplification strategies
<i>COI</i>	LCO1490	GGTCAACAAATCATAAAGATATTGG	LCO1490 + HCO2198 (Folmer et al. 1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
<i>CAD</i>	CAD4_Pyr_F	GAAGAAGCATTTCAAAAAGC	CAD4_Pyr_F + CAD4_Pyr_R (Haines and Rubinoff 2012)
	CAD4_Pyr_R	CKRTCACCTCATGTCRTA	
<i>wg</i>	LepWg1	GARTGYAARTGYCAYGGYATGTCTGG	LepWg1 + LepWg2 (Brower and Desalle 1998)
	LepWg2	ACTICGCARACCARTGGAATGTRCA	

\* PCR amplifications condition

- *COI*: 5-min 95 °C; 35 cycles: 30-s 95 °C, 25-s 48 °C, 45-s at 72 °C; 5-min 72 °C.

- *CAD*: 2-min 94 °C, 1-min 50 °C, 1-min 72 °C; 34 cycles: 1-min 94 °C, 1-min 50 °C, 1-min at 72 °C; 12-min 72 °C.

- *wg*: 2-min 94 °C, 1-min 56 °C, 1-min 72 °C; 34 cycles: 1-min 94 °C, 1-min 56 °C, 1-min at 72 °C; 12-min 72 °C.

model (Kimura 1980) for nucleotide substitutions. Bootstrap support values for each node were also evaluated via MEGA X with 1000 replicates. Parsimony analyses (PA) with bootstrap were conducted in TNT 1.5 (Goloboff and Catalano 2016) using search strategies described by Song and Ahn (2018).

Intra- and inter-specific distances in different taxonomic levels were calculated using the uncorrected pairwise distance method (Srivathsan and Meier 2012). To explore molecular diagnostic characters for the *Galleria* species, we used the “list common synapomorphies” function of TNT and then examined thoroughly listed characters in the alignment file.

## Results

### Molecular character analysis

A total of 21 new sequences was generated from four specimens of *Galleria similis* and 17 specimens of *G. mellonella* (524–650 bp of partial *COI* barcode region, 613 bp of partial *CAD*, and 432 bp of partial *wg* gene region). All new sequences were uploaded to GenBank (Table 1). The DNA barcodes (*COI*) were compared to those of 72 DNA barcodes in 16 countries (*G. mellonella*), one Australian specimen (*Galleria* sp.) and seven lesser wax moths (*Achroia grisella* Fabricius) downloaded from BOLD systems v4 (Fig. 7).

Genetic divergence of *COI* using uncorrected *p*-distance among the *Galleria* and *Achroia* species ranged from 5.3% to 12.0%, while intraspecific divergence ranged from 0% to 2.2% (Table 3). All four species were strongly supported as a single lineage on both NJ and PA trees (Figs 7, 8). The molecular analyses (*p*-distance, NJ and PA analyses) revealed that *G. mellonella* was closely related to *G. similis* (Table 3; Figs 7, 8). The maximum difference among populations within *G. mellonella* was 2.2%, and within *G. similis* was 0% (Table 3). For these two species, it is difficult to correctly delimit each species, due to their extreme similarities in external morphological characters (see taxonomy section below). In contrast to morphological characters, however, genetic divergence strongly supported the separation of *G. mellonella* and *G. similis*. The minimum inter-specific difference between the two species (5.3%) was much higher than the maximum intraspecific difference of *G. mellonella* (2.2%) (Table 3). Furthermore, molecular diagnostic characters for the *Galleria* species, *G. mellonella* and *G. similis* contained 15 characters for *COI*, one character of *CAD* and four characters of *wg* gene regions (Table 4).

**Table 3.** Inter- and intraspecific genetic differences in the two genera *Galleria* and *Achroia* species for *COI* (658 bp) calculated using *p*-distance.

	<i>G. mellonella</i>	<i>G. similis</i>	<i>Galleria</i> sp.	<i>A. grisella</i>
<i>G. mellonella</i>	0–0.022			
<i>G. similis</i>	0.053–0.066	0		
<i>Galleria</i> sp.	0.112–0.119	0.114	0	
<i>A. grisella</i>	0.107–0.116	0.117–0.119	0.116–0.120	0–0.003

**Table 4.** List of 20 molecular diagnostic characters used to determine the genetic distinctiveness of two cryptic species *Galleria mellonella* and *G. similis* based on mtDNA partial *COI*, nuDNA partial *CAD* and *wg* gene region. Numbers indicate nucleotide sites in the sequenced 658 bp portion of the *COI* gene, 613 bp portion of the *CAD* gene and 432 bp portion of the *wg* gene. Number position follows *G. mellonella*: MT439366 (*COI*), MT447104 (*CAD*) and MT447124 (*wg*).

Species	Genes									
	<i>COI</i>									
	16	34	109	197	232	259	271	274	280	307
<i>G. mellonella</i>	T	A	A	T	T	T	T	T	T	T
<i>G. similis</i>	C	T	G	C	C	C	C	C	C	C
Species	<i>COI</i>					<i>CAD</i>	<i>wg</i>			
	385	391	403	424	470	319	129	241	343	379
<i>G. mellonella</i>	T	T	C	T	T	G	A	T	C	C
<i>G. similis</i>	C	C	T	C	C	A	C	C	T	T

**Table 5.** List of three molecular diagnostic characters used to determine the molecular distinctiveness of two cryptic species *Galleria mellonella* and *G. similis* based on amino acid sequences of partial *COI*, *CAD*, and *wg* protein region. Numbers indicate amino acid site in the sequenced 201 amino acid (aa) portion of the *COI* protein, 204 aa portion of the *CAD* protein and 143 aa portion of the *wg* protein. Number position follows *G. mellonella*: the translated amino acid sequences of MT439366 (*COI*), MT447104 (*CAD*) and MT447124 (*wg*).

Species	Proteins		
	<i>COI</i>	<i>CAD</i>	<i>wg</i>
	152	107	43
<i>G. mellonella</i>	V	A	E
<i>G. similis</i>	I	T	A

We also found three distinct differences in the amino acid sequences of each protein (Table 5). In particular, the transition from G (guanine) to A (adenine) at the 319 site of *CAD* protein led to a change from a hydrophobic amino acid (Alanine, A) to a hydrophilic amino acid (Threonine, T), and the transversion from A to C (cytosine) at the 129 site of the *wg* protein led to a change from a hydrophilic amino acid (Glutamate, E) to a hydrophobic amino acid (A). The molecular characters provided further evidence that new species *G. similis* was distinct and valid.

## Taxonomic accounts

### Genus *Galleria* Fabricius, 1798

*Galleria* Fabricius, 1798: 419, 462. Type species: *Phalaena cereana* Blom, 1764, by subsequent designation by Latreille (1810: 441).

*Cericlepta* Sodoffsky, 1837: 93. Type species: *Galleria mellonella* Linnaeus, 1758, by original designation.

*Vindana* Walker, 1866: 1706. Type species: *Vindana obliquella* Walker, 1866, by monotypy.

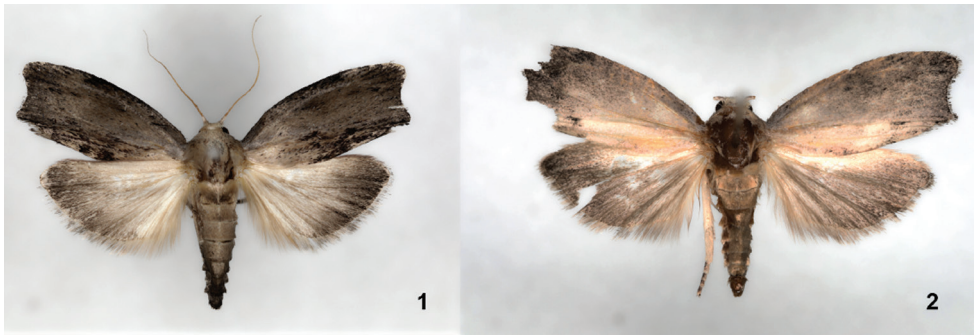
***Galleria similis* Roh & Song, sp. nov.**

<http://zoobank.org/DD9DF8D5-D3D5-4235-80AE-294C9B731EAB>

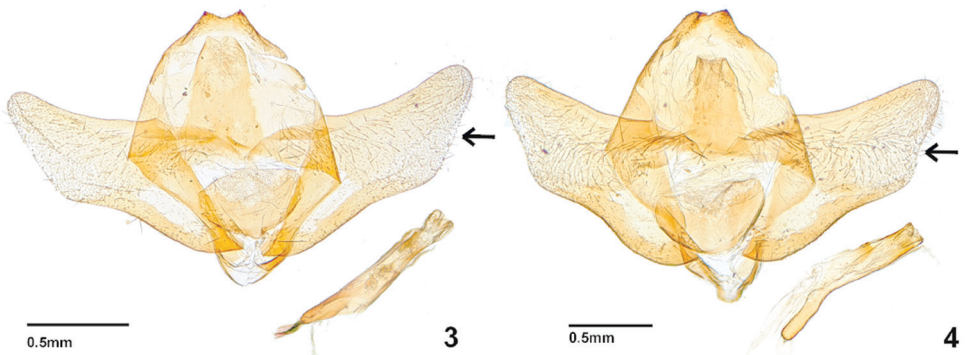
Figures 2, 4, 6

**Type material.** *Holotype*. ♂, **Korea**: Wanju-gun, 14.xi.2014, 35°49'45.64"N, 127°02'27.20"E, leg. H.S. Shim, genitalia slide no. 15315, DNA barcode GenBank accession no. MT447100 (NAS). *Paratypes*. 3♂, **Korea**: Tongyeong, 17.i.2020, 34°50'58.58"N, 127°26'51.79"E, leg. J.-H. Song, genitalia slide no. 21366–21968, DNA barcode GenBank accession no. MT447101, MT447102, and MT447103 (NAS).

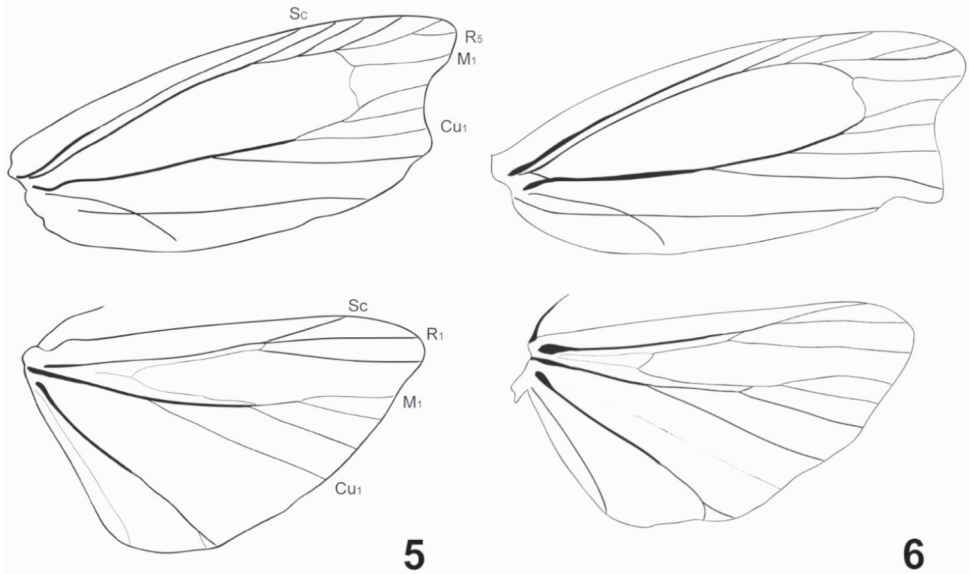
**Diagnosis.** *Galleria similis* sp. nov. (Figs 2, 4, 6) is very similar to *G. mellonella* (Figs 1, 3, 5) but can be distinguished by a square discal cell of its hindwing venation (Fig. 6) and the different shape of male genitalia (Fig. 4, *G. similis*: valva shorter and wider, concave at outer margin). *Galleria similis* sp. nov. had 15, one and four diagnostic characters from 658 bp of partial *COI*, 613 bp of partial *CAD* and 423 bp of partial *wg* gene region, respectively (Table 4). Our study showed that morphological and molecular characters can be used to resolve the status of cryptic species, *G. mellonella*



**Figures 1, 2.** Adults of *Galleria* species. **1** Male of *G. mellonella* **2** male of *G. similis*, holotype.



**Figures 3, 4.** Male genitalia of *Galleria* species. **3** *G. mellonella* (slide no. 21364) **4** *G. similis*, paratype (slide no. 21367).



**Figures 5, 6.** Male wing venation of *Galleria* species. **5** *G. mellonella* **6** *G. similis*, paratype.

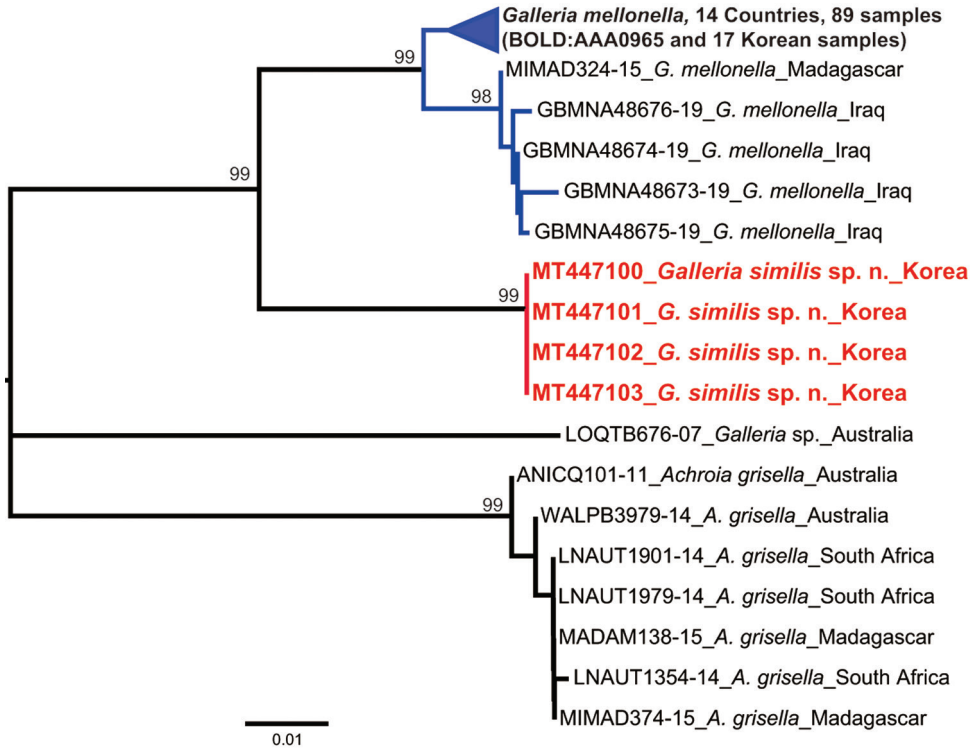
and *G. similis*. A cryptic species was suggested by the unusually high genetic distances within specimens originally identified as *G. mellonella*.

**Description. Adult. Male** (Fig. 2). Head: vertex densely clothed with gray hair-like scales; labial palpus three-segmented. Thorax: Light brown; notum covered with gray scales. Legs with femora, tibiae, and tarsi clothed with light gray piliform scales; tarsi apical and medial spurs covered dark-brown scales. Wingspan 21.5–32.0 mm. Forewing (Fig. 6) narrow, costa straight at base and gently curved beyond 4/5, termen concave; tornus pointed, 9 separate veins originating at the discal cell; Sc terminating at 4/5 costa; R<sub>5</sub> originated at R<sub>4</sub>, M<sub>1</sub> and M<sub>2</sub> parallel; M<sub>2</sub>, M<sub>3</sub> originating at distal corner of discal cell; Cu<sub>1</sub> and Cu+A<sub>1</sub> parallel, ground color yellowish white with gray and some dark overscaling. Hindwing (Fig. 6) discal cell square, L/W ratio 1.72; costa straight, apex straightly curved to termen; Sc straight to 3/5 costa; R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> present; R<sub>1</sub> and R<sub>2</sub> terminating at apex; M<sub>2</sub> originating at 1/5 M<sub>3</sub>; CuA<sub>1</sub> and CuA<sub>2</sub> parallel; A<sub>1</sub> originating at 4/5 Cu<sub>2</sub>. Hindwing covered with dark-brown scales; postmarginal part present with short light brown hairs. Abdomen: Male genitalia (Fig. 4) with uncus concave and hooked; tegumen wide at base; gnathos long; valva short and wide, costa straight, termen relatively concave, small setae present sparsely on outer and inner surface; vinculum narrower than gnathos; juxta heart shaped; saccus very short and slender; phallus slightly short and thick, vesica with short setae, ductus ejaculatorius present.

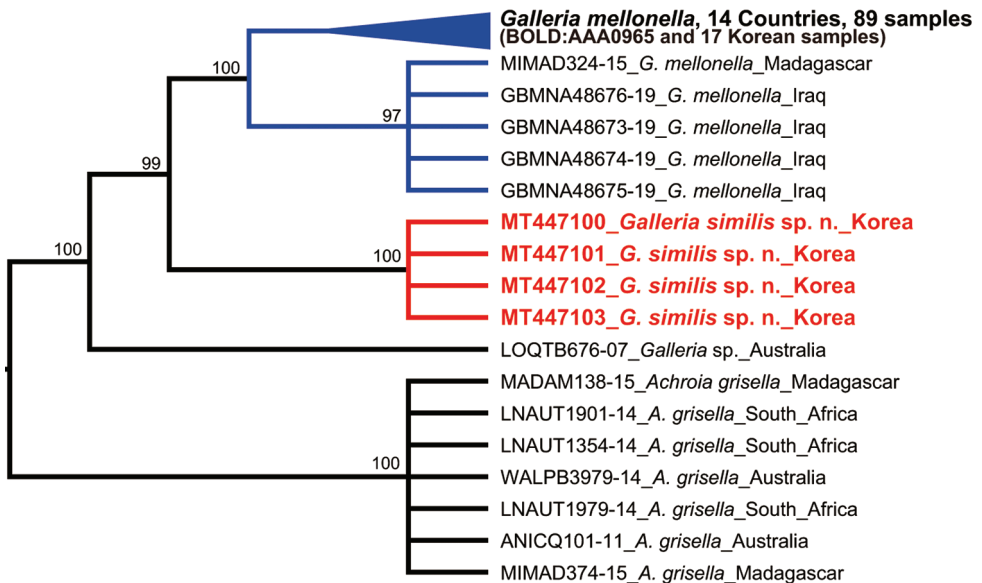
**Female.** Unknown.

**Distribution.** Korea.

**Etymology.** Named from the Latin *similis* meaning “similar”, which refers to the similar morphological characters with *G. mellonella*.



**Figure 7.** Neighbor-Joining tree based on partial *COI* gene sequences with bootstrap values. Scale bar indicates the expected number of substitutions per site.



**Figure 8.** Strict consensus tree of equally parsimonious cladograms based on partial *COI* gene sequences with bootstrap values.



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