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A new species of *Pristiphora* Latreille (Hymenoptera, Tenthredinidae), a defoliator of *Picea meyeri* in China

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Abstract

In this paper, a new species of *Pristiphora* is described and illustrated, *P. brevitheca* **sp. nov.** collected in Hebei Province from China. Based on the morphological characteristics of females and males and the maximum likelihood tree analysis based on COI, it is determined that this new species belongs to the *P. abietina* group. Partial mitochondrial genome and 21 tRNA secondary structures of the new species are described. The partial mitochondrial genome (16,187 bp) contains 36 genes: 13 protein-coding genes, 21 tRNAs, and 2 rRNAs. Notably, trnQ and the control region (CR) are missing from the assembly. The A+T content of *P. brevitheca* mitochondrial genome is 80.9%. Phylogenetic analysis showed that *P. brevitheca* belongs to the *P. abietina* group, and this also supports the monophyly of the group. The consumption of *Picea meyeri* (Pinaceae) needles by the new species results in the complete defoliation of some trees. Some biological characteristics of the new species are also provided.

Keywords

China, mitochondrial genome, Nematinae, sawfly, taxonomy

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Introduction

Pristiphora Latreille, 1810, belongs to the family Tenthredinidae (Hymenoptera) and the subfamily Nematinae, as defined by Prous et al. (2014), including about 240 species (Taeger et al. 2010; Prous et al. 2017). In China, there are records of 34 species belonging to the genus *Pristiphora* (Rohwer 1916; Wong 1977; Xiao 1990; Zhou and Zhang 1993; Nie and Wei 1998; Wei and Nie 1998, 2003; Wei et al. 1999; Wei 2002; Wei et al. 2006; Haris 2012; Li and Wei 2012; Wei and Xia 2012).

In the Saihanba Forest Farm in Hebei Province, China, a new species of *Pristiphora* was discovered. Its larvae feed on the needles of *Picea meyeri*, a plant endemic to China, and can cause complete defoliation of individual branches. Given that the body color, ovipositor sheath, and lancet morphological characteristics of the female, as well as the structure of the male's penis valve, are similar to those of males and females in the *P. abietina* group, it is therefore determined that this new species belongs to the *P. abietina* group (Benson 1958; Wong 1975; Prous et al. 2017). The maximum likelihood tree analysis based on COI also supports this result.

The morphological characteristics of adults and biological features, such as larval morphology, are described and illustrated, including distinguishing features from the closely related species *P. jacutica* Beneš, 2014. Additionally, partial mitochondrial genome and 21 tRNA secondary structures of the species as molecular characteristics and a phylogenetic tree based on the COI gene are provided.

Material and methods

Morphological methods

The specimens were examined with a Motic-SMZ-171 stereomicroscope. Images of the imagines were taken with a Nikon D700 digital camera and a Leica Z16APO separately. The genitalia were examined with a Motic BA410E microscope, and images of the genitalia were taken with Motic Moticam Pro 285A. The series of images produced were focus-stacked using Helicon Focus (HeliconSoft, Kharkiv, Ukraine) and further processed with Adobe Photoshop CS 11.0.

The terminology of genitalia follows Ross (1945) and that of general morphology follows Viitasaari (2002). For a few terms, including middle fovea, lateral fovea, and lateral walls, we follow Takeuchi (1952).

Specimens examined in this study are deposited in the Asian Sawfly Museum, Nanchang, China (**ASMN**); Biohazard Prevention and Control Center, National Forestry and Grassland Administration, Shenyang, China (**BPCC**); Saihanba Machinery Forest Farm, Chengde, China (**SMFF**).

Abbreviations used in the text and illustrations are as follows:

OCL The distance between a lateral ocellus and the occipital carina, or the hind margin of the head where this carina would be if it were developed (Benson 1954).
OOL The distance between an eye and a lateral ocellus.
POL The distance between the mesal margins of the 2 lateral ocelli.

Molecular methods

The larvae specimen (CSCS-Hym-MC0851) was collected from the Beimandian Substation of Saihanba Mechanical Forest Farm, Weichang autonomous County of Man and Mongolia minority, Hebei, China on July 13, 2022; the accession number is PQ789806. Whole genomic DNA was extracted from the integument muscle of the larvae using the TIANamp Genomic DNA Kit (Tiangen, Beijing, CN). Genomic DNA was sequenced by the high-throughput Illumina Hiseq 6000 platform, yielding a total of 214,164,390 raw reads (SRR32393833). DNA sequences were assembled by MitoZ v2.3 with the parameters --threads 16 (parallel computation) and --genetic_code 5 (invertebrate mitochondrial code) (Meng et al. 2019), and further verified by Geneious Prime v2024.0.7 (https://www.geneious.com) through comparisons to related species (*P. abietina*). Annotations were initially generated using the MITOS web server (Bernt et al. 2013). When degenerate bases were detected, ambiguous positions were resolved in Geneious Prime using a majority rule threshold (>50% read frequency), with unresolved positions marked as "N". The tRNA secondary structures were drawn by using VARNA (Darty et al. 2009) and Rnaviz (De Rijk et al. 2003).

COI sequences (810 bp) of *Pristiphora* species and *Nematus pumila* Liu, Li & Wei, 2019 used in the phylogenetic analyses are available in GenBank, and their accession numbers are shown in Table 1. The sequences were aligned using MAFFT 7.313 (Katoh & Standley, 2013). The best-fitting DNA substitution model for the alignment sequences was selected using jModelTest 2.1.7 (Darriba et al. 2012). Maximum likelihood (ML) phylogenies were inferred using IQ-TREE webserver (http://iqtree.cibiv.univie.ac.at/) (Nguyen et al. 2015) with 1000 ultrafast bootstrap replicates. In the phylogenetic analysis, *N. pumila* was selected as the outgroup.

Results

Taxonomy

Pristiphora abietina group

Diagnosis and remarks refers to Prous et al. (2017, P17).

Key to species of *Pristiphora abietina* group

Females

1 Head, thorax, abdomen and all femora black; body length 9–11 mm. Europe ..... ***P. robusta*** **(Konow, 1895)**

– Head, thorax, abdomen and all femora at least partly pale; body length not more than 9 mm **2**

2 In lateral view, posterior margin of valvula 3 about as long as ventral margin; in dorsal view, valvula 3 slightly tapering from base to apex **3**

– In lateral view, posterior margin of valvula 3 shorter than ventral margin; in dorsal view, valvula 3 not tapering from base to apex **6**

3 In posterior view, valvula 3 without invagination. Europe ... ***P. gerula*** **(Konow, 1904)**

– In posterior view, valvula 3 with small invagination **4**

4 Mesepimeron usually pale, abdomen mostly testaceous with separated black dorsal spots; lancet with distinct ctenidia. Europe ..... ***P. saxesenii*** **(Hartig, 1837)**

– Mesepimeron entirely black, abdominal terga 1–7 dorsally black; lancet lacks distinct ctenidia **5**

5 Antennae with scape and pedicel at least dorsally black, flagellum dorsally piceous, ventrally brownish; stigma brownish yellow to yellowish brown; antennomere 3 subequal to antennomere 4; valvula 3 about 0.3 × as long as valvifer 2, in dorsal view, cercus about as long as valvula 3; the two basal annuli nearly perpendicular. East Siberia ..... ***P. jacutica*** **Beneš, 2014**

– Antennae entirely black; stigma black-brown; antennomere 3 longer than antennomere 4; valvula 3 0.7 × as long as valvifer 2, in dorsal view, cercus shorter than valvula 3; the two basal annuli strongly oblique. China (Hebei) ..... ***P. brevitheca*** **sp. nov.**

6 Valvula 3 lacks distinct invagination **7**

– Valvula 3 with distinct invagination **8**

7 Vein C of the forewing pale brown; metatarsomere 1 as long as combined length of metatarsomeres 2–4; sawsheath about 1/2 as long as basal plate; the angle between inner tooth and outer tooth of the claw relatively small. Japan ..... ***P. harai*** **Togashi, 1989**

– Vein C of the forewing black; metatarsomere 1 as long as combined length of metatarsomeres 2–3; sawsheath about as long as basal plate; the angle between inner tooth and outer tooth of the claw relatively large. Japan ..... ***P. ezomatsuvora*** **Togashi, 1977**

8 Hypopygium posteriorly excised **9**

– Hypopygium posteriorly not excised **10**

9 Mesopleuron usually extensively pale; lancet with 14 serrulae, annular suture 1 slightly curved; sutures with distinct setae bands; ventral margin of lancet and its annuli more distinctly curved upwards. Europe ..... ***P. decipiens*** **(Enslin, 1916)**

– Mesopleuron usually black or slightly pale; lancet with 13 serrulae, annular suture 1 straight; sutures with few setae bands; ventral margin of lancet and its annuli less distinctly curved upwards. Europe ..... ***P. compressa*** **(Hartig, 1837)**

10 Body length 4.0–6.5 mm; head and mesopleuron black or slightly pale; lancet with 16 serrulae, ventral margin of lancet apically strongly curved upwards; sutures with distinct setae bands; radix 1.2 × as long as lamnium. Europe ..... ***P. abietina* (Christ, 1791)**

– Body length 6.0–9.0 mm; head and mesopleuron extensively pale; lancet with 14 serrulae, ventral margin of lancet not curved upwards; sutures with few setae bands; radix 1.7 × as long as lamnium. Europe..... ***P. pseudodecipiens* Beneš & Krístek, 1976**

Males

1 Body length 8.0–9.0 mm; valvispina small, more or less straight, and almost simple; paravalva broad..... ***P. robusta* (Konow, 1895)**

– Combination of characters not as above..... **2**

2 Body length 3.5–5.0 mm; paravalva and pseudoceps relatively narrow, valvispina distinctly bent, and with thin and non-serrate dorsal margin..... ***P. abietina* (Christ, 1791)**

– Combination of characters not as above..... **3**

3 Paravalva narrow at the anterior and broad at the posterior, valvispina strongly bent ..... ***P. ezomatsuvora* Togashi, 1977**

– Combination of characters not as above..... **4**

4 Valvispina simple, the dorsal margin of paravalva long and nearly equal in width from anterior to posterior..... **5**

– Combination of characters not as above..... **6**

5 Valvispina relatively thick and angled in middle..... ***P. decipiens* (Enslin, 1916)**

– Valvispina relatively thin and not angled in middle ..... ***P. harai* Togashi, 1989**

6 Paravalva relatively broad and abruptly narrowing at apex..... **7**

– Paravalva slightly narrower and more gradually narrowing at apex..... **10**

7 Apical margin of paravalva with concave part followed by convex part, the dorsal margin of paravalva short; valvispina slightly bent..... ***P. saxesenii* (Hartig, 1837)**

– Apical margin of paravalva more or less entirely convex, the dorsal margin of paravalva long; valvispina more or less straight or slightly bent **8**

8 Valvispina only with dorsal serrate margin; the apex of paravalva arched and convex ..... ***P. compressa* (Hartig, 1837)**

– Valvispina with dorsal and ventral serrate margins; the apex of paravalva nearly truncate..... **9**

9 Antennae with scape and pedicel testaceous, flagellum dorsally light brown, ventrally light amber yellow; all femora yellowish; hind tarsi yellowish; the middle of the apex of paravalva slightly concave..... ***P. jacutica* Beneš, 2014**

– Antennae with scape and pedicel black, flagellum dorsally black brown, ventrally yellowish-white to light brown; all femora with narrow black stripes on ventral sides; hind tarsi black brown; the middle of the apex of paravalva not concave..... ***P. brevitheca* sp. nov.**

10 Valvispina with dorsal and ventral thinned and serrate margins; paravalva more elongate .....***P. gerula*** **(Konow, 1904)**
– Valvispina only with dorsal thinned and serrate margin; paravalva less elongate ... . ***P. pseudodecipiens*** **Beneš & Krístek, 1976**

Pristiphora brevitheca **Liu & Wei, sp. nov.**
https://zoobank.org/12CEB5C4-AC76-4615-B0B3-16E3769B025E
Figs 1, 2, 3

Material examined. ***Holotype*****,** female, China • Hebei Province, Weichang autonomous county of Man and Mongolia minority, Beimandian Substation of Saihanba Mechanical Forest Farm, 42°28'28"N, 117°27'16"E, 1850 m, 13 July 2022, leg. Tao Li. ***Paratypes*****,** 1 female 4 males, China • Hebei Province, Weichang autonomous county of Man and Mongolia minority, Beimandian Substation of Saihanba Mechanical Forest Farm, 42°28'28"N, 117°27'16"E, 1850 m, 13 July 2022, leg. Tao Li; 26 females 15 males, China • Hebei Province, Weichang autonomous county of Man and Mongolia minority, Sidaogou Town, Baliang, 42°28'34"N, 117°27'43"E, 1867 m, 5–10 June 2023, leg. Guoquan Yi & Shuang Li; 5 females 6 males, China • Hebei Province, Weichang autonomous county of Man and Mongolia minority, Sidaogou, Baliang, 42°28'34"N, 117°27'43"E, 1867 m, 6 June 2022, leg. Tao Li; 65 females 40 males, China • Hebei Province, Weichang autonomous county of Man and Mongolia minority, Dahuanqi Town, Sandaoyingzi, 42°11'03"N, 117°27'58"E, 1490 m, 2 June 2023, leg. Tao Li.

Diagnosis. Body mostly black, stigma usually dark (Fig. 1A); in lateral view, posterior margin of valvula 3 about as long as ventral margin (Fig. 2H); in dorsal view, valvula 3 slightly tapering from base to apex (Fig. 2I); in posterior view, valvula 3 with small invagination; lancet with 9 serrulae, sutures without distinct ctenidia (Fig. 3A); tangium slightly long elliptic; ventral margin of lancet curved upwards (Fig. 3B); valvispina with dorsal and ventral serrate margins; paravalva abruptly narrowing at apex, narrow at the anterior and broad at the posterior (Fig. 3D).

Description. Holotype, female. Body length approximately 6.5 mm (Fig. 1A).

Color**.** Body black. Labrum, most parts of clypeus, pale parts of supraclypeal area and facial orbit, stripe of posterior eye, pronotum, tegula, most parts of propleuron, the sides of terga 1–8, tergum 10, most parts of sterna, most of the apical parts of all coxae, all trochanters, most parts of fore and middle femora, the basal small part of hind femur, fore and middle tibiae, basal 2/3 of hind tibia, most parts of fore and middle tarsi, cenchrus and most parts of valvifer 2 yellowish-white to yellow. Wings hyaline, without smoky macula, apex of vein C pale, stigma and most parts of veins black-brown (Fig. 1A, B).

Head**.** Base of labrum elevated, apex rounded, approx. 2.2 × as wide as long; clypeus short, the median length approximately as long as diameter of the median ocellus, base slightly elevated, apex nearly truncate, with almost no notch, lateral corners indistinct; labrum and clypeus shiny, punctures faint and very sparse, microsculpture indistinct.

Table 1. GenBank accession numbers of COI sequences in this work.

Species	GenBank Accession Number	Species	GenBank Accession Number
Nematus pumila	MK190422	*P. gerula*	HM237384
P. abietina	DQ302227	*P. glauca*	KY698082
P. albilabris	KY698097	*P. incisa*	KX602589
P. albitibia	MF426916	*P. insularis*	KY698110
P. alpestris	DQ302228	*P. laricis*	HM237441
P. amphibola	KY698078	*P. lativentris*	KY698101
P. angulata	HM237442	*P. leucopodia*	HM237437
P. aphantoneura	KX602531	*P. leucopus*	HM237401
P. appendiculata	KY698058	*P. luteipes*	KY698035
P. armata	KX602569	*P. maesta*	HM237368
P. astragali	KX602574	*P. malaisei*	KY698127
P. atripes	KY698086	*P. melanocarpa*	KY698052
P. beaumonti	KX602545	*P. mollis*	HM237374
P. bifida	KY698049	*P. nigra*	HM237395
P. biscalis	HM237407	*P. nigriceps*	HM237432
P. bivittata	HM237398	*P. opaca*	KX602552
P. borea	KC972848	*P. pallida*	HM237415
P. brevis	KY698063	*P. pallidiventris*	KY698108
P. bufo	HM237417	*P. parva*	KY698079
P. cadma	KX602583	*P. pseudocoactula*	KY698105
P. caraganae	MF426917	*P. pusilla*	KX602557
P. carinata	KY698093	*P. ruficornis*	KY698051
P. chlorea	DQ302230	*P. rufipes*	KY698060
P. cincta	KY698069	*P. retusa*	HM237409
P. coactula	DQ302229	*P. reuteri*	HM237416
P. condei	KY698116	*P. saxesenii*	HM237431
P. confusa	KY698047	*P. sermola*	KY698089
P. compressa	HM237440	*P. siskiyouensis*	HM237421
P. conjugata	KY698130	*P. staudingeri*	KY698037
P. cretica	KY698118	*P. subbifida*	KY698125
P. dasiphorae	KY698128	*P. subopaca*	KY698043
P. dedeara	MF426918	*P. thalictri*	HM237406
P. erichsonii	DQ302224	*P. testacea*	HM237372
P. euxantha	KY698080	*P. tetrica*	KY698123
P. friesei	KY698077	*P. thalictrivora*	KY698064
P. fulviceps	KY698114	*P. wesmaeli*	HM237423
P. geniculata	KX602585		

Malar space 0.8 × as long as diameter of median ocellus. Inner margins of eyes slightly convergent downward in frontal view, distance between eyes 1.9 × as long as height of eyes (Fig. 2B). In dorsal view, inner margins of eyes subparallel, the length of temple 0.3 × as long as length of eyes; middle fovea longitudinal groove-like and deep; Frontal area flat, less shiny, punctures coarse and dense, with wrinkles; anterior wall faintly elevated, no notched medially, lateral walls very low and blunt. Interocellar and postocellar furrows indistinct, circumocellar furrow indistinct; POL : OOL : OCL = 2.0 : 1.7 : 1.0 (Fig. 2A). Vertex and postocellar area slightly shiny, punctures small and slightly



Figure 1. Whole body of *P. brevitheca* sp. nov. **A** female, in dorsal view **B** female, in lateral view **C** male, in dorsal view **D** male, in lateral view. Dorsal and lateral views of the adult specimens were taken at the same scale.

dense, with fine microsculpture and some wrinkles; postocellar area slightly convex, middle furrow indistinct, approx. 2.5 × as wide as long, lateral postocellar furrows narrow and shallow, divergent backward (Fig. 2A). Antenna filamentous, shorter than thorax and abdomen together, antennomere 3 slightly compressed; relative length of antennomere 3 : antennomere 4 : antennomere 5 = 1.2 : 1.0 : 1.0 (Fig. 2E).

Thorax. Mesonotum slightly shiny, the anterior part with coarse and dense punctures, and with wrinkles, the posterior part with small and slightly dense punctures, microsculpture indistinct; median mesoscutal groove shallow and thin; mesoscutellum shiny, with fine and slightly sparse punctures, without microsculpture, and slightly elevated, middle ridge faint, the length as long as wide; mesoscutellar appendage shiny, with fine and sparse punctures, microsculpture faint, about 0.2 × length of scutellum, middle ridge low and blunt (Fig. 2C). Distance between cenchri 1.6 × as long as breadth of a cenchrus. Mesepisternum shiny, with fine setigerous punctures, without microsculpture; mesepimeron



Figure 2. Partial characteristics of *P. brevitheca* sp. nov. **A** female, head, dorsal view **B** female, head, anterior view **C** female, thoracic notum **D** female, mesopleuron and metapleuron **E** female, antenna, lateral view **F** male, antenna, lateral view **G** female, hind tarsus and claw **H** female, ovipositor sheath, lateral view **I** female, ovipositor sheath, dorsal view **J** male, head, dorsal view **K** male, head, anterior view.

smooth and shiny, punctures and microsculpture indistinct; metepisternum shiny, posterior part with some fine punctures, microsculpture indistinct; metepimeron shiny and smooth, punctures and microsculpture indistinct (Fig. 2D). Vein M of forewings longer than vein R+M, cross-vein cu-a joining cell 1M at basal 0.6, cell 2Rs 2.4 × as long as cell 1R1, petiole of anal cell of hindwing 1.8 × as long as cu-a, and cu-a curved.

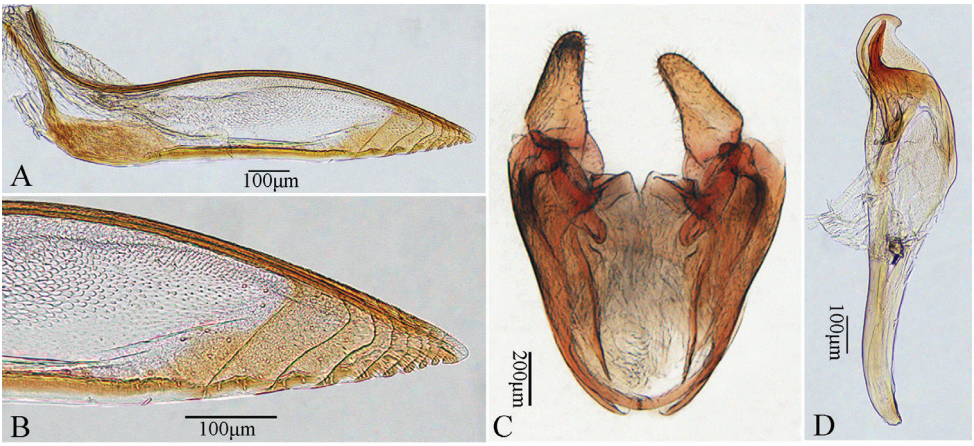


Figure 3. A female, lancet **B** female, lamnium **C** male, gonoforceps **D** male, penis valve.

Abdomen. All abdominal terga shiny, with some small and shallow punctures, microsculpture fine and weak. Ovipositor sheath smooth and shiny, punctures and microsculpture indistinct; sheath 1.7 × as long as metatarsomere 1 and 0.9 × as long as front tibia, valvula 3 0.7 × as long as valvifer 2; in lateral view, posterior margin of valvula 3 truncate and about as long as ventral margin (Fig. 2H); in dorsal view, valvula 3 with small invagination, cercus shorter than valvula 3, angle between most lateral setae of valvula 3 about 80° (Fig. 2I). Lancet with 9 serrulae and ventral margin curved upwards, serrula almost without denticle (Fig. 3A); annular suture 1 oblique and straight, all sutures without setae bands; cypsellae indistinct; tangium long and 4.8 × as long as annulus 1, radix 2.5 × as long as lamnium, the basal angle rounded and obtuse, with a protruding dorsal edge (Fig. 3A, B).

Legs. Protarsomere 1 0.8 × as long as combined length of tarsomeres 2–4; hind tibia 1.2 × as long as hind tarsus, inner apical spur of hind tibia 0.5 × as long as metatarsomere 1, metatarsomere 1 0.6 × as long as combined length of metatarsomeres 2–5; tarsal claw with inner tooth very short (Fig. 2G).

Male. Body length approximately 5.0 mm. Body color and structure similar to the female except for following characters (Figs 1C, D, 2J, K): labrum, clypeus, supraclypeal area, most parts of facial orbit, most parts of genal orbit, antennae with flagellum dorsally black brown, ventrally yellowish-white to light brown; large spot on the anterior part of mesepisternum, the sides of all abdominal tergites and all femora except for black stripes on the ventral sides yellowish-brown; antennomere 3 strongly compressed (Fig. 2F); gonoforceps as shown in Fig. 3C, harpes 1.3 × as long as wide at the base, apical margin roundish; paravalva slightly broad and abruptly narrowing at apex, pseudoceps slightly narrow, valvispina slightly bent, with dorsal and ventral thinned and serrate margins (Fig. 3D).

Distribution. China (Hebei).

Variation. Body length 6.5–7.0 mm in the females; pale parts of the facial orbit yellowish white to yellowish brown; stripe of posterior eye yellowish brown to brown; mesepisternum with brown spots in some individuals; the Rs vein in the forewing vestigial or absent. Body length 4.5–5.0 mm in the males.



Figure 4. Host plants: *Picea meyeri*.

Host plants. *Picea meyeri* (Fig. 4).

Remarks. The new species is similar to *P. jacutica* Beneš, 2014, but it can be distinguished from the latter by its body color and characteristics of the external genitalia: In the female of *P. jacutica*, antennae with scape and pedicel at least dorsally black, flagellum dorsally piceous, ventrally brownish; stigma brownish-yellow to yellowish-brown; mesoscutellum laterally with small inconspicuous paler spots; antennomere 3 subequal to antennomere 4; valvula 3 about 0.3 × as long as valvifer 2, in dorsal view, cercus about as long as valvula 3; the two basal annuli nearly perpendicular. In the male, antennae with scape and pedicel testaceous, flagellum dorsally light brown, ventrally light amber yellow; all femora yellowish; hind tarsi yellowish; the middle of the apex of paravalva slightly concave.

Biology

Under natural conditions, adults of *P. brevitheca* began to emerge around May 20, 2024. Eggs appeared around May 25, were elongated oval in shape, and milky white in color, with an incubation period of 5–6 days. After hatching, the first instar larvae had a light dirty brown head and a light grass-green thorax and abdomen, with the instar lasting 2–3 days. The second instar larvae had a dirty brown head and a light grass-green thorax and abdomen, with the instar lasting 2–3 days. The third instar larvae had a clay brown head and a grass-green thorax and abdomen. The apical of tibiae, tarsi and claws of the thoracic legs were black. The 1–7 pairs of abdominal prolegs had black spots. The surpedal lobes had 2–3 small black spots, and the subspiracular lobes had 4–6 small black spots. This instar lasted 3–4 days. The fourth instar larvae had a clay brown head and a green thorax and abdomen, with two light black longitudinal stripes on the sides of the back. The coxae of the thoracic legs had black spots, the apical of tibiae, tarsi and claws were brown to black. The 1–8 pairs of abdominal prolegs had black spots. The surpedal lobes had black strip-like spots, and the subspiracular lobes had a relatively larger oval black spot. This instar lasted 4–5 days. Around June 16, mature larvae began to spin cocoons, which changed in color from white to clay yellow to dark brown (Fig. 5).



Figure 5. The life history of *P. brevitheca* sp. nov. **A** egg **B** the 1st instar larvae **C** the 2nd instar larvae **D** the 3rd instar larvae **E** the 4th instar larvae **F** the pupa, from initiation to maturity.

Mitochondrial genome and phylogenetic analyses

The partial mitochondrial genome sequence of *P. brevitheca* that we assembled was 16,187 bp. It was further examined by reassembly using and *P. abietina* (PV021568) as references. The partial mitochondrial genome assembly comprises 13 protein-coding genes, 21 tRNAs, and 2 rRNAs, but lacks trnQ and the control region. It was annotated and visualized as a circular map using Geneious Prime (Fig. 6). The A+T content of the *P. brevitheca* mitochondrial genome is 80.9% (A: 43.4%, T: 37.5%, C: 11.2%,

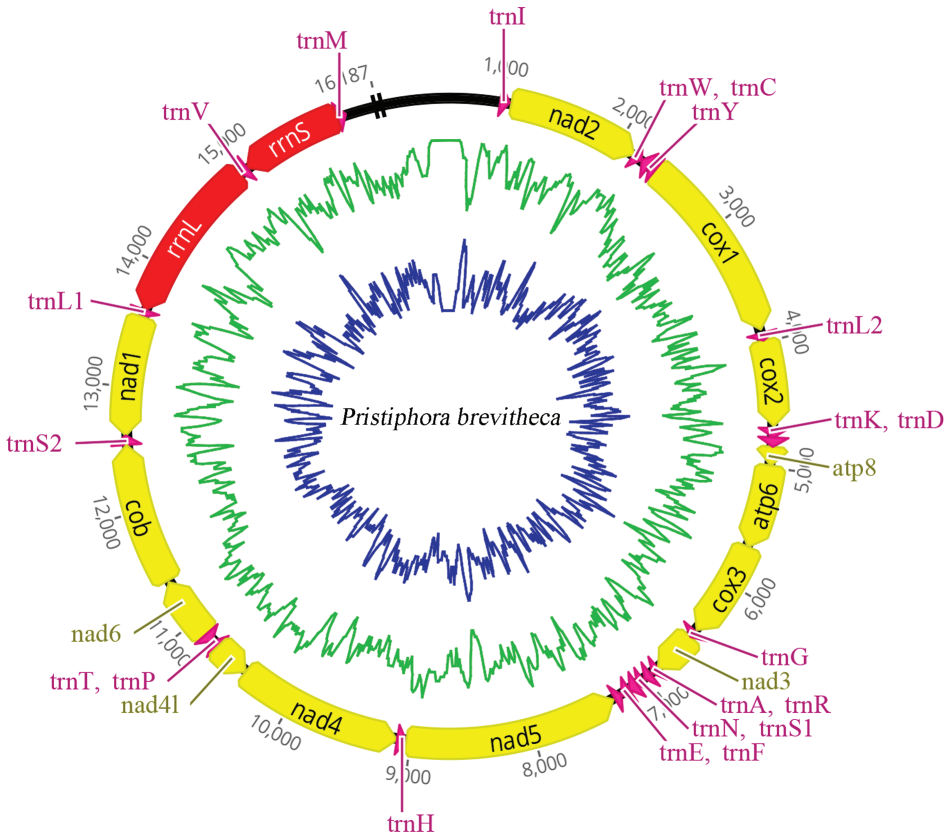


Figure 6. Circular map of the annotated partial mitochondrial genome of *P. brevitheca* sp. nov. (PQ789806). Outer ring: Protein-coding genes (yellow), tRNAs (pink), rRNAs (red). Inner ring: GC (bule) / AT (green) content.

G: 8.0%). All PCGs initiated by ATN codons and most PCGs ended with TAA stop codons, except nad4 using TA as a stop codon. Six tRNAs (trnC, trnY, trnF, trnH, trnP, trnV), two rRNAs and four PCGs (nad1, nad4, nad4L, and nad5), were located on the N-strand, while the J-strand encoded the remaining. The 21 tRNA secondary structures were shown in Fig. 7. The best-fitting model for the COI alignment was TPM1uf+I+G. Phylogenetic analysis showed that the *P. brevitheca* is sister to *P. gerula* (*P. saxesenii* + *P. compressa*), and together they form a clade that is sister to *P. abietina*. The monophyletic *P. abietina* group is sister to the *P. erichsonii* group (Fig. 8).

Discussion

Both morphological classification and phylogenetic analyse results indicate that the *P. brevitheca* has been established as a new species, belonging to the *P. abietina* group. The *P. abietina* group was first defined by Benson (1958). Wong (1975) further divided

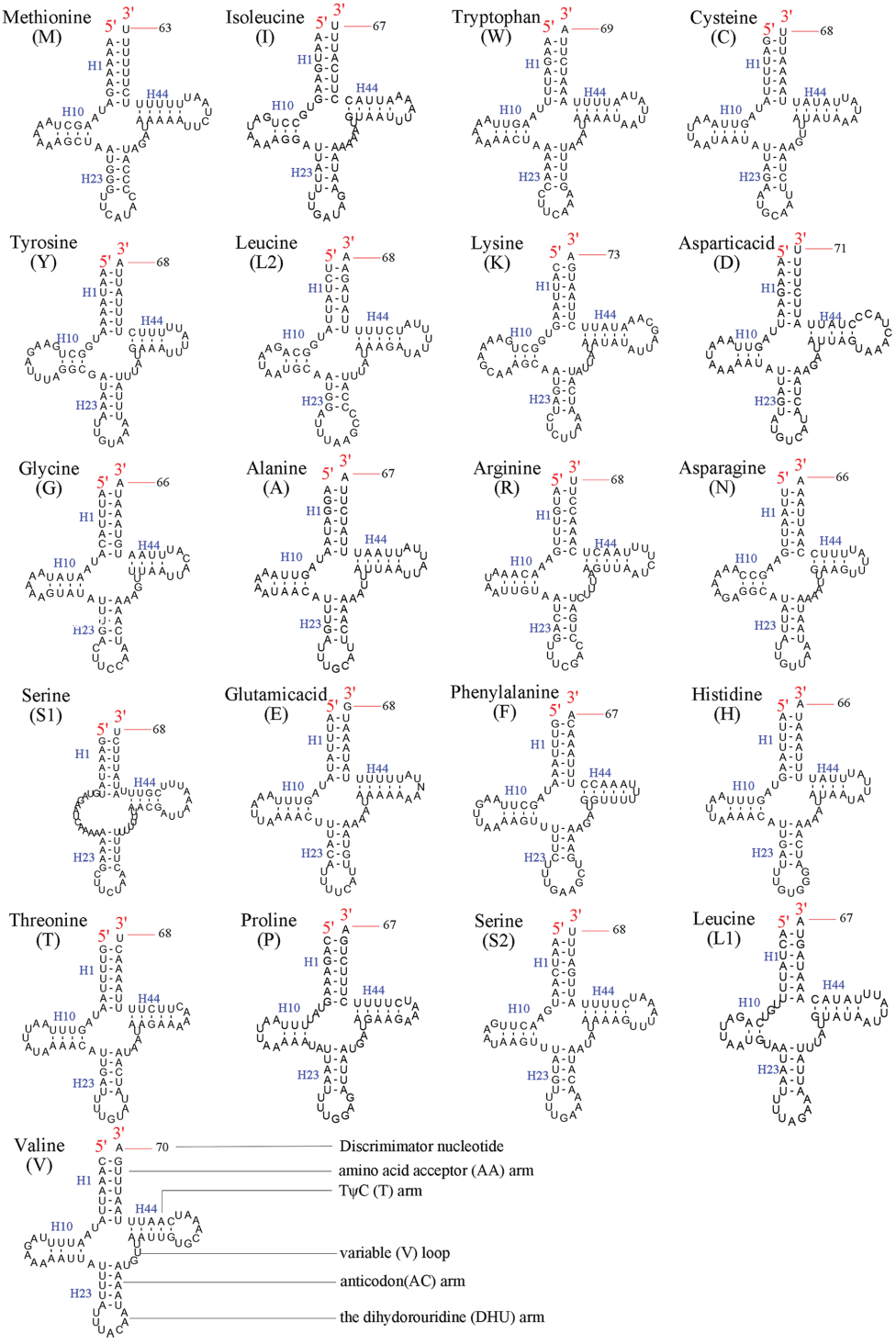


Figure 7. The 21 tRNA secondary structures of *P. brevitheca* sp. nov.

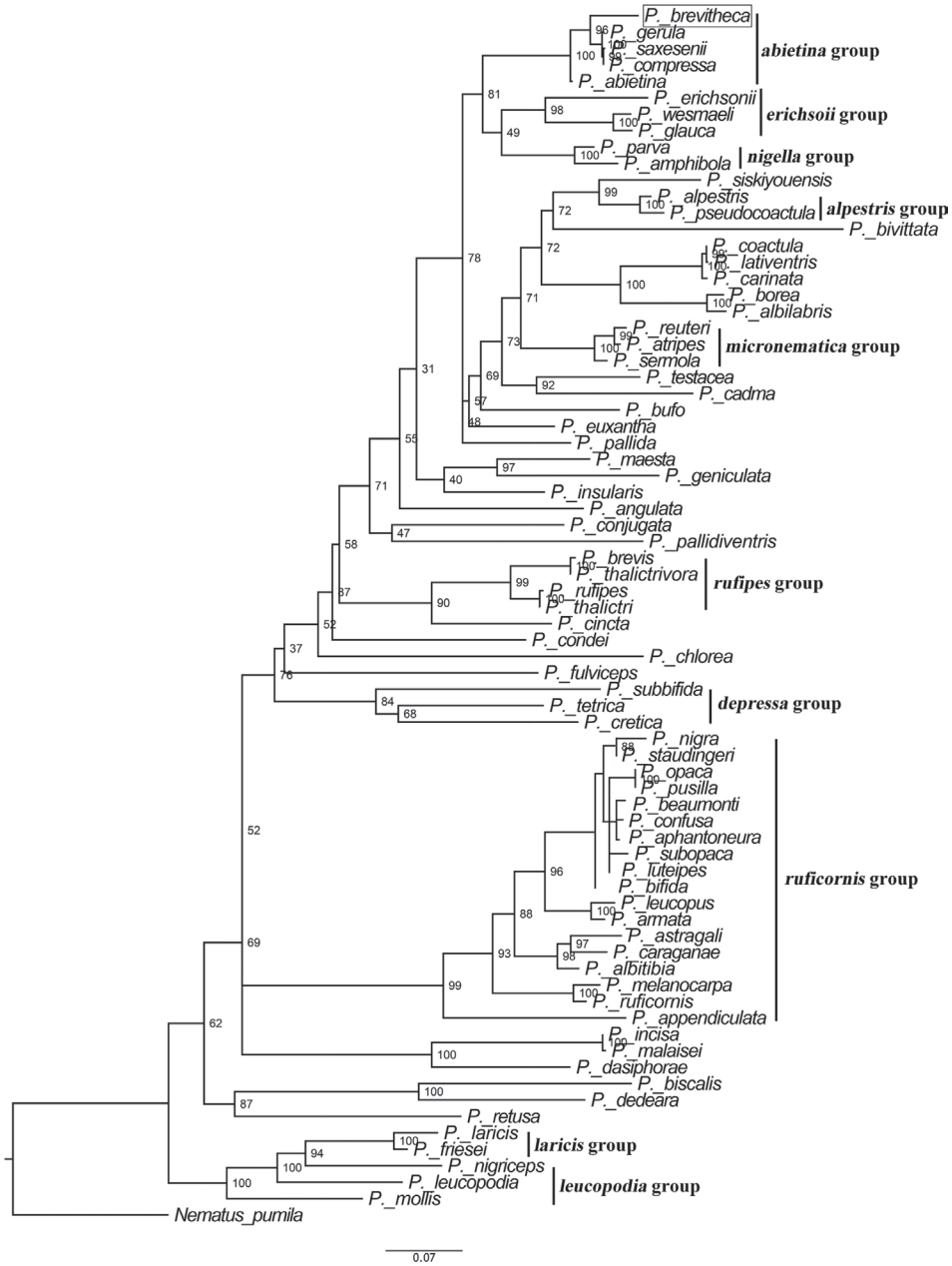


Figure 8. Maximum likelihood tree of *Pristiphora* and *Nematus pumila* based on COI genes (810 bp).

this species group into the *compressa* complex, *pallida* complex, and *wesmaeli* complex. Prous et al. (2017) excluded the *erichsonii* group (the *wesmaeli* complex) and the *pallida* group (the *pallida* complex) from the *abietina* group (the *compressa* complex) based on the results of phylogenetic analyses. The *P. brevitheca* belongs to the

P. abietina group, redefined by Prous et al. (2017), which includes ten species (Togashi 1977, 1989; Beneš 2014). Phylogenetic analysis showed that *P. brevitheca*, along with *P. gerula*, *P. saxesenii*, and *P. compressa*, formed a clade that is sister to *P. abietina*. It was confirmed that *P. brevitheca* belongs to the *P. abietina* group, and this also supports the monophyly of the group. However, not all species in this group have COI data available, and we based our analysis solely on the available data.

In this study, the mitochondrial genome of the *P. brevitheca* was partially assembled from short-read Illumina data. The final annotation included 13 protein-coding genes, 21 tRNAs, and 2 rRNAs, but lacked trnQ and the control region, comparisons to complete mitogenomes of congeneric species (*P. abietina*, GenBank: PV021568).

Despite encompassing more than 1,200 described species, the subfamily Nematinae is genomically underrepresented, with only a few complete mitochondrial genomes having been reported (Tan et al. 2021; Olli et al. 2024). However, the genomes assembled with only Illumina short-reads might not in fact be complete (Olli et al. 2024). In published mitochondrial genomes, longer control regions are typically assembled using long-read sequencing (e.g., PacBio), while shorter CRs primarily rely on short-read Illumina data. The actual lengths of CRs may exceed those assembled from short reads. In subsequent studies, we will employ long-read sequencing to assemble the mitochondrial genome of *P. brevitheca*, aiming to obtain a complete mitochondrial genome assembly for further comparative and phylogenetic analyses.

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