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Three new species of *Pteromalus* (*Habrocytus*) *sequester* group (Hymenoptera: Pteromalidae) from China based on morphology and DNA barcodes

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Simple Summary: *Pteromalus* Swederus, 1795 is the largest genus in the Pteromalinae (Hymenoptera: Chalcidoidea:Pteromalidae), and is widely distributed in the world. Previously, the *Pteromalus* has not been investigated extensively and deeply from Xinjiang, northwest China. However, many of these belong to cryptic species complexes with hardly any morphologically distinguishing features. And molecular data are rarely generated and used to these small parasitoids. Thus, we evaluated the efficacy of morphological taxonomy and DNA barcoding for the identification of the *Sequester* group. Three new species of the *Sequester* group, including *Pteromalus* (*Habrocytus*) *boleensis* Yan *et* Li sp. nov., *Pteromalus* (*Habrocytus*) *longepedicelus* Yan *et* Li sp. nov., and *Pteromalus* (*Habrocytus*) *robustus* Yan *et* Li sp. nov. are described here for the first time. Molecular results showed that ITS2 is effective in identifying the *Sequester* group in China.

Abstract: Three new species of the *Sequester* group of *Pteromalus* (*Habrocytus*) (Hymenoptera: Pteromalidae: Pteromalinae), *Pteromalus* (*Habrocytus*) *boleensis* Yan *et* Li sp. nov., *Pteromalus* (*Habrocytus*) *longepedicelus* Yan *et* Li sp. nov., and *Pteromalus* (*Habrocytus*) *robustus* Yan *et* Li sp. nov. are described and illustrated based on adult morphology and molecular data from Xinjiang, China. The DNA barcodes (ITS2) of *Pteromalus* (*Habrocytus*) *boleensis* sp. nov. and *Pteromalus* (*Habrocytus*) *robustus* sp. nov. have been generated and compared with all existing sequences of the *Sequester* species group. The results of morphological taxonomy and molecular identification are consistent. A key to all known species of the *Sequester* group in the world is provided.

Keywords: New taxon; morphological characteristics; ITS2; *Medicago sativa*

1. Introduction

As the largest genus of the subfamily Pteromalinae (Hymenoptera: Pteromalidae), *Pteromalus* Swederus contains 488 valid species worldwide, with the majority (371 species) having been described from Europe [1], whereas only 15 species were described from China [2,3]. Many of these belong to cryptic species complexes with hardly any morphologically distinguishing features [4]. To simplify and recapitulate the huge diversity of *Pteromalus* and the very similar genus *Habrocytus* Thomson, which differs from the former only by the number of teeth on the left mandible (3 in *Habrocytus* versus 4 in *Pteromalus*, Graham (1969) established a framework of species groups, including the *Sequester* group of the current subgenus *P.* (*Habrocytus*), as treated by Bouček and Graham [4,5].

Graham described four species in the *Sequester* group [4]. Dzhanokmen provided a key to *Pteromalus* from Kazakhstan including *P.* (*Habrocytus*) *sequester* in 1998 [6] and also a detailed description of *P.* (*Habrocytus*) *sequester* in 2001 [7]. Ye et al. (2012) and Klimmek & Baur (2018) described *P.* (*Habrocytus*) *sequester* in this species group [1,8]. Herein, three new species of the *Sequester* group from Xinjiang Uyghur Autonomous Region (hereafter Xinjiang) China are studied based on morphological taxonomy and DNA barcoding (Non-genic, internal transcribed spacers 2, ITS2). At present, seven species of the *Sequester* group are known in the world, including three new taxon described herein. A key to all the known species of the *Sequester* group in the world is provided.

2. Materials and Methods

2.1. Specimens collected and Morphology

All the specimens were collected by sweeping with a net in Xinjiang from 2020 to 2022 and preserved in 100% ethanol. They were subsequently air-dried, point-mounted, and examined with a Nikon SMZ 745T stereomicroscope. The images were taken with a Nikon DS-Fi3 connected to a Nikon SMZ 25 stereomicroscope. All images were stacked with NIS-Elements software and arranged in plates by using Adobe Photoshop. All specimens are deposited in the insect collection of the College of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang, China (ICXU).

Morphological terminology follows that of Graham (1969) [4]. The description of the new species is based on the holotype, and variation is also noted for the paratypes. Body length, including the ovipositor sheaths, is measured in millimeters (mm); other measurements are given as ratios. Abbreviations of morphological terms used are: OOL= ocell ocellar line; POL= posterior ocellar line; fun = funicular segment; MV= length of marginal vein; PMV= length of postmarginal vein; STV= length of stigmal vein; Gtn = gastral tergum.

2.2. DNA barcoding

Three specimens of *P*. (*Habrocytus*) *boleensis* sp. nov. and three specimens of *P*. (*Habrocytus*) *robustus* sp. nov. were extracted total genomic DNA by using the TIANamp Genomic DNA Kit (China). Due to repeated failures of amplification of the Cytochrome c oxidase I Folmer fragment (COI, standard barcode) for most specimens, we used a non-genic internal transcribed spacers 2 and a region of 28s rDNA. In addition, three *P*. (*Habrocytus*) *sequester* ITS2 sequences and two outgroup ITS2 sequences were downloaded from GenBank (download from: https://www.ncbi.nlm.nih.gov/) used in the phylogenetic analyses (Table 1). For DNA amplification and sequencing, we followed the protocols described by Yao [9]. Forward and reverse sequences were assembled and reciprocally edited with ContingExpress. MEGA X was also used to perform genetic distances under the Kimura 2-parameter model of base substitution [10]. The phylogenetic tree is constructed by PhyloSuite v1.2.3 software (download from: https://github.com/dongzhang0725/PhyloSuite/releases) [11]. Meanwhile, ITS2 sequences were performed to best evolutionary model analysis with 10,000 bootstrap replicates in the BI. The resulting phylogenetic trees were visualized and beautified through the Figtree. All sequences were deposited in the Genbank database (NCBI).

Table 1. Accession numbers of ITS2 molecular for the phylogenetic analysis of the Pteromalidae from GenBank.

Number	Species	GenBank accession number
1	*P*. (*Habrocytus*) *sequester*	MZ646309.1
	P. (*Habrocytus*) *sequester*	MZ646310.1
	P. (*Habrocytus*) *sequester*	MZ646311.1
2	*Nasonia giraulti* (outgroup)	U02953.1
	Nasonia giraulti (outgroup)	U02955.1

3. Results

3.1. Morphological Taxonomy

3.1.1. P. (Habrocytus) sequester species group

Diagnosis. The anterior margin of clypeus deeply incised; propodeal nucha represented only by a narrow, transversely aciculate to smooth strip; fore wing with basal cell bare or virtually so, basal vein usually bare; lower surface of costal cell with hairs usually widely broken in the middle [4].

Hosts. Most species of this group parasitize Diptera and Coleoptera. For example, *P.* (*Habrocytus*) *sequester* was reared from galls of A*sphondylia mayeri* Liebel (Diptera: Cecidomyiidae) on *Sarothamnus scoparius* (Fabaceae) and also from larvae of *Apion fuscirostre* (F.) (Coleoptera: Apionidae) in seeds of *Sarothamnus* sp.; *P.* (*Habrocytus*) *cionobius* (Erdös) was reared from cocoons of *Cionus thapsi* (F.) (Coleoptera: Curculionidae) in fruits of *Verbascum phlomoides* (Scrophulariaceae) [4].

Distribution. *P.* (*Habrocytus*) *sequester* is a cosmopolitan species; *P.* (*Habrocytus*) *cionobius* is known from Hungary; the two undetermined, undescribed *P.* (*Habrocytus*) spp. (I and J) are from Slovakia [4].

3.1.2. Key to the world species of P. (Habrocytus) sequester group (females)

1. Propodeum with plicae as sharp carinae on the sides of nucha, and foveae at the base of propodeum, but effaced in the middle; flagellum stout, distinctly stouter proximally than pedicel in dorsal view; proximal segments of funicle subquadrate, distal segments at least slightly transverse................................... *P.* (*Habrocytus*) *cionobius* (Erdös)
- Propodeum with plicae complete, sometimes not sharp in the middle but always distinct three; flagellum sometimes less stout; proximal segments of funicle sometimes slightly longer than broad 2
2. Antenna with scape extending to the level of lower edge of median ocellus ....*P.* (*Habrocytus*) *sequester* (Walker)
- Antenna with scape hardly extending to the level of lower edge of median ocellus... 3
3. Lower edge of torulus slightly above the level of ventral edge of eye; pedicel about 3.0× as long as first funicular segment; pronotal collar very long, medially 1/3 as long as mesoscutum .... *P.* (*Habrocytus*) *longepedicelus* Yan *et* Li, sp. nov.
- Lower edge of torulus distinctly above the level of ventral edge of eye; pedicel at most 1.5× as long as first funicular segment; pronotal collar shorter, medially 1/7 to 1/4 as long as mesoscutum 4
4. Body size 2.2–2.8 mm; posterior margin of Gt1 at most slightly curving backwards but medially not produced; POL 2.0–2.4× OOL; metafemur about 4.1× as long as broad ... . *P.* (*Habrocytus*) *boleensis* Yan *et* Li sp. nov.
- Body size 3.1–3.5 mm; posterior margin of Gt1 curving backwards and strongly produced medially; POL 1.7–1.8× OOL; metafemur about 3.6× as long as broad ....*P.* (*Habrocytus*) *robustus* Yan *et* Li sp. nov.

1. *P.* (*Habrocytus*) *boleensis* Yan *et* Li sp. nov.

Description. FEMALE (holotype, Figures 1 A–F). Body length 2.8 mm. Body (Figures 1A and 1F) metallic green with weak blue metallic reflections; eye bare; antenna brown except scape yellow; fore wing hyaline, veins brown; coxae identical to body in color; femora brownish-black, brown in apical sixth; tibiae slightly infuscate, apical tibial segments and tarsus white except apical tarsomere dark brown.

Head in frontal view (Figure 1B) about 1.2× as wide as high; Antennal sockets slightly closer to anterior margin of clypeus than to median ocellus; scape short, 6.7× as long as broad, hardly extending to the level of lower margin of median ocellus; pedicel 2.0× as long as broad, 0.7× as long as fu1; funicular segments connected tightly to each other and each with 2 rows of sensilla; fu1 to fu5 about 1.2× as long as wide, fu6 as long as wide, clava about 2.5× as long as broad; pedicel and flagellum combined about 0.9× as long as head width. In lateral view, eye height about 1.6× eye width, malar space about 0.4× eye height. In dorsal view, head (Figure 1C) about 2.3× as broad as long; POL about 2.0× OOL; temple about 0.3× as long as eye.

Mesosoma in dorsal view (Figure 1D) as long as head width; pronotum collar slightly narrower than mesoscutum width (about 0.9×), pronotal collar medially 1/5 as long as mesoscutum; mesoscutum about 0.6× as long as broad. Propodeum (Figure 1E) median area smooth, with a row of deep foveae at base, sides of propodeum smooth, about 0.3× as long as scutellum. In lateral view, prepectus about 0.7× as long as tegula; entire mesosomal pleura regularly reticulate except upper mesepimeron smooth. MV as long as PSV and about 1.4× as long as STV. Metacoxa in dorsal view bare, with fine reticulate sculpture, metafemur about 4.4× as long as broad.

Gaster (Figure 1G) about 1.8× as long as broad, as long as head plus mesosoma; Gt1 smooth, with posterior margin obviously curved backward medially, about 0.3× as long as gaster.

Male. Unknown.

Diagnosis. Body smaller (2.2–2.8 mm); body metallic green with weak blue metallic reflections; fore wing veins brown; antennal sockets slightly closer to anterior margin of clypeus than to median ocellus; POL 2.0–2.4× OOL; propodeum with a row of deep foveae at base; posterior margin of Gt1 at most slightly curving backward but medially not produced; metafemur about 4.1× as long as broad.

Variation (paratypes, ♀). Body length 2.2–2.8 mm. Head in frontal view 1.2–1.25× as wide as high; pedicel and flagellum combined 0.83–0.85× as long as head width; malar space 0.4–0.5× eye height; head 2.3–2.5× as broad as long; POL 2.0–2.4× OOL; pronotal collar medially obviously narrower than mesoscutum width (1/7–1/5); mesoscutum about 0.6× as long as broad; scutellum about 0.9× as broad as long; MV 1.2–1.3× PSV; MV 1.5–1.6× STV; prepectus about 0.7× as long as tegula; metafemur about 4.1× as long as broad; gaster 0.95–1.0× as long as head plus mesosoma.

Remarks. The new species resembles *sequester* (Walker) but in general body shape and size. However, it can be distinguished from the existing species by the markings on its antennal sockets location, propodeum shape, and posterior margin shap of Gt1.

Etymology. The specific name is derived from the collection locality of its holotype.

Type material. Holotype f# [ICXU], air-dried on card point: China, Xinjiang, Bole, Alashankou-Tianshan route, sweeping on Asteraceae plants, 44°52'5.61"N, 82°2'45.65"E, 542 m, 26.vi.2022, Q. Li research group. Paratypes 21 f#, same data as holotype.

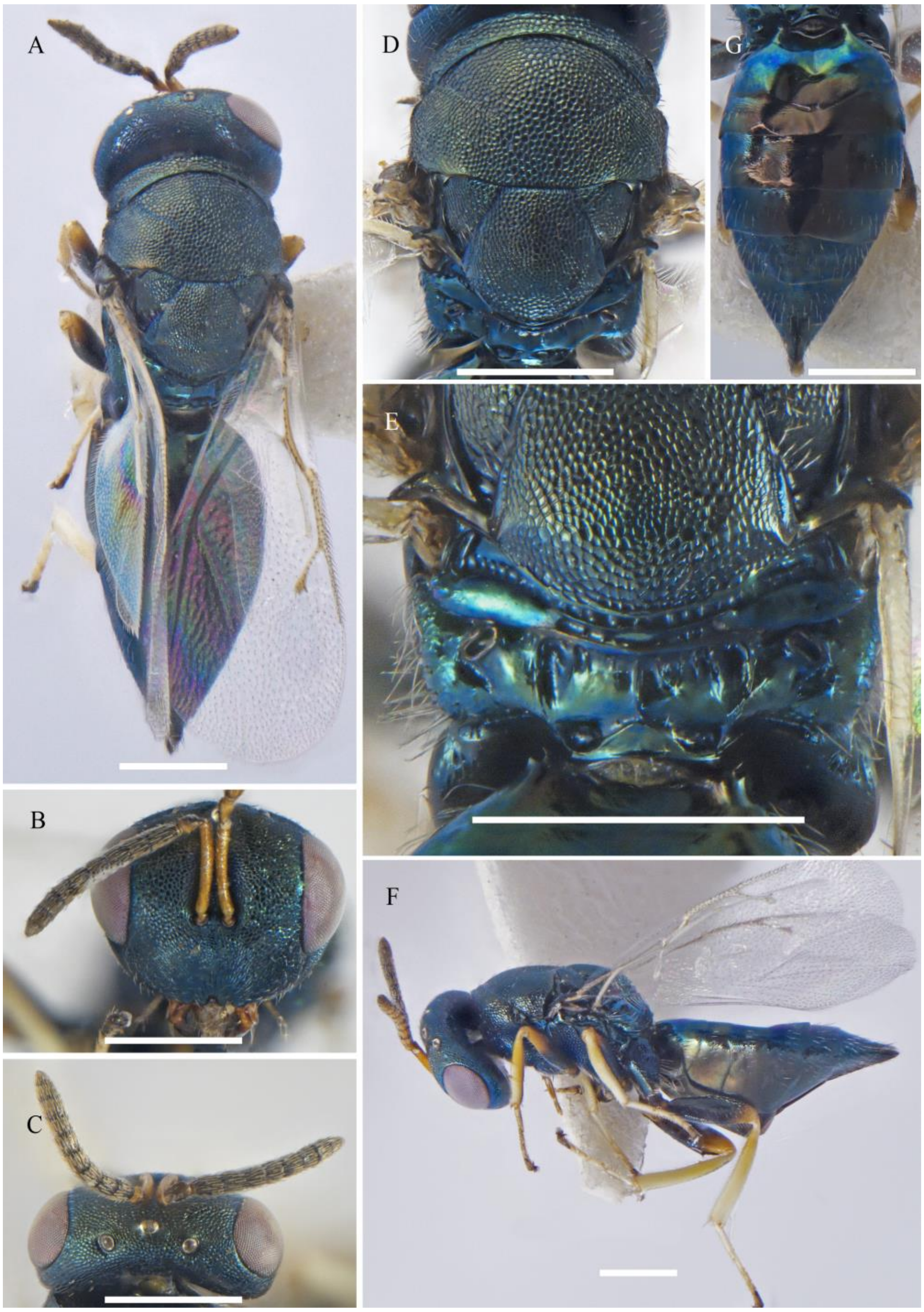

Figure 1. *P*. (*Habrocytus*) *boleensis* sp. nov. holotype (A–G female): A body, dorsal B head, frontal C head, dorsal D mesosoma, dorsal E propodeum, dorsal F body, lateral G gaster, dorsal. Scale bars: 0.5 mm.

2. *P*. (*Habrocytus*) *longepedicelus* Yan *et* Li sp. nov.

Description. FEMALE (holotype, Figures 2 A–F). Body length 2.3 mm. Body (Figure 2A) metallic dark green with shine; eye bare; antenna bright yellow except pedicel, anelli and flagellum brownish-yellow; fore wing hyaline, veins pale brown; coxae identical with body color; femora brown, slightly brown in apical sixth; tibiae and white, telotarsi dark brown.

Head in frontal view (Figure 2B) about 1.2× as wide as high; Antennal sockets (Figure 2B) situated obviously closer to anterior margin of clypeus than to median ocellus; scape hardly extending to the level of lower margin of median ocellus; pedicel about 2.0× as long as broad, about 3.0× as long as fu1; funicular segments connected tightly to each other and each with 1 row of sensilla; fu1 to fu6 nearly as long as wide (2:2.3, 3:2.5, 3:3, 3:3, 3:3, 3:3), clava about 2.3× as long as broad; pedicel and flagellum combined about 0.8× as long as head width. In lateral view, eye height about 1.4× eye width, malar space about 0.5× eye height. In dorsal view, head (Figure 2C) about 2.2× as broad as long; ocellus very small, POL about 1.8× OOL; temple about 0.5× as long as eye.

Mesosoma in dorsal view (Figure 2D) narrower than head width (about 0.9×); pronotum collar slightly narrower than mesoscutum width (about 0.9×), pronotal collar very long, medially 1/3 as long as mesoscutum; mesoscutum about 0.53× as long as broad; scutellum with strong raised reticulate sculpture, about 0.95× as long as broad, frenal area absent. Propodeum (Figure 2E) median area with slightly oblique rugae, sides of propodeum with irregular and weak rugae, about 0.5× as long as scutellum; nucha triangular and small. In lateral view, prepectus about 0.6× as long as tegula. MV about 1.9× as long as PMV and about 1.9× as long as the STV. Metacoxa in dorsal view bare, with fine reticulate sculpture, metafemur about 4.1× as long as broad.

Gaster (Figure 2F) elongate, about 2.1× as long as broad, about as long as head plus mesosoma; Gt1 smooth, with posterior margin medially curved backward medially, about 0.6× as long as gaster.

Male. Unknown.

Diagnosis. Antennal sockets obviously closer to anterior margin of clypeus than to median ocellus; pedicel about 2.0× as long as broad, about 3.0× as long as fu1; funicular segments with 1 row of sensilla; fu1 to fu6 nearly as long as wide; ocellus very small; pronotal collar medially obviously narrower than mesoscutum width (nearly 1/4–1/3); propodeum median area with slightly oblique rugae, sides of propodeum with irregular and weak rugae.

Variation (paratypes, ♀). Body length 2.41–2.42mm. Head in frontal view about 1.0–1.2× as wide as high; malar space about 0.48–0.54× eye height; head about 2.1–2.3× as broad as long; POL about 1.7–1.9× OOL; pronotal collar medially obviously narrower than mesoscutum width (nearly 1/4–1/3); mesoscutum about 0.66–0.70× as long as broad; scutellum about 1.0–1.1× as broad as long; MV 1.67–1.68× PSV; MV 1.67–1.68× STV; prepectus about 0.6–0.7× as long as tegula; gaster elongate about 0.9–1.2× as long as head plus mesosoma.

Remarks. The new species resembles *P*. (*Habrocytus*) *sequester* (Walker), both green with golden reflections; proximal segment is slightly longer than broad; propodeum median carina and plica complete, median area usually smooth. *P*. (*Habrocytus*) *longepedicelus* sp.nov. differs from *P*. (*Habrocytus*) *sequester* (Walker) by the following characters: Antennal sockets obviously closer to anterior margin of clypeus than to median ocellus (Antennal sockets obviously closer to median ocellus than to anterior margin of clypeus); Pedicel 3.0× as long as fu1(Pedicel 1.2× as long as fu1); funicular segments with 1 whorl of sensilla (funicular segments with 2 rows of sensilla); ocellus very small (ocellus is smaller than *P*. (*Habrocytus*) *longepedicelus* sp.nov.).

Etymology. The specific name refers to the long pedicel of the holotype.

Type material. Holotype f# [ICXU], air-dried on card point: China, Xinjiang, Emin County, sweeping on Poaceae, 46°21'43.91"N, 83°53'17.52"E, 645 m, 28.vi.2021, Q. Li research group. Paratypes 1f#, air-dried on card point: China, Xinjiang, Tekes County, 43°9'19.98"N, 81°47'23.76"E, 1184 m, 9.vii.2021, Q. Li research group; 1f#, air-dried on card point: China, Xinjiang, Fuyun County, 47°0'53.64"N, 89°32'4.90"E, 1360 m, 11.vii.2020, Q. Li research group.

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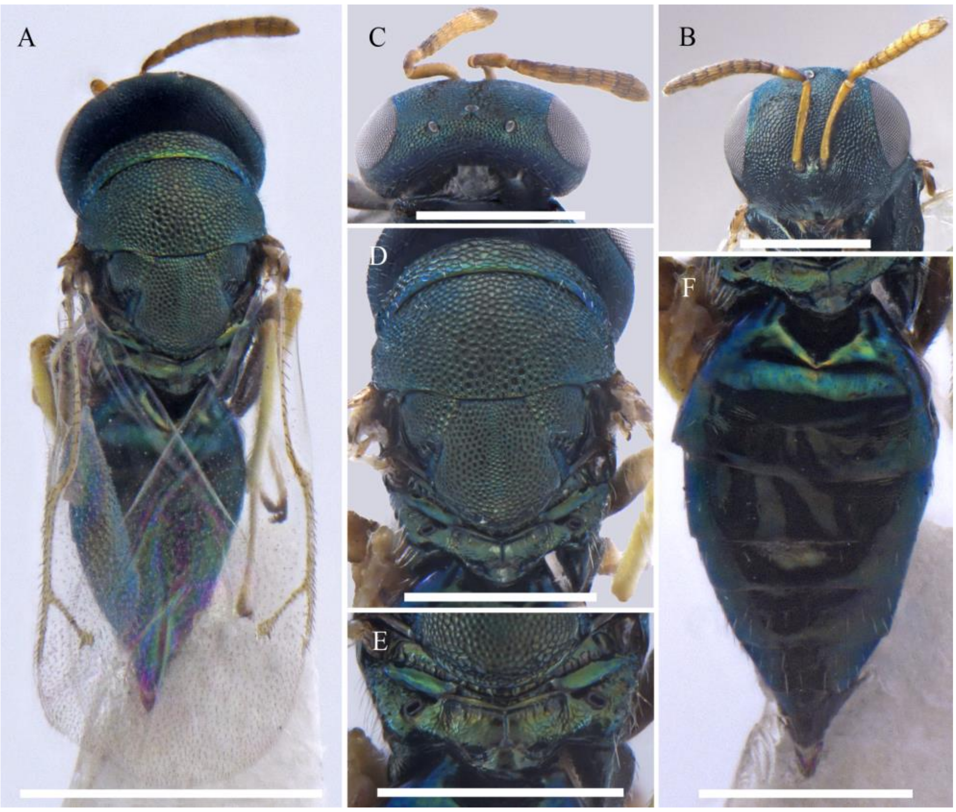

Figure 2. *P*. (*Habrocytus*) *longepedicelus* sp. nov., holotype (A–F female): A body, dorsal; B head, frontal; C head, dorsal; D head, lateral; E mesosoma, dorsal; F propodeum, dorsal G body, lateral H metasoma, dorsal. Scale bars: 0.5 mm.

3. *P*. (*Habrocytus*) *robustus* Yan *et* Li, sp. nov.

Description. FEMALE (holotype, Figures 3 A–E). Body length 3.0 mm. Body (Figures 3A and 3E) metallic bright green with shine; eye bare; antenna pale brown except scape and pedicel yellow; fore wing hyaline, veins white; coxae identical with body color; femora dark brow in basal 2/3 white, femora brown with basal, ventral margin 2/3 and apical 1/3; tibiae and tarsus white; telotarsi dark brown.

Head in frontal view (Figure 3B) about 1.3× as wide as high, face with metallic reflections and regularly raised reticulations; anterior margin of clypeus deeply emarginate in the middle. Antennal sockets (Figure 3B) situated closer to clypeus anterior of margin than to median ocellus; scape short, 7.0× as long as broad, hardly extending to the level of lower margin of median ocellus; pedicel about 1.5× as long as broad, about as long as fu1; funicular segments connected tightly to each other and each with 2 rows of sensilla; fu1 to fu3 about 1.3× as long as wide, the following three funicular segments about 0.80× as long as wide, clava about 2.0× as long as broad; pedicel and flagellum combined about 0.76× as long as head width. In lateral view, eye height about 1.4× eye width, malar space about 0.7× eye height. In dorsal view, head (Figure 3C) about 2.5× as broad as long; POL about 1.7× OOL; temple about 0.3× as long as eye.

Mesosoma in dorsal view (Figure 3A), narrower than head width (about 0.8×); pronotum slightly narrower than mesoscutum width (about 0.9×), ponotal collar 1/5 as long as mesoscutum; mesoscutum about 0.7× as long as broad; scutellum with strong raised reticulate sculpture, about 1.0× as long as broad, frenal area absent. Propodeum (Figure 3D) median area smooth, with a row of shallow foveae at base, sides of propodeum median area smooth, about 0.4× as long as scutellum. In lateral view, prepectus about 0.5× as long as tegula. MV about 1.3× as long as PMV and about 1.5× as long as STV. Metacoxa in dorsal view bare, with fine reticulate sculpture, metafemur about 3.6× as long as broad.

Gaster (Figure 3A) about 1.8× as long as broad, about 1.0× as long as head plus mesosoma; Gt1 smooth, with posterior margin obviously curved backwards medially, about 0.4× as long as gaster.

Male. Unknown.

Diagnosis. Body larger (3.1–3.5 mm); body metallic bright green with shine, veins white; the antennal sockets slightly closer to anterior margin of clypeus than to median ocellus; POL 1.7–1.8× OOL; Gt1 with posterior margin obviously curved backward medially.

Variation (paratypes, ♀). Body length 3.0–3.5 mm. Head in frontal view about 1.2–1.3× as wide as high; pedicel and flagellum combined about 0.7–0.8× as long as head width; malar space about 0.4–0.5× eye height; head about 2.3–2.4× as broad as long; POL about 1.7–1.8× OOL; pronotal collar medially obviously narrower than mesoscutum width (1/5–1/4); mesoscutum about 0.61–0.63× as long as broad; scutellum about 0.87–0.91× as broad as long; MV 1.0–1.1× PSV; MV 1.3–1.4× STV; prepectus about 0.70–0.72× as long as tegula; gaster elongate about 1.1–1.2× as long as head plus mesosoma.

Remarks. The new species strong resembles that of *boleensis* sp. nov. but is larger (3.0–3.5 mm). Through the integrative taxonomy based on DNA barcoding and morphological evidence, the result showed that *P*. (*Habrocytus*) *robustus* sp. nov. is a valid species (Figures 4).

Etymology. The specific name refers to the body of this species being robust.

Type material. Holotype f# [ICXU], air-dried on card point: China, Xinjiang, Tacheng Perfecture, Tacheng City, sweeping on Poaceae, 46°43'2.69"N, 83°4'12.54"E, 544 m, 23.vi.2022, Q. Li research group. Paratypes 11 f#, air-dried on card point: China, Xinjiang, Kizilsu Kirgiz Autonomous Prefecture, Atushi City, sweeping on Poaceae plants, 39°43'23.03"N, 76°9'4.76"E, 1349 m, 28.vii.2022, Q. Li group; 2m# [ICXU], air-dried on card point: China, Xinjiang, Ili Kazakh Autonomous Prefecture, Xinyuan County, sweeping on Medicago or Salvia, 43°24'12.16"N, 83°34'53.73"E, 1044 m, 11.vii.2021, Q. Li research group.

Figure 3. *P*. (*Habrocytus*) *robustus* sp. nov., A–C, E holotype, D paratypes (A–E female): A body, dorsal B head, frontal C head, dorsal D propodeum, dorsal E body, lateral. Scale bars: 0.5 mm.

3.2. DNA barcoding

There were also no differences among any of the *Sequester* group taxa in the 28srDNA regions flanking the ITS2. So molecular results only showed that ITS2 efficacy of the *Sequester* group.

The ITS2 barcode sequence data of the two obtained of the *Sequester* group species were submitted to the GenBank database under the following accession numbers: *P*. (*Habrocytus*) *boleensis* sp. nov. (NCBI Accession OQ942216-OQ942218) and *P*. (*Habrocytus*) *robustus* sp. nov. (NCBI Accession OQ942211, OQ942214, and OQ942215). The pairwise genetic distances within and between the *Sequester* species group are shown in Table 2. The mean interspecific genetic distance between the *Sequester* group ranged from 4.06% to 5.43%, whereas intraspecific genetic distance ranged from 0% to 0.41%. The highest genetic distance among species was between the *Sequester* group of *P*. (*Habrocytus*) *sequester* and *P*. (*Habrocytus*) *boleensis* sp. nov.

Table 2. Kimura 2-parameter genetic distances calculated within (in bold) and between each species of Pteromalidae.

	1	2	3	4	5	6	7	8	9	10	11
1	—	0.0029	0.0041	0.0103	0.0103	0.0103	0.0117	0.0117	0.0117	0.0252	0.0255
2	0.0040		0.0029	0.0099	0.0099	0.0099	0.0111	0.0111	0.0111	0.0258	0.0261
3	0.0081	0.0041		0.0093	0.0093	0.0093	0.0104	0.0104	0.0104	0.0251	0.0255
4	0.0494	0.0450	0.0406		0.0000	0.0000	0.0112	0.0112	0.0112	0.0254	0.0256
5	0.0494	0.0450	0.0406	0.0000		0.0000	0.0112	0.0112	0.0112	0.0254	0.0256
6	0.0494	0.0450	0.0406	0.0000	0.0000		0.0112	0.0112	0.0112	0.0254	0.0256
7	0.0543	0.0492	0.0441	0.0520	0.0520	0.0520		0.0000	0.0000	0.0258	0.0260
8	0.0543	0.0492	0.0441	0.0520	0.0520	0.0520	0.0000		0.0000	0.0258	0.0260
9	0.0543	0.0492	0.0441	0.0520	0.0520	0.0520	0.0000	0.0000		0.0258	0.0260
10	0.2416	0.2474	0.2390	0.2364	0.2364	0.2364	0.2358	0.2358	0.2358		0.0042
11	0.2442	0.2500	0.2415	0.2389	0.2389	0.2389	0.2360	0.2360	0.2360	0.0112	—

* The diagonal row of values (in bold) indicate intraspecific distances, the values below the diagonal indicate mean interspecific distances and values above the diagonal indicates SE estimates obtained by bootstrap procedure (1000 replicates) as implemented in MEGA X. 1. OQ942216; 2. OQ942217; 3. OQ942218; 4. OQ942211; 5. OQ942214; 6. OQ942215.; 7-9. MZ646309.1-MZ646311.1 P. (*Habrocytus*) *sequester*; 10. U02955.1 *Nasonia giraulti*; 11. U02953.1 *Nasonia giraulti* (7-11 download sequence from Genbank).

An ML phylogenetic analysis revealed three distinct *P*. (*Habrocytus*) clusters, including the *P*. (*Habrocytus*) *boleensis* sp. nov. cluster, the *P*. (*Habrocytus*) *sequester* cluster and the *P*. (*Habrocytus*) *robustus* sp. nov. cluster (Figure 4). The result strongly visualized that *P*. (*Habrocytus*) *boleensis* sp. nov., *P*. (*Habrocytus*) *robustus* sp. nov. and *P*. (*Habrocytus*) *sequester* samples were grouped into the same cluster based on similarity with the sequences of both the *Sequester* group species.

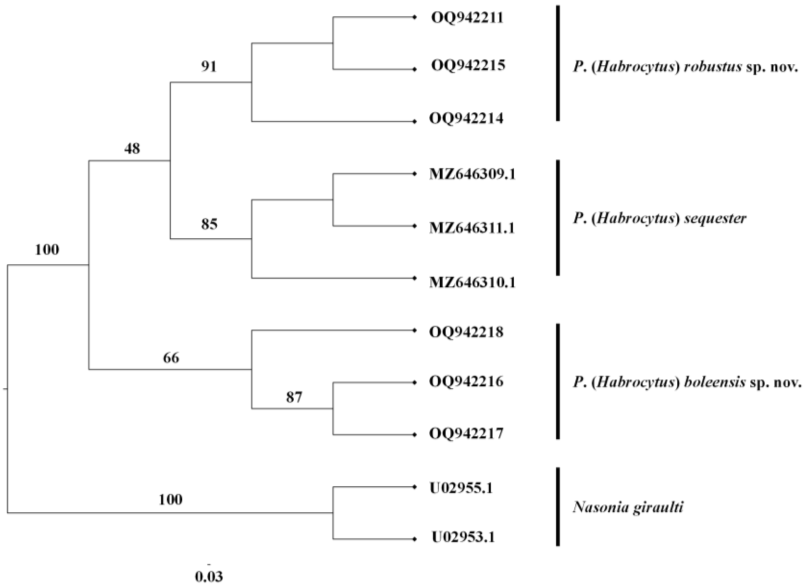

Figure 4. ML tree of the *Sequester* group based on ITS2 sequence Three sequences from the GenBank database (accession numbers MZ646309.1-MZ646311.1) were used as reference *P.* (*Habrocytus*) *sequester*. *Nasonia giraulti* (accession number: U02953.1, U02955.1) was used as an outgroup.

4. Discussion

Although the ITS2 sequence were amplification from *P.* (*Habrocytus*) *boleensis* sp. nov. and *P.* (*Habrocytus*) *robustus* sp. nov., and *P.* (*Habrocytus*) *longepedicelus* sp. nov., two specimens (all paratypes) of *P.* (*Habrocytus*) *longepedicelus* sp. nov. did not yield any results. In this study, we only evaluated the efficacy of the DNA barcoding based on ITS2 sequence analysis to identify two the *Sequester* group species in China: *P.* (*Habrocytus*) *boleensis* sp. nov. and *P.* (*Habrocytus*) *robustus* sp. nov. We found that *P.* (*Habrocytus*) *robustus* sp. nov. was genetically more closely related to *P.* (*Habrocytus*) *sequester* than *P.* (*Habrocytus*) *boleensis* sp. nov., with low genetic differences between species groups. In this study, barcodes based on the nuclear ITS2 sequence successfully identified the *Sequester* group specimens. This proves that there is a large intraspecific genetic variation in ITS2 in the *Sequester* group.

Including three new species in this study, 7 species of the *Sequester* group are known from the world with two indet species [4]. However, hosts of most species are unknown except for *P.* (*Habrocytus*) *sequester* and *P.* (*Habrocytus*) *cionobius* [4]. We also collected 45 specimens of *P.* (*Habrocytus*) *sequester* from Xinjiang: Urumqi, Jeminay County, Wensu County, Kuqa County separately. Current evidence indicates that *P.* (*Habrocytus*) *sequester* a larvae parasite of the *Sarothamnus* sp. (Fabaceae) seed pest *Apion fuscirostre* (F.) (Coleoptera: Apionidae) [4]. According to our field observations, *P.* (*Habrocytus*) *sequester* was swept from *Medicago sativa* (Fabaceae) in all collecting sites in Xinjiang. Therefore, we plan to focus on *M. sativa* to find out whether this species is phytophagous or parasitic. This would be very meaningful because *M. sativa* is an important forage plant in animal husbandry.

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Data Availability Statement: DNA sequence data are available on GenBank under accession num bers OQ942216–OQ942218, OQ942211, OQ942214, and OQ942215. Other data of this research were deposited in the College of Life Science and Technology, Xinjiang University, Urumqi, Xinjiang, China.

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