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A new feather mite species of the genus *Proctophyllodes* Robin, 1877 (Astigmata: Proctophyllodidae) from the Long-tailed Tit *Aegithalos caudatus* (Passeriformes: Aegithalidae)—morphological description with DNA barcode data

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Abstract

A new feather mites species, *Proctophyllodes valchukae* **sp. n.**, is described from the Long-tailed Tit, *Aegithalos caudatus* (Linnaeus, 1758) (Passeriformes: Aegithalidae), captured in the Primoriye (Russian Far East). The new species belongs to the *tricetratus* species group and is most closely related to *P. stachyris* Atyeo et Braasch, 1966. For the first time for feather mites the standard morphological description is supplemented by sequence data of the mitochondrial cytochrome *c* oxidase subunit I gene fragment (COI) and nuclear D2 region of 28S rDNA.

Key words: Astigmata, feather mites, Proctophyllodidae, new species, DNA barcoding, COI, D2, 28S rRNA, nondestructive DNA extraction

Introduction

The feather mite genus *Proctophyllodes* Robin, 1877 (Astigmata: Analgoidea: Proctophyllodidae: Proctophyllodinae) is the most species-rich genus among all recently recognized feather mite families (Gaud & Atyeo 1996) and currently includes about 155 species (Fritsch 1961; Atyeo & Braasch 1966; Gaud & Fain 1990; Mironov & Kopij 1996; Mironov & Galloway 2002; Badek *et al*. 2008). In the plumage of their avian hosts, these mites, as for most proctophyllodids, inhabit the flight feathers and greater coverts of the wings and also the tail feathers, where they are located in narrow corridors on the ventral surface of the vanes. Mites of the genus *Proctophyllodes* are predominantly distributed on birds of the order Passeriformes and have been recorded so far from representatives of about 35 families; just a few species were reliably recorded from hosts of the orders Apodiformes and Charadriiformes (Atyeo & Braasch 1966).

The systematics and species identification of the genus *Proctophyllodes* are mainly based on the structure of the body terminus and genital region in males, while females of this genus are rather uniform morphologically. In the world revision of this genus, Atyeo and Braasch (1966) arranged all species they had at their disposal into ten morphological groups. Although these authors constructed a clear key to groups, they did not give uniform diagnoses to groups and characterized them in a rather free format listing only main diagnostic features. Besides, these authors clearly acknowledged that some recognized groups are artificial because they were based on arbitrary characters. Subsequently, two more species groups were established (Gaud & Fain 1990; Mironov & Kopij 1996) by splitting some species off groups previously created by Atyeo and Braasch.

A recent preliminary study of phylogenetic relationships in the family Proctophyllodidae (Knowels & Klimov 2011) based on molecular data from four nuclear genes showed that the genus *Proctophyllodes* in the current taxonomic concept is paraphyletic in relation to the two derived genera, *Joubertophyllodes* Atyeo et Gaud, 1971 and *Monojoubertia* Oudemans, 1905.

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In the present work we describe a new *Proctophyllodes* species from the Long-tailed Tit *Aegithalos caudatus* (Linnaeus, 1758).

Material and methods

The material used in the present study was collected by SVM from a Long-tailed tit at the Bird Banding Station of the Amur-Ussurian Centre for Biodiversity of Birds (Institute of Biology and Soil Sciences of the Russian Academy of Sciences, Vladivostok) in the southern part of the Primorsky Kray (Russia) in October of 2008. Mites were removed manually from live birds with a needle under a dissecting microscope and preserved in 96% ethanol. Specimens mounted in Faure medium (Evans 1992) were investigated under an Olympus BX51 light microscope with differential interference contrast (DIC) illumination. Drawings were made using a camera lucida drawing attachment.

Several mite individuals from the sample were subjected to DNA extraction and further also mounted in Faure medium. Total genomic DNA was extracted from individual specimens using a nondestructive method as described by Dabert et al. (2008). A 670-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified with primers bcdF05 (5'-TTTTCTACHAAYCATAAAGATATTGC-3') and bcdR04 (5'-TATAAACYTCDGGATGNCCAAAAAA-3') (Dabert *et al*. 2008), and an 850-bp fragment of the nuclear 28S rDNA, including the D2 region, was amplified with primers developed in this study: 28SF0001 (5'-ACCCVCYNAATTTAAGCATAT-3') and 28SR0990 (5'-CCTTGGTCCGTGTTTCAAGAC-3'). PCRs were carried out in 10 μl reaction volumes containing 5 μl Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany), 0.5 μl of each primer, and 4 μl of DNA template using a thermocycling profile of one cycle of 5 min at 95 °C followed by 35 steps of 30 sec at 95 °C, 90 sec at 50 °C, 1 min at 72 °C, with a final step of 5 min at 72 °C. After amplification, the PCR were diluted with 10 μl of water and 5 μl of the diluted PCR reaction was analyzed by electrophoresis on a 1% agarose gel. Samples containing visible bands were directly sequenced in the forward direction by using 1 μl of the PCR reaction and 40 pmoles of primer. Sequencing was performed with a BigDye Terminator v3.1 on an ABI Prism 3130XL Analyzer (Applied Biosystems). Contigs were aligned and manually assembled in ChromasPro v. 1.32 (Technelysium Pty Ltd.) and GeneDoc v. 2.7.000 (Nicholas and Nicholas 1997). Pairwise distance calculations between sequences were computed using the Kimura two parameter (K2P) distance model (Kimura 1980) for all codon positions with MEGA 5 (Tamura *et al*. 2011).

The morphological description generally follows the descriptive scheme proposed by Atyeo and Braasch (1966). However this standard scheme of description is supplemented by characters relating to the details of the gnathosoma and legs, as proposed by recent works on *Proctophyllodes* species (Mironov & Kopij 1996; Mironov & Galloway 2002; Badek *et al*. 2008). All measurements are given in micrometers (μm). The length of the gnathosoma was measured from the distal tips of palpi to the basal margin of the subcapitulum. The width of the gnathosoma was measured at the base of the subcapitulum. Idiosomal length was measured from the anterior margin of the prodorsum to the posterior end of body excluding terminal hyaline appendages (females) or opisthosomal lamellae (males). Idiosomal width was measured as the greatest width at level of humeral shields. The body and leg chaetotaxy follow that of Gaud and Atyeo (1996). Latin and English names of birds as well as the higher-level classification of birds are those of Dickinson (2003).

The holotype and half of the paratypes are deposited in the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (ZISP); other paratypes (including samples for extracting DNA) are in the collection of the Faculty of Biology of the Adam Mickiewicz University, Poznan, Poland (AMUMD).

Results

Family Proctophyllodidae Mégnin et Trouessart, 1884

Genus *Proctophyllodes* Robin, 1877

***Proctophyllodes valchukae* sp. n.**
(Figs. 1–3)

Type material. Male holotype (ZISP-4702), 7 male and 10 female paratypes ex *Aegithalos caudatus* (L.) (Aegithelidae), Russia, Primoriye, Partizanskii District, Novolitovsk, 9 km N, 42º51'40"N; 132º53'5.5"E, 8 October 2008, leg. S.V. Mironov. Holotype, 4 male and 4 female paratypes—ZISP, remaining paratypes—AMUMD.

DNA vouchers. Male paratype: AMUMD016, GenBank Acc. JN936875 (COI), JN936876 (D2), 4 female paratypes: AMUMD001-4, GenBank Acc. JN936871-74 (COI).

Description. *Male* (Figs. 1A, B) (holotype, measurements for 5 paratypes in parentheses): gnathosoma length 44 (42–47), greatest width 36 (35–38). Idiosoma length 276 (270–280), width 146 (135–150). Prodorsal shield: setae *vi* rudimentary (scarcely distinct), anterior-lateral extensions acute, lateral margins entire, posterior margin slightly concave, posterior angles rounded, greatest length 77 (72–80), greatest width 102 (100–108), surface of shield without lacunae. Distances between scapular setae: *se-se* 57 (50–60). Scapular shields narrow. Humeral shields well developed, fused with epimerites III, encompassing setae *cp*. Setae *c2* in antero-mesal angle of humeral shield. Subhumeral setae *c3* lanceolate, 13 (12–14) long, 3.5 (3.5–4) wide. Hysteronotal shield: anterior margin straight or slightly concave, anterior angles acute, length 179 (170–185), width at anterior margin 97 (95–100), median part with small sparsely disposed pit-like lacunae. Supranal concavity opened terminally, anterior end extending beyond level of setae *e2*, length from bases of setae *ps1* 46 (45–52). Posterior margin of opisthosoma between setae *h2* slightly convex. Terminal lamellae tongue-shaped, straight, not overlapping, with pennate venation; length of lamellae 52 (50–55), maximal width 18 (16–20). Setae *ps1* as long as distance between them. Distances between hysteronotal setae: *c2:d2* 55 (53–60), *d2:e2* 75 (71–80), *e2:h3* 45 (44–50), *d1:d2* 18 (16–20), *e1:e2* 31 (25–32), *h1:h2* 15 (15–20), *h2:h2* 55 (51–56), *h3:h3* 31 (30–34), *ps2: ps2* 66 (65–70).

Epimerites I fused into a narrow U with weak connection, without lateral extensions. Setae *3a* situated distinctly posterior to inner tips of epimerites IIIa. Genital arch of moderate size, 31 (30–32) in length, 24 (24–26) in width, its base situated at midlevel of trochanters IV, apex extending to midlevel between trochanters III and IV. Aedeagus sword-shaped, directed backward from genital arch apex, extending to midlevel between setae *g* and *ps3*, 51 (47–52) in length, 4 (4–4.5) in width at base; genital sheath poorly sclerotized, approximately as long as two thirds of aedeagus (Fig. 2A). Setae *4a* at midlevel of genital arch. Paragenital and pregenital apodemes absent, genital papillae not connected. Opisthogastric shield represented by two pairs of sclerites: anterior (genital) sclerites longitudinal, with anterior ends adjoining to tips of genital arch, with posterior ends acute and slightly divergent; posterior (adanal) sclerites oblique, with anterior ends directed antero-medially and bearing setae *ps3*. Bases of setae *g* and *ps3* in trapezoidal arrangement, setae *g* filiform, setae *ps3* narrowly lanceolate, distances between these setae: *g:g* 9 (8–9), *g:ps3* 18 (18–20), *ps3:ps3* 27 (26–32). Distance from genital arch apex to level of setae *ps1* 112 (110–115). Anal suckers cylindrical, 17 (15–18) in length, 13 (12–13) in width, corolla with 15–18 small teeth. Postero-lateral areas of ventral opisthosoma with 3–4 well pronounced bow-shaped striae.

Femora I, II with narrow ventral crests. Tarsus IV 27 (25–27) long, seta *d* at midlevel of this segment (Fig. 2C). Genual solenidion σIII situated approximately at midlevel of segment (Fig. 2B). Length of genual solenidia: σ*1*I 29 (26–29), σIII 12 (11–13).

Female (Figs. 3A, B) (range for 5 paratypes): Gnathosoma shaped as in males, length 60–66, width 54–56. Length of idiosoma 425–455, width 190–208. Prodorsal shield shaped as in males, except for straight posterior margin, length 98–105, width 93–107. Distances between scapular setae *se* 86–90. Scapular shields narrow. Humeral shields fused with epimerites III, encompassing bases of setae *cp*, setae *c2* at anterior margin of this shield. Subhumeral setae *c3* lanceolate, 13–16 long, 3.4–4 wide. Lobar region of opisthosoma distinctly separated from remaining part of hysterosoma, hysteronotal shield split into anterior and lobar parts by narrow transverse furrow. Anterior hysteronotal shield roughly rectangular, with anterior margin straight or shallowly concave, with posterior margin slightly sinuous, surface with poorly expressed small sparsely disposed lacunae, greatest length 235–245, width at anterior margin 120–135. Distance between hysteronotal and lobar shields 5–8. Lobar shield 70–85 in length, 90–95 in width at level of extensions bearing setae *h2*. Opisthosomal lobes straight, slightly attenuate apically, nearly twice longer than wide; terminal cleft U-shaped, parallel-sided, length 40–47 in length, 20–22 in width at level of setae *ps1*. Setae *h1* on posterior margin of hysteronotal shields. Setae *ps1* on lateral margins of terminal cleft. Setae *h2* with spindle-like basal enlargement and with long filiform distal part; setae *h3* 16–24 long, about 1/6 of terminal appendages. Distance between dorsal setae: *c2:d2* 68–72, *d2:e2* 118–122, *e2:h2* 70–74, *h2:h3* 35–40, *d1:d2* 17–20, *e1:e2* 42–48, *h1:h2* 30–32, *h2:ps1* 9–11, *h1:h1* 38–44, *h2:h2* 80–83.

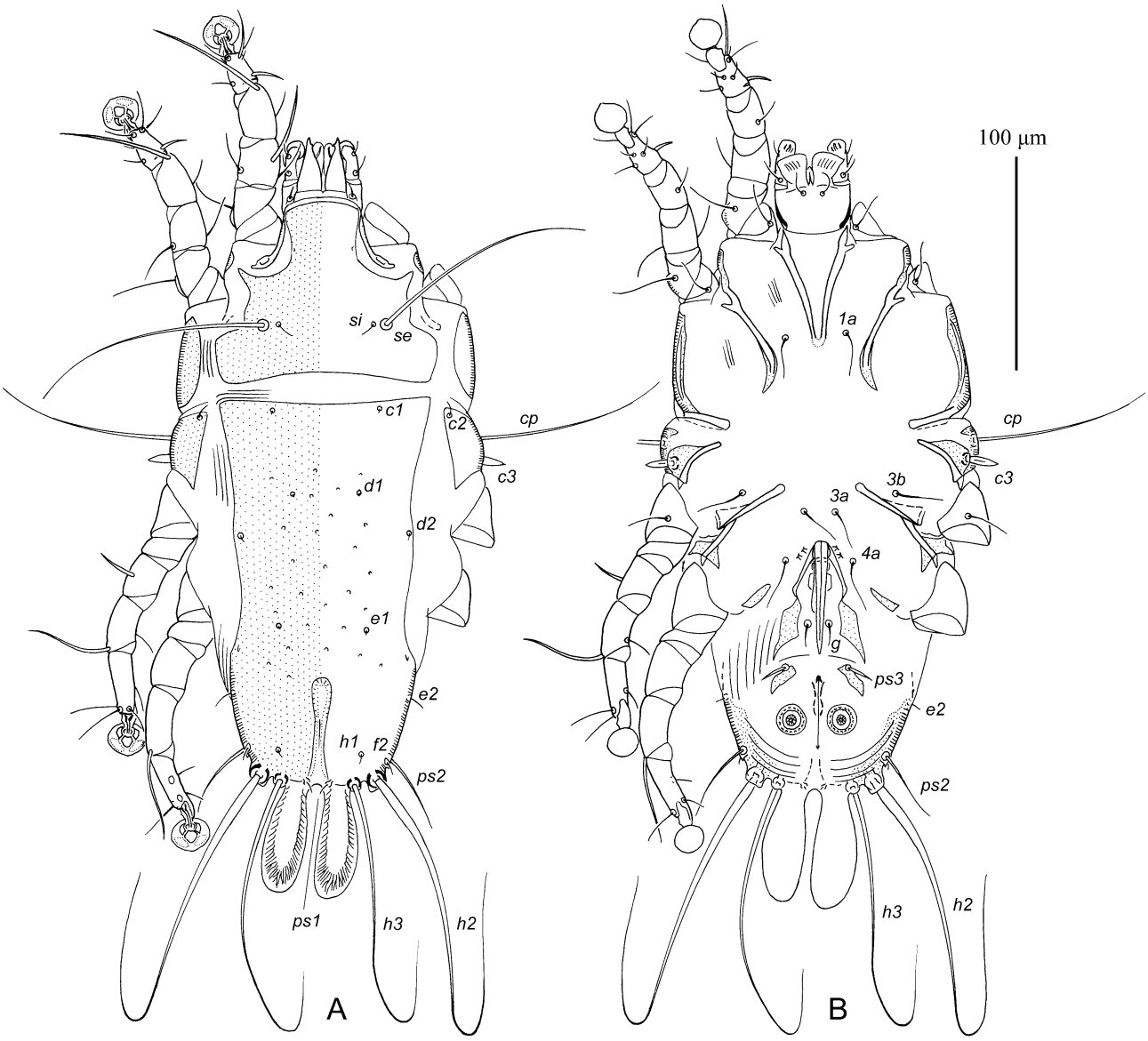

FIGURE 1. *Proctophyllodes valchukae* **sp. n.**, male. A—dorsal view, B—ventral view.

Epimerites shaped as in males. Epigynum short bow-shaped, with thickened lateral parts, not extending to level of genital papillae, length 28–33, width 68–74. Apodemes of oviporus thin. Copulatory opening situated immediately posterior to anal opening and covered with heavily sclerotized semicircular plate-like extension (Fig. 2D). Translobar apodemes wide, connected to each other anterior to terminal cleft. Genital setae *g* anterior to level of setae *3b*. Setae *ps2* at level of posterior end of anal opening, widely separated from each other.

Femora I, II with ventral crest as in male. Solenidion σ of genu III situated at midlevel of segment or slightly distal to it. Length of genual solenidia: $\sigma 1$I 37–40, σIII 15–17.

Differential diagnosis. The new species *Proctophyllodes valchukae* **sp. n.** belongs to the *tricetratus* species group, of which males are mainly characterized by having a relatively short aedeagus (not extending to the bases of terminal lamellae) and the reduced sclerotization of the central part of the opisthogastric shield (Atyeo & Braasch 1966). Because the opisthogastric shield varies in this group, being represented by one or two pairs of sclerites, or one unpaired genital fragment and two adanal fragments, this species group might be artificial. Within this group, the new species is close to *P. stachyris* Atyeo et Braasch, 1966 from the Greay-headed Babbler, *Stachyris poliocephala* (Temminck, 1836) (Timaliidae), by having relatively small tongue-shaped terminal lamellae and an opisthogastric shield split into two pairs of sclerites.

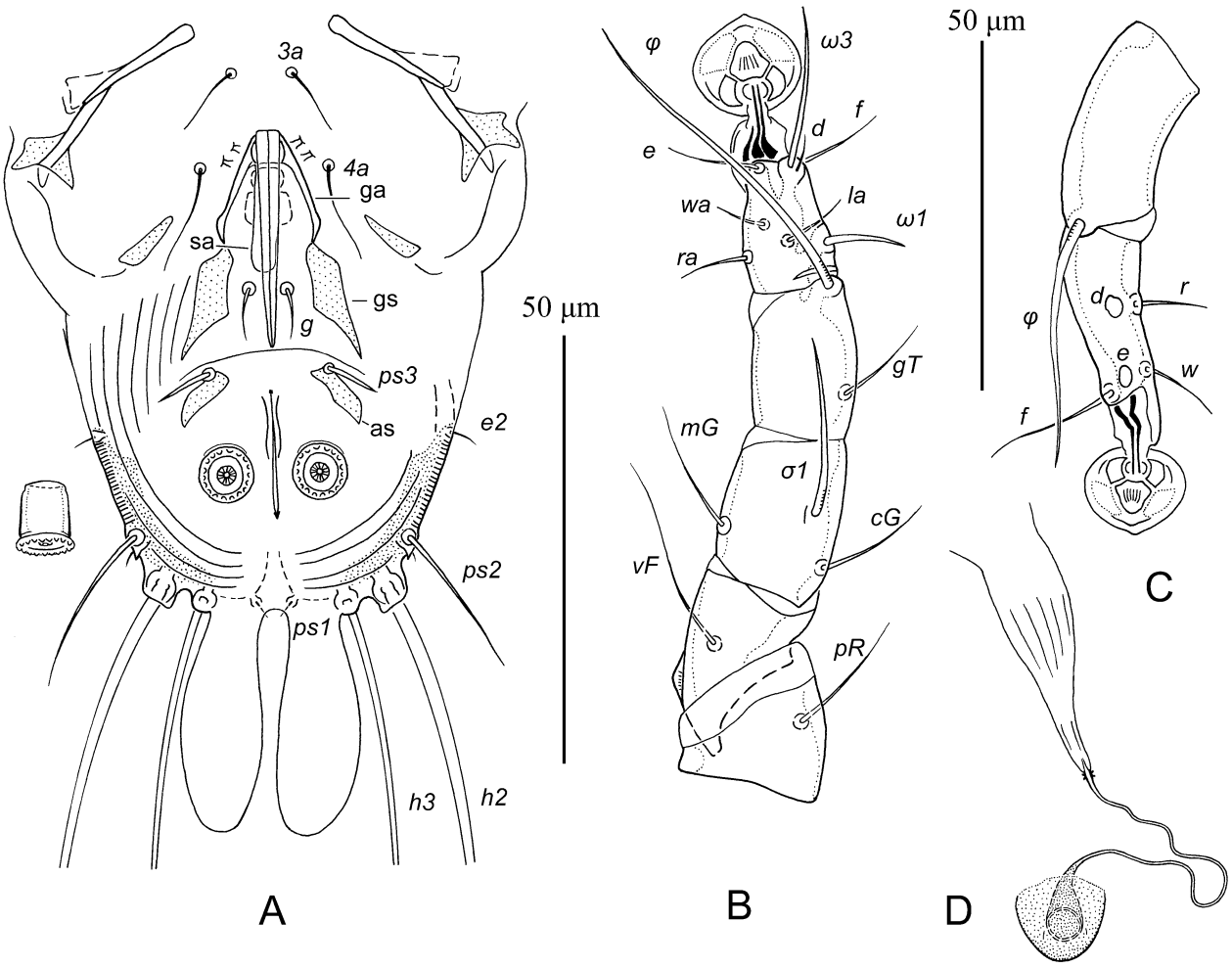

FIGURE 2. *Proctophyllodes valchukae* **sp. n.**, details. A—ventral view of male opisthosoma, B—leg I of male, C—Tibia and tarsus IV of male, D—spermatheca and spermaducts. Abbreviations: as—adanal fragment of opisthogastric shield, ga—genital arch, gs—genital fragment of opisthogastric shield, sa—sheath of aedeagus.

Proctophyllodes valchukae **sp. n.** differs from that species by the following features: in males, the aedeagus (in normal position) extends approximately to the midlevel between setae *g* and *ps3*, and the terminal lamellae are distinctly longer (52–55); in females, the terminal cleft is parallel-sided and the anterior end of this cleft lacks strong sclerotization. In males of *P. stachyris*, the aedeagus (in normal position) scarcely extends to the level of setae *g*, and the terminal lamellae are about 30 long; in females, the terminal cleft is V-shaped, and the cuticular wall around the anterior end of this terminal cleft is strongly thickened and heavily sclerotized.

DNA barcode. We sequenced a 613-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene (DNA barcode region chosen by the Consortium for the Barcode of Life, http://barcoding.si.edu) and 788-bp of the nuclear 28S rDNA, containing D2 region (Sonnenberg *et al*. 2007) for one male paratype (Acc. JN936875, JN936876) and 4 female paratypes (Acc. JN936871-4). The average genetic distance (K2P) within the *P. valchukae* COI sequences was 0.72% (SE 0.25). Because most of the nucleotide substitutions were synonymous, they resulted only in one amino acid change (substitution of glycine 189 with serine). No intraspecific variability in the *P. valchukae* D2 region of 28S rDNA was detected.

Etymology. The new species is named in honour of Dr. Olga P. Valchuk (Institute of Biology and Soil Sciences of the Russian Academy of Sciences, Vladivostok), the head of the bird banding station of the Amur-Ussurian Centre for Biodiversity of Birds.

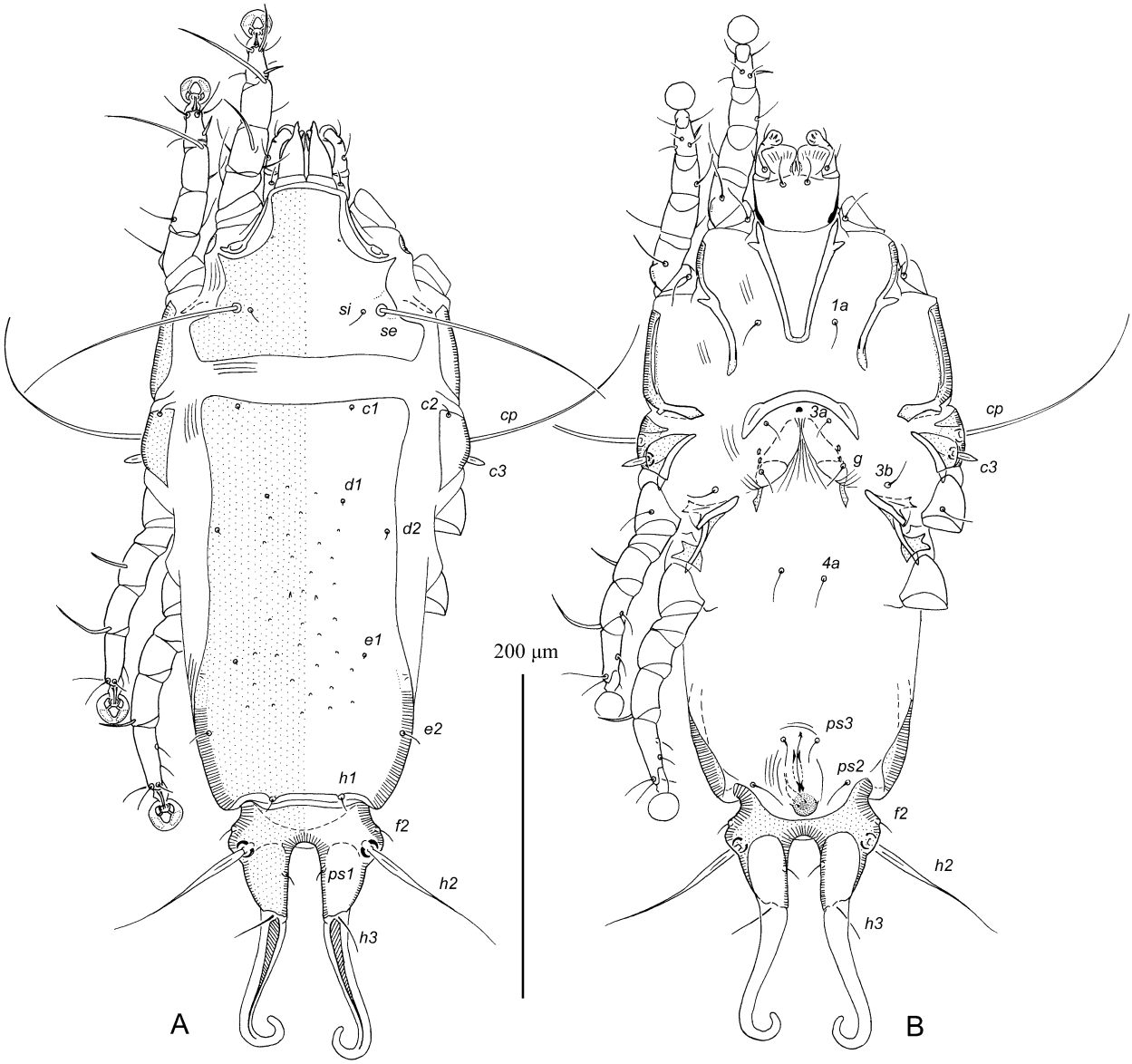

FIGURE 3. *Proctophyllodes valchukae* **sp. n.**, female. A—dorsal view, B— ventral view.

Discussion

The Long-tailed Tit (*Aegithalos caudatus*) was previously reported as a host of two *Proctophyllodes* species, *Proctophyllodes clavatus* Fritsch, 1961 (Černý 1977; Dabert 1997) and *P. sylviae* Gaud, 1957 (Dolfus 1961) in Europe. The record of the second species is perhaps a misidentification and actually represents *P. clavatus*, which was not yet described at that time. Nevertheless, both mite species belong to the *pinnatus* species group and are very common on warblers of the genus *Sylvia* Scopoli, 1769. *Proctophyllodes sylviae* is common on *S. atricapilla* (Linnaeus, 1758), while *P. clavatus* lives on *S. curruca* (Linnaeus, 1758), *S. borin* (Boddaert, 1783), *S. nisoria* (Bechstein, 1795) and also occurs on the genera *Acrocephalus* Naumann et Naumann, 1811 and *Locustella* Kaup, 1829 (Frisch 1961; Atyeo & Braasch 1966; Černý 1977, 1979; Mironov 1996, 1997). Therefore it is quite obvious that the presence of *P. clavatus* and *P. sylviae* on *Aegithalos caudatus* is the result of a natural transfer of these species from warblers. The record of the new species *P. valchukae* belonging to the *tricetratus* group on *Aegithalos caudatus* in its most eastern part of its range is an interesting subject for speculations. Cases of an avian species bearing different feather mite species of the same genus in different parts of its host range are relatively rare (Gaud

& Atyeo 1976; Dabert & Mironov 1999). This can have various causes, for instance by sympatric speciation of descendants of one parasite species or by invasion of a mite species from another host and subsequent dispersion in a host population. The *tricetratus* group is very different from the *pinnatus* group, therefore this is not a case of sympatric speciation. In relation to the origin of *P. valchukae* **sp. n.**, perhaps this species either represents a primary feather mite fauna inherited by *A. caudatus* from its *Aegithalos* ancestors and retained only in the eastern part of its range, or was also transferred from still unknown avian hosts from the Oriental region, for instance some babblers, on which representatives of the *tricetratus* group do occur.

In this paper we apply for the first time for feather mite description the DNA barcodes from both mitochondrial and nuclear genomes. Moreover, genomic DNA has been extracted from single individuals of paratype mites. Our experiences have enabled us to conclude that the Type-it Microsatellite PCR Kit (Qiagen) is the most effective reagent for PCR amplification of barcode sequences from the extracts contained very low amounts of template DNA.

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