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Two new species of the feather mite genus *Analges* Nitzsch, 1818 (Analgoidea: Analgidae) from accentors (Passeriformes: Prunellidae) — morphological descriptions with DNA barcode data

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Abstract

Two new species of the genus *Analges* (Astigmata: Analgoidea) are described from two species of accentors (Passeriformes: Prunellidae): *Analges himalayanus* sp. nov. from the Altai Accentor *P. himalayana* (Blyth), and *A. slovakiensis* sp. nov. from the Alpine Accentor *Prunella collaris* (Scopoli). Both species are closely related to *A. bidentatus* Giebel, 1871 described from the Dunnock *Prunella modularis* (Linnaeus). We extended the standard morphological descriptions of feather mites by species delimitation analyses carried out on DNA barcode sequences.

Keywords: Species delimitation methods, molecular taxonomy, morphology

Introduction

Feather mites of the genus *Analges* Nitzsch, 1818 (Analgoidea: Analgidae) live on the down feathers or downy parts of contour feathers of passerine birds. All representatives of this genus are rather uniformly shaped with strongly developed hook-like lateral apophyses on femora I and II used for clasping hair-like barbs, strongly hypertrophied legs III in males, and poorly sclerotized hysteronotum usually lacking of any shields in females (Gaud & Atyeo 1996).

Analges is the largest genus of the family with over 50 described species, mostly from European birds (Gaud 1974; Mironov 1985, 1996; Sohn 1995; Mironov & Kopij 1996; Su *et al.* 2013; Pedroso & Hernandes 2018). Traditional morphological systematics of this genus is complicated by the strong and continuous polymorphism of males, mainly expressed in the body size and structure of hypertrophied legs III, on the one hand, and extreme morphological uniformity of females, on the other hand. Reliable identification of many known species can be made only with heteromorphic males (Mironov & Kopij 1996).

In the present paper we describe two new species of *Analges* from accentors (Passeriformes: Prunellidae), the Alpine Accentor *Prunella collaris* (Scopoli) and the Altai Accentor *P. himalayana* (Blyth). We compared these new species with the most phenotypically similar and probably closely related *Analges bidentatus* Giebel 1871, which inhabits the third species of accentors, the Dunnock *Prunella modularis* (Linnaeus). For the first time in the descriptions of *Analges* species, we augment the standard morphological analysis with statistical delimitation methods based on DNA barcode markers. We propose this procedure as a new formal standard for feather mite descriptions in the cases when DNA barcode sequences are available.

Material and methods

Mites from *Prunella collaris* were collected by M.J. in Slovakia in 2015 while the material from *P. himalayana* was collected in Kyrgyzstan in 2012. The type material is deposited at the Department of Animal Morphology, Adam Mickiewicz University, Poznan, Poland (AMU). For comparative material we used *Analges bidentatus* from *P. modularis*, represented by slide-mounted specimens (ZISP 1393, Russia, Kaliningrad province, Curonian Spit, Biological station Rybachy, 55°05'18"N, 20°44'03"E, 23 May 1980, S.V. Mironov) and alcohol-preserved specimens (SVM-12-0420-2, Rybachy, 20 April 2012, S.V. Mironov, and AMU01766, Germany, near Osnabrück, 1991, J. Dabert). We had also a DNA isolate from *A. bidentatus* collected by J.D. from *P. modularis* in Osnabrück, Germany, in 1991 (AMU 01765).

Morphological descriptions of the new species follow the recent standards applied for *Analges* and related genera (Mironov 1985; Mironov & Kopij 1996). The idiosomal and leg chaetotaxy follow Gaud and Atyeo (1996); nomenclature for coxal setation was used with corrections proposed by Norton (1998). Drawings were made with a Leica DM5500 B microscope with DIC illumination and equipped with a camera lucida. All measurements are in micrometres and were assessed using images taken by a Leica DFC450 digital camera and calculated by LAS v.4.6.1 software (2105, Leica Microsystems CMS GmbH). Measuring standards for particular structures are as follows: (i) length of idiosoma is measured from the anterior margin of the propodosoma to the posterior margin of the opisthosoma, excluding the terminal lamella in males; width of idiosoma is measured at the level of the humeral setae *cp*; (ii) hysterosoma length is measured from the level of the posterior margins of the scapular shields to the posterior margin of the opisthosoma, excluding the terminal lamella in males; (iii) distance between idiosomal setae of the same pair is the distance between their bases; distance between different pairs of setae is the distance between the their bases of either side of the body (the mean of two measures is taken for each specimen).

The standard morphological descriptions of new species are supplemented with barcode data of the mitochondrial cytochrome c oxidase subunit I gene 661 bp fragment (COI) and 808 bp of the nuclear D2 region of 28S rDNA. The sequencing protocol follows Mironov *et al.* (2012). Statistical species delimitation by barcode data was carried out on COI alignment by four methods: (1) comparison of pairwise Kimura 2-parameter (K2P) distances was computed in MEGA 7.0 (Kumar *et al.* 2016); intergroup and intragroup distances and 10 fold species screening threshold were estimated according to Hebert *et al.* (2004), (2) gap analysis between putative species was carried by the ABGD method (Puillandre *et al*. 2011) with default settings, (3) gene genealogies were estimated in TCS 1.21 (Clement *et al.* 2000) using statistical parsimony networks (SP) (Templeton *et al.* 1992). The 95% connection limit for species boundary was applied for assessing putative species (Hart & Sunday 2007), (4) the posterior probability (PP) of species distinctiveness was estimated by Bayesian method based on the multispecies coalescent model as implemented in STACEY package for BEAST 2 (Jones 2017).

Morphological descriptions

Family Analgidae Trouessart et Mégnin, 1884
Subfamily Analginae Trouessart et Mégnin, 1884
Genus *Analges* Nitsch, 1918

***Analges himalayanus* sp. nov. (Figs. 1–3, 5A, D, 7C)**

Description. HETEROMORPH MALE (Figs. 1, 3A, B, E, 5A). Idiosoma, length × width, 398 in holotype (349–398 in two paratypes) × 250 (206–231), hysterosoma length 314 (263–311). Prodorsal shield longitudinally trapezoidal with a pair of median ridges, length excluding posterior processes 83 (75–83), width at posterior margin 84 (71–84). Posterior suprategumental processes of prodorsal shield long triangular with acute tips, length 15 (15–16) (Fig. 1B). Postero-medial part of scapular shields with triangular suprategumental extensions. Hysteronotal shield: gradually expanded anteriorly with maximum width at anterior angles; anterior margin deeply concave; concavity about ¼ of shield length; maximum length 251 (216–256), maximum width 184 (136–168); surface uniformly dotted. Posterior margin of opisthosoma rounded, in some paratypes a small rectangular lamella present. Supranal concavity small, slit-like, 28 (23–35) long. Setae *vi* relatively long, nearly as long as prodorsal shield. Scapular macrosetae *se* and setae *si* situated on postero-lateral extensions of prodorsal shield; setae *se* separated by 70 (60–71) and extending beyond level of cupules *im*, setae *si* extending beyond level of setae *d1*. Setae *c2* situated on anterior margins of narrow and long humeral shields and approximately as long as idiosoma. Short setae *d1* set close to anterior margin of hysteronotal shield. Bases of setae *d2* and *e2* adjoining and set on anterior angles of the hysteronotal shield; setae *d2* approximately equal to body length, setae *e2* about 1.5 times longer than body. Setae *f2* longer than the distance between their bases. Distances between dorsal setae and pores: *c2*:*e2* 65 (61–63), *d1*:*d1* 78 (62–77), *e1*:*e1* 76 (62–77), *e1*:*e2* 146 (123–139), *e1*:*f2* 92 (79–86), *h2*:*h2* 43 (35–37), *e1*:*gl* 25 (27–29), *gl*:*im* 28 (25–27).

Epimerites I fused as a Y, sternum long with bifurcate end, area between free parts of epimerites sclerotized (Fig. 1A). Posterior ends of epimerites II expanded, truncate. Coxal fields III with a pair of small humeral spurs set laterally at bases of trochanters III. Epiandrum bow-like; length × width 26 (28–38) × 47 (41–44). Length of aedeagus 24 (17–20)[1*]. Adanal shield shaped as a longitudinal plate, with slightly narrowed anterior part and concave anterior margin, posterior branches extending to the midway level between adanal suckers and setae *ps2*. Maximum length of adanal shield 99 (96–100), maximum width 61 (57–59), posterior cleft of this shield 49 (35–50) in length. Adanal suckers 14 (12–14) in diameter. Cupules *ih* set at level of setae *ps2*. Setae *4a* situated at level of setae *g*. Setae *4b* extending beyond bases of setae *ps3*; setae *3a* extending posteriorly far beyond tarsi III. Distances between ventral setae: *4b*:*g* 43 (43–50), *g*:*ps*3 106 (98–105), *ps*3:*ps2* 46 (42–43).

Femur I with large spatuliform lateral apophysis, femur II with small triangular lateral apophysis (Figs. 3A, B). Femur III enlarged, about twice as wide in apical part than in basal one; inner margin with three distal spines (Fig. 5A); medial spine long and straight, directed mesally, two smaller triangular spines (dorsal and ventral ones) set at its base; dorsal spine acute, ventral spine with rounded tip. Genu III two times wider than long. Tibia about 1.5 times longer than wide at base. Tarsus III with vestigial ambulacral stalk and ambulacrum; inner margin without projection. Setae *cG* on genua I and II similar in shape, thickened basally with distal long filiform part (Figs. 3A, B). Setae *sR* on femur III and *f* on tarsus III approximately as long as the whole leg III. Setae *e* and *d* of tarsus IV button-like, seta *r* fine, shorter than the segment (Fig. 3E). Legs IV with tarsi extending beyond level of body terminus.

HOMEOMORPH MALE (one paratype, Figs. 5D, 7C). Only features different to heteromorph male are presented. Idiosoma, length × width, 352 × 195, hysterosoma length 263. Length of prodorsal shield excluding posterior processes 79, width at posterior margin 69. Maximum length of the hysteronotal shield 222, maximum width 135. Posterior margin of opisthosoma regularly

1. * All analysed specimens had genital apparatus rotated backwards and the dimensions of the genital arch were not measured.

rounded with small rounded terminal lamella. Supranal concavity 30 long. Scapular macrosetae *se* separated by 59 and reaching the body terminus, setae *si* not reaching the level of setae *d1*. Setae *d2* and *e2* both approximately equal to body length. Distances between dorsal setae and pores: *c2*:*e2* 55, *d1*:*d1* 61, *e1*:*e1* 62, *e1*:*e2* 119, *e1*:*f2* 81, *h2*:*h2* 39, *e1*:*gl* 22, *gl*:*im* 19.

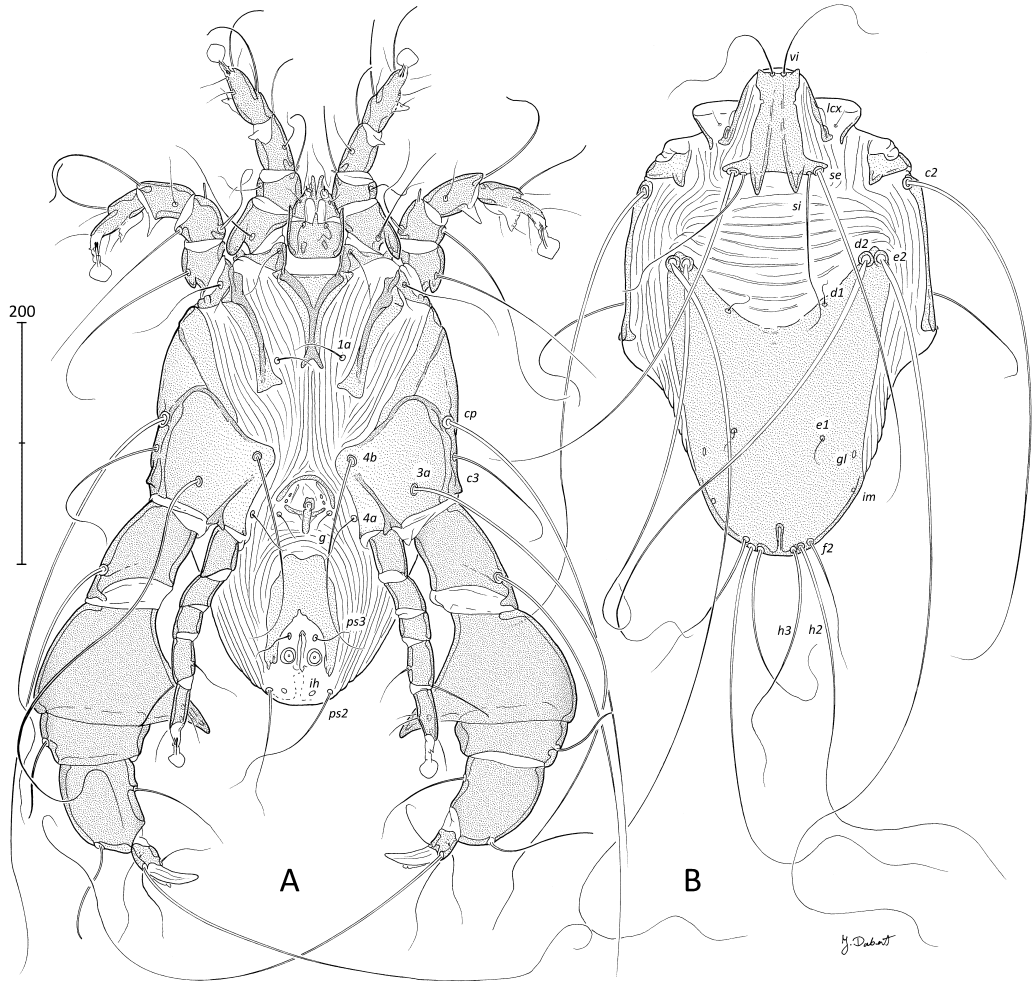

FIGURE 1. ***Analges himalayanus*** **sp. nov., male**. A—ventral view, B—dorsal view.

Epiandrum, length × width, 25 × 42. Length of aedeagus 16. Adanal shield similar in shape to one of heteromorph male (Fig. 7C). Maximum length of adanal shield 86, maximum width 53, posterior cleft of this shield 44 in length. Adanal suckers 12 in diameter. Setae *3a* reaching at most the tips of tarsi III. Distances between ventral setae: *4b*:*g* 40, *g*:*ps*3 91, *ps*3:*ps2* 43.

All podomeres of the leg III of similar width (except small tarsus) and lacking apophyses. (Fig. 5D). Genu III as wide as long.

FEMALE (3 paratypes, Figs. 2, 3 C, D). Idiosoma, length × width, 418–528 × 164–229, length of hysterosoma 317–427. Prodorsal shield shaped as in male, with exception of long postero-lateral angles extending well beyond level of setae *se* (Fig. 2B), length excluding posterior processes 90–92, width at posterior margin 93–103, length of posterior suprategumental processes 18–20. Scapular

shields shaped as in male. Opisthosoma bluntly rounded. Hysteronotal shield absent. Scapular setae *se* and setae *c2* extending beyond level of setae *d2*; setae *si* filiform, length not exceeding distance between their bases. Setae *d2* reaching the bases of setae *e2*. Setae *e2* not longer than distance between them. Setae *f2* short filiform. Distance between dorsal setae *se*:*se* 74–77, *c2*:*c2* 145–159, *c2*:*d2* 90–103, *d2*:*d2* 121–134; *d2*:*e2* 116–131, *e2*:*e2* 120–136, *e2*:*h2* 129–138, *h2*:*h2* 79–96.

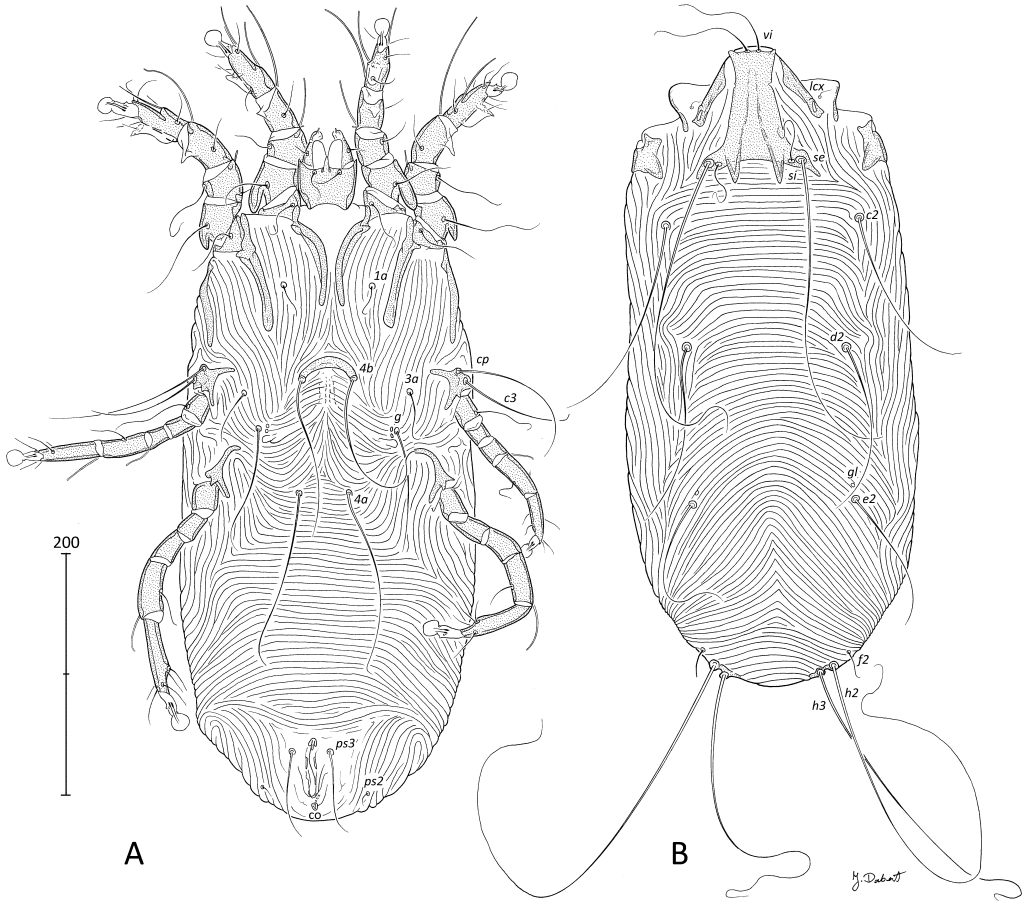

FIGURE 2. ***Analges himalayanus*** **sp. nov., female**. A—ventral view, B—dorsal view, co—copulatory opening.

Epimerites I free. Epigynum bow-shaped, 19–22 long, 45–47 wide, setae *4b* situated on tips of epigynum (Fig. 2A). Apodemes of oviporus as inverted V. Copulatory opening set ventrally, immediately posterior to the anal slit. Setae *g* set near ends of oviporal apodemes, genital papillae mesal to *g*. Setae *1a* short, not reaching the epigynum. Setae *4b* reaching the bases of setae *4a*; setae *4a* longer than half the distance *4a*:*ps3*. Setae *3a* reaching the bases of setae *g*, set slightly posterior to level of setae *4b*. Distances between ventral setae: *4b*:*g* 42–44, *4b*:*4b* 39–43, *3a*:*3a* 120–138, *g*:*g* 100–116, *4a*:*4a* 40–44, *g*:*4a* 46–52.

Legs I, II shaped as in male. Legs IV not reaching level of setae *ps3*. Tarsi III and IV with small ventral extension bearing seta *s* III and *w* IV, respectively (Figs. 3C, D).

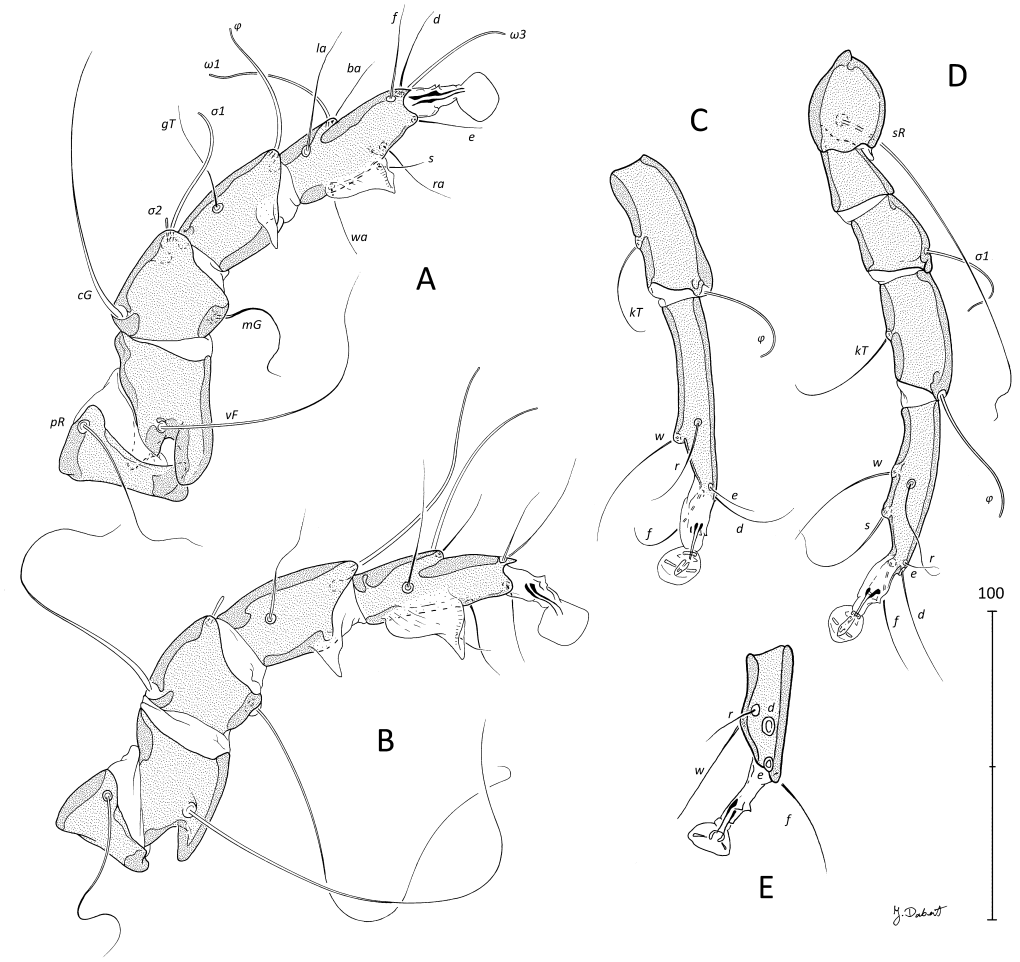

FIGURE 3. ***Analges himalayanus*** **sp. nov., legs**. A—leg I, heteromorph male, B—leg II, heteromorph male, C—tibia and tarsus IV, female, D—leg III, female, E—tarsus IV, heteromorph male. A, B—ventral view, C–E—dorsal view.

Type material. Heteromorph male holotype, 2 heteromorph male, 1 homeomorph male, and 3 female paratypes (AMU 01763) from *Prunella himalayana*, adult, **KYRGYZSTAN**: Chuy Region, Panfilov District, Ala-Bel Pass, 42°14′47″N, 73°03′08″E, 2 July 2012, coll. M. Janiga.

Type deposition. Holotype and paratypes in the Department of Animal Morphology, AMU, Poznan, Poland.

DNA barcode. GenBank accession numbers: MH593806–MH593810 (COI). Sequencing of the nuclear DNA failed.

Etymology. The specific epithet is derived from the specific name of the type host.

Differential diagnosis. *Analges himalayanus* sp. nov. is close to *A. bidentatus* in having well-developed lateral apophysis in femur II in both sexes (Figs. 2A, 3B, 6A). In heteromorph males of these species, the median spine of the femur III is distinctly longer than two remaining spines, and the base of femur III is as wide as tibia III (Figs. 5A, C). The new species differs from *A. bidentatus* by the following features: in heteromorph males, the hysteronotal shield is uniformly dotted (Fig. 1B), the adanal shield is longer than wide (Fig. 1A), femur of leg III (excluding spines) is nearly as

long as wide and genu is about 2–2.5 times wider than long and (Fig. 5A). In heteromorph males of *A. bidentatus*, the hysteronotal shield has wavy longitudinal striae in antero-medial part (Fig. 8), the adanal shield is as long as wide (Fig. 7A), the femur of leg III is distinctly longer than wide and genu is slightly wider than long (Fig. 5C). In homeomorph males of *A. himalayanus* sp. nov., the adanal shield is distinctly narrower in the anterior than in the posterior part; in *A. bidentatus,* the anterior part of the adanal shield is laterally expanded and much wider than the posterior part (Figs. 7C, D).

Analges slovakiensis sp. nov. (Figs. 4, 5B, 6B-G, 7B)

Description. HETEROMORPH MALE (Figs. 4, 5B, 6B–G). Idiosoma, length × width, 425 in holotype (405–440 in four paratypes) × 267 (247–316), hysterosoma length 321 (308–342). Prodorsal shield longitudinally trapezoidal with a pair of median ridges, length excluding posterior processes 91 (89–92), width at posterior margin 94 (88–98). Posterior suprategumental processes of prodorsal shield long triangular with rounded tips, length 17 (16–17) (Fig. 4B). Postero-mesal part of scapular shields with triangular suprategumental extensions. Hysteronotal shield: gradually expanded anteriorly with maximum width at level of setae *d1*; anterior margin of shield deeply concave; concavity about ¼ of the shield length; maximum length 269 (251–275), maximum width 186 (179–197). Hysteronotum uniformly dotted. Posterior margin of opisthosoma rounded, with small rectangular lamella. Supranal concavity small, keyhole-like, 32 (31–38) long. Setae *vi* relatively long, nearly as long as prodorsal shield. Scapular macrosetae *se* and setae *si* situated on lateral extensions of prodorsal shield; setae *se* separated by 79 (73–81) and reaching level of cupules *im*, setae *si* extending beyond level of setae *d1*. Setae *c2* situated on anterior margins of narrow and long humeral shields and approximately as long as idiosoma. Setae *d1* short, set close to anterior margin of hysteronotal shield. Bases of setae *d2* and *e2* adjoining and set on anterior corners of the hysteronotal shield; setae *d2* shorter than body length, setae *e2* about 1.5 of body length. Setae *f2* longer than distance between their bases. Distances between dorsal setae and pores: *c2*:*e2* 71 (70–91), *d1*:*d1* 78 (62–77), *e1*:*e1* 76 (62–77), *e1*:*e2* 165 (150–162), *e1*:*f2* 88 (85–91), *h2*:*h2* 44 (40–48), *e1*:*gl* 32 (31–32), *gl*:*im* 19 (16–22).

Epimerites I fused as a Y; sternum long, with bifurcate posterior end, area between free parts of epimerites sclerotized (Fig. 4A). Posterior ends of epimerites II expanded, slightly concave. Coxal fields III with a pair of small humeral spurs set laterally near trochanters III. Epiandrum bow-like, length × width 32 (28–29) × 46 (47–51). Length of aedeagus 28 (22–28)[2*]. Adanal shield roughly shaped as inverted longitudinal trapezium with anterior part distinctly wider than posterior one; anterior margin of this shield rounded and convex, posterior branches extending to the halfway point between adanal suckers and setae *ps2*; maximum length of 119 (110–122), maximum width 83 (94–97), posterior cleft 58 (49–51) in length. Adanal suckers 15 (14–15) in diameter. Cupules *ih* set posterior to the level of setae *ps2*. Setae *4a* situated at level of setae *g* or slightly posterior. Setae *4b* extending beyond bases of setae *ps3*; setae *3a* not extending beyond tarsi III. Distances between ventral setae: *4b*:*g* 52 (44–64), *g*:*ps*3 104 (100–112), *ps*3:*ps2* 50 (48–55).

Femur I with large spatuliform lateral apophysis (Fig. 4A), femur II without lateral apophysis or apophysis vestigial (Figs. 6D–G). Femur III enlarged, about 1.5 times wider in apical part than in basal one; inner margin with three triangular spines of similar size (Fig. 5B); medial spine directed adaxially, and dorsal and ventral spines at its base directed perpendicularly to the axis of medial spine. Genu III about two times wider than long. Tibia III 1.6–1.7 times longer than wide at base. Tarsus IV with vestigial ambulacral stalk and ambulacrum; inner margin without projection. Setae

2.* All analysed specimens had genital apparatus rotated backwards and the dimensions of the genital arch were not estimated.

cG on genua I and II thickened basally, with distal long filiform part, seta *cG*II with minute tongue-like membrane at base of filiform part (Fig. 6D). Setae *sR* on femur III approximately as long as the whole leg III, setae *f* on tarsus III shorter, not longer than 2/3 the length of *sR*III. Setae *e* and *d* of tarsus IV button-like, setae *r* subequal in length to this segment. Legs IV with basal parts of tarsi extending to level of body terminus.

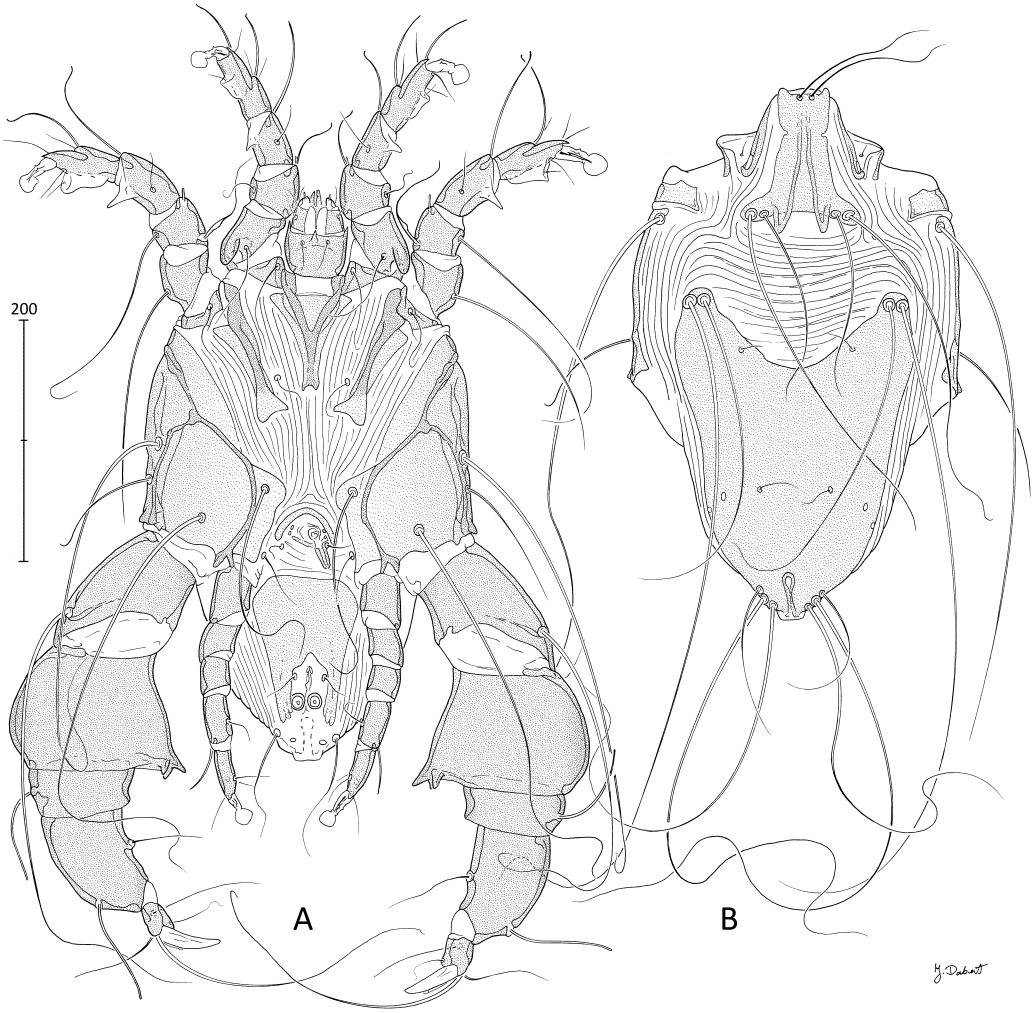

FIGURE 4. ***Analges slovakiensis*** **sp. nov., male**. A—ventral view, B—dorsal view.

HOMEOMORPH MALE (one paratype, Fig. 7B). Only features different to heteromorph male are presented. Idiosoma length × width, 347 × 188, hysterosoma length 267. Length of prodorsal shield excluding posterior processes 76, width at posterior margin 67. Maximum length of the hysteronotal shield 212, maximum width 114. Posterior margin of opisthosoma regularly rounded with small rounded terminal lamella. Supranal concavity 33 long. Scapular macrosetae *se* separated by 56 and reaching the body terminus, setae *si* not reaching the level of setae *d1*. Setae *d2* and *e2* both approximately equal to body length. Distances between dorsal setae and pores: *c2*:*e2* 66, *d1*:*d1* 50, *e1*:*e1* 65, *e1*:*e2* 108, *e1*:*f2* 83, *h2*:*h2* 41, *e1*:*gl* 23, *gl*:*im* 17.

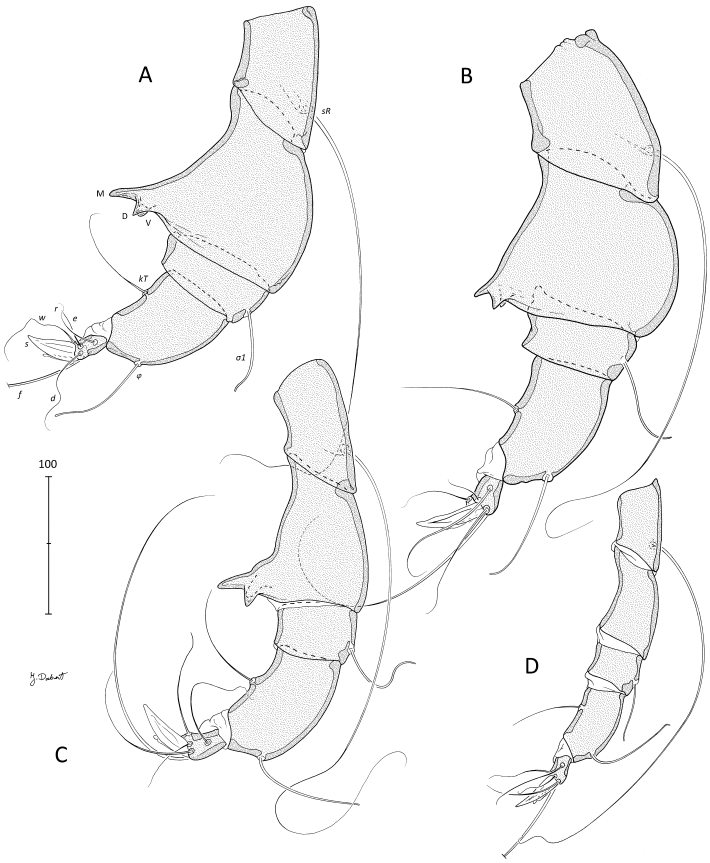

FIGURE 5. Legs III of *Analges* males, dorsal view. A—*A. himalayanus* sp. nov., heteromorph, B—*A. slovakiensis* sp. nov., heteromorph, C—*A. bidentatus* Giebel, 1871, heteromorph, D—*A. himalayanus* sp. nov., homeomorph.

Epiandrum, length × width, 25 × 41. Length of aedeagus 17. Adanal shield different in shape to that of heteromorph male, regularly trapezoidal, wider at base than in anterior part (Fig. 7B). Maximum length of adanal shield 83, maximum width 56, posterior cleft of this shield 32 in length. Adanal suckers 10 in diameter. Setae *3a* extending posteriorly far beyond the tips of tarsi III. Distances between ventral setae: *4b*:*g* 39, *g*:*ps*3 97, *ps*3:*ps2* 40.

All podomeres of leg III of similar width (except small tarsus) and lacking apophyses. Genu III as wide as long.

FEMALE (3 paratypes, Figs. 6B, C). Idiosoma, length × width, 420–518 × 192–218, length of hysterosoma 318–416. Prodorsal shield shaped as in male, with exception of postero-lateral corners that extending as triangular extensions well beyond the bases of setae *se*, length excluding posterior processes 94–98, width at posterior margin 93–113, length of posterior suprategumental processes 18–19. Scapular shields shaped as in male. Opisthosoma bluntly rounded. Hysteronotal shield absent. Scapular setae *se* and setae *c2* extending posteriorly beyond the level of setae *d2*, setae *si* minute. Setae *d2* reaching the bases of setae *e2*. Setae *e2* as long as distance between them or longer. Setae *f2* minute. Distance between dorsal setae *se*:*se* 77–81, *c2*:*c2* 158–173, *c2*:*d2* 80–98, *d2*:*d2* 120–136; *d2*:*e2* 100–115, *e2*:*e2* 118–142, *e2*:*h2* 102–145, *h2*:*h2* 81–87.

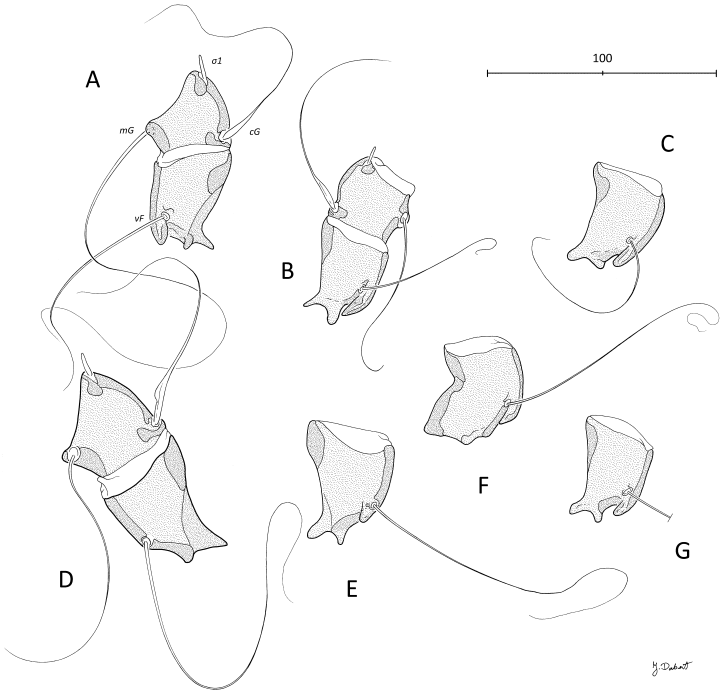

FIGURE 6. Femur and genu II of *Analges* males, ventral view. A—*A. bidentatus*, male, B–G—*A. slovakiensis* sp. nov., B,C—females, D–G—males.

Epimerites I free. Epigynum bow-shaped with slightly enlarged tips, 18–22 long, 41–48 wide, setae *4b* situated on tips of epigynum. Apodemes of oviporus as inverted V. Copulatory opening set ventrally immediately posterior to the anal slit. Setae *g* set near ends of oviporal apodemes, genital papillae mesal to *g*. Setae *1a* short, not reaching the epigynum. Setae *4b* reaching bases of setae *4a*; setae *4a* longer than half the distance between *4a*:*ps3*. Setae *3a* set posterior to level of setae *4b*. Distances between ventral setae: *4b*:*g* 44–53, *4b*:*4b* 36–41, *3a*:*3a* 99–138, *g*:*g* 79–107, *4a*:*4a* 36v37, *g*:*4a* 42–64.

Legs I, II shaped as in male. Lateral apophyses on femur II always present, generally better developed than in males (Figs. 6B, C). Legs IV, depending on individual, may reach or not reaching the level of setae *ps3*. Tarsi III and IV with small ventral extension bearing seta *s* III and *w* IV, respectively.

Type material. Heteromorph male holotype, 4 heteromorph male, 1 homeomorph male, 3 female paratypes (AMU 01764) from *Prunella collaris* nestling, **SLOVAKIA**, Low Tatra mountains, Chopok, 8 July 2015, coll. M. Janiga.

Type deposition. Holotype and paratypes in the Department of Animal Morphology, AMU, Poznan, Poland.

DNA barcode. GenBank accession numbers: MH593800–MH593805 (COI), MH593798 (D2)

Etymology. The specific epithet is derived from Slovakia, the country of the type material origin.

Differential diagnosis. *Analges slovakiensis* sp. nov. is close to males of *A. himalayanus* sp. nov. in having a uniformly dotted hysteronotal shield (Figs. 1B, 4B). In heteromorph males, genu III is at least two times wider than long (Figs. 5A, B) and the adanal shield is distinctly longer than wide (Figs. 1A, 4A). In homeomorph males of both species the adanal shield is distinctly narrower in the

anterior part than in the posterior (Figs. 7B, C). These features clearly distinguish these two species from *A. bidentatus*, in which the hysteronotal shield is striated antero-medially (Fig. 8) and the adanal shield is expanded laterally in anterior part (Figs. 7A, D) in both homeo- and heteromorph males; heteromorph males have genu III at most 1.5 times wider than long (Fig. 5C). *Analges slovakiensis* differs from *A. himalayanus* by the following characters: in heteromorph males, all spines of femur III are of similar size (Fig. 5B) and the adanal shield is much wider in the anterior than in the posterior part (Fig. 4A). In heteromorph males of *A. himalayanus,* the median spine of femur III is distinctly longer than two remaining spines (Fig. 5A) and the adanal shield has almost parallel lateral margins and distinctly narrowed anterior part (Fig. 1A). In homeomorph males of *A. slovakiensis* sp. nov., the adanal shield is shaped as a narrow and long trapezoid being slightly wider at the base than in the anterior part, and the length of the posterior cleft of this shield is *ca* one–third the total shield length (Fig. 7B); in *A. himalayanus,* this shield is narrowed in the anterior half only and the posterior cleft is over the half of the total shield length (Fig. 7C).

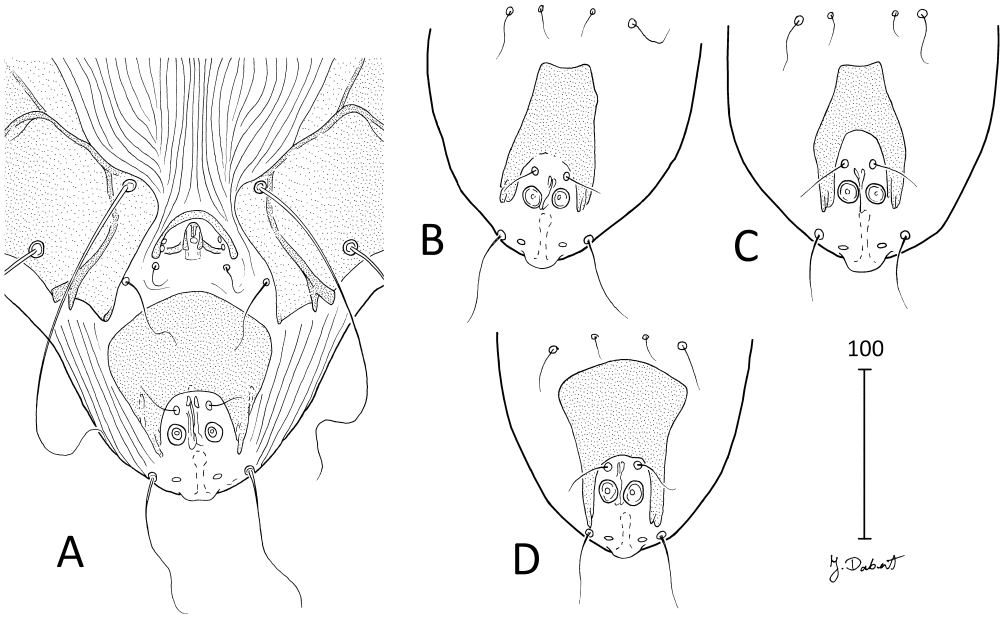

FIGURE 7. Adanal shield of *Analges* males. A—*Analges bidentatus*, heteromorph, B—*A. slovakiensis* sp. nov., homeomorph, C—*A. himalayanus* sp. nov., homeomorph, D— *A. bidentatus*, homeomorph.

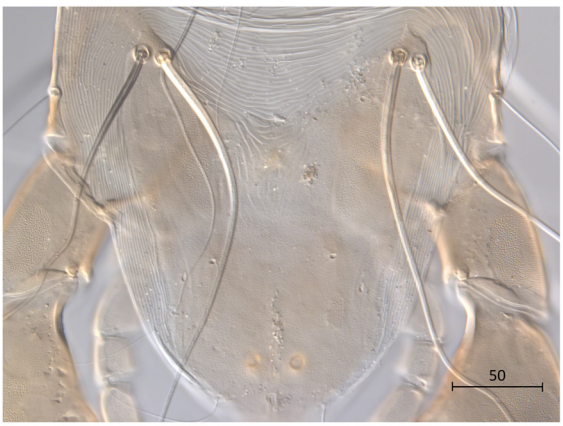

FIGURE 8. *Analges bidentatus*, heteromorph male, sculpture of hysteronotum.

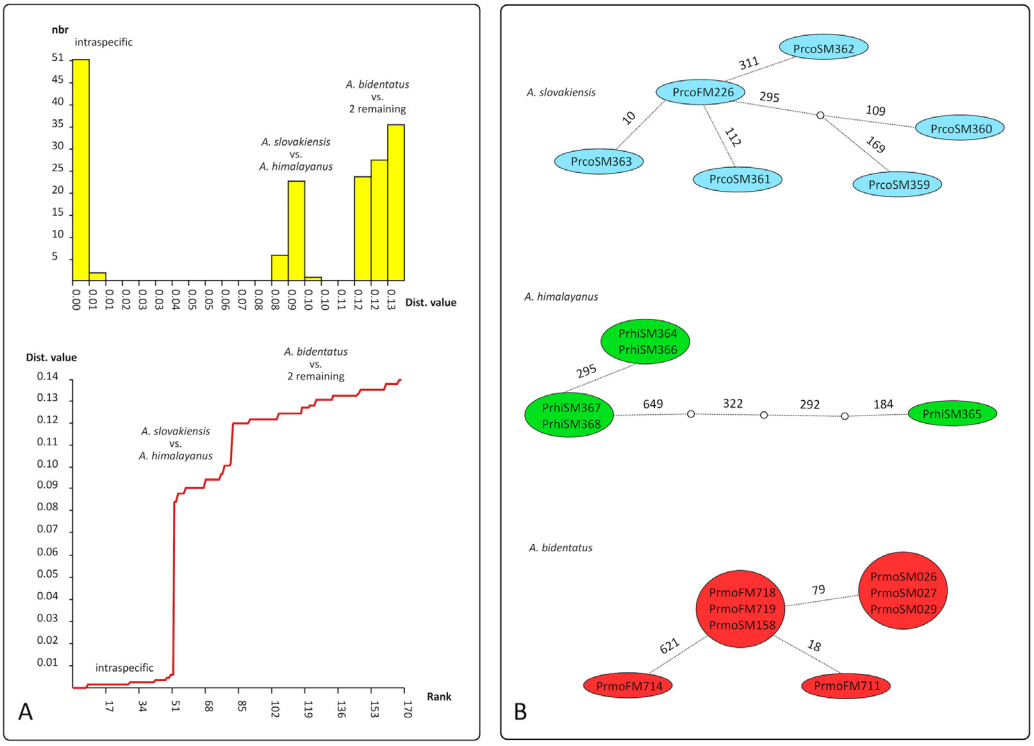

FIGURE 9. *Analges* species delimitation results for COI. A—Automatic Barcode Gap Discovery, distribution of pairwise differences (above) and ranked pairwise differences (below). The analysis supports the specific status of three *Analges* populations and also points on more close relationship between two new species than either of them with *A. bidentatus*. B – Statistical parsimony network broken at 95% connection limit. The three subnetworks correspond to three *Analges* species. Empty circles—hypothetical haplotypes, numbers—mutated positions in COI sequence. Abbreviations for host species in mite haplotypes: Prmo (red)— *Prunella modularis*, Prco (blue)—*P. collaris*, Prhi (green)—*P. himalayana*.

Molecular species delimitation

All species delimitation methods applying COI barcode sequences univocally supported species-level status of each of the *Analges* populations inhabiting particular prunellid hosts: *A. bidentatus* Giebel, 1871 from *Prunella modularis*, *A. himalayanus* sp. nov. from *P. himalayana*, and *A. slovakiensis* sp. nov. from *P. collaris*. The intraspecific K2P distances for all sequences were 0.0-0.7%, while the distances between groups were much higher: *A. himalayanus* vs *A. slovakiensis*—8.8–9.8%, *A. bidentatus* vs *A. himalayanus*—11.9–12.3%, and *A. bidentatus* vs *A. slovakiensis*—12.1–13.0% (Supplementary data Tab. S1). Thus in all cases the minimal value for interspecific distance was greater than 10× maximal observed intraspecific distance. However the intraspecific distances may be underestimated due to small number of independent populations of the same species. The ABGD gap analysis reconstructed three groups of sequences corresponding to three *Analges* species and discovered a distinct gap between intraspecific and interspecific distances (Fig. 9A). The statistical parsimony network (TCS) was broken into three pieces corresponding to three analysed species (Fig. 9B). Finally, the coalescent method implemented in STACEY package showed significant posterior probability PP=0.64 for three species as true clustering; the best alternative clustering was ten times less probable (PP=0.05) (Supplementary data Tab. S2).

Species delimitation based on nuclear region D2 was possible only for *Analges slovakiensis* and *A. bidentatus*; we failed to obtain these data for *A. himalayanus*. Pairwise distance between sequences of *A. slovakiensis* (MH593798) and *A. bidentatus* (MH593799) was 0.027 (SD 0.006) that support the specific level of divergence between both compared species.

Discussion

Classical phenotypic species definition in the genus *Analges* is a complicated task because of great intraspecific morphological variability expressed in continuous polymorphism of males, and extremely uniform appearance of females of different species. This problem is amply evident in both new species described here. The observed intraspecific variation concerns morphometric characters (e.g. body dimensions or setal distances in both sexes) as well as discrete features (e.g. shape of femoral apophyses on legs II and presence/absence of a terminal lamella in males). Females of three analysed species are phenotypically indistinguishable, not only in discrete features but also in morphometric characters. However, it is possible that further analyses of more representative number of specimens may reveal some statistically supported differences in morphometric data (e.g. setal lengths or length proportions).

Despite their phenotypic similarity, closely related *Analges* species from prunellids can be distinguished by a few discrete characters in males, such as the shape of hypertrophied legs III in heteromorphs, the shape of adanal shield and the sculpture of hysteronotal shield in heteromorphs and homeomorphs. But the strongest evidence for species definition in this case turned out the molecular data. COI DNA barcode sequences, analysed by different methods of species delimitation, univocally indicate three species that are strongly genetically separated. We suggest adding this method to morphological analysis as a standard (or quite desirable) procedure in future species descriptions in all cases when suitable DNA material is available.

According to molecular analyses, *Analges himalayanus* seems to be a sister-species of *A. slovakiensis*, while *A. bidentatus* is more distant from these pair of species. This relationship is concordant with the phylogenetic relationships of the birds (Liu *et al.* 2017), which suggests cospeciation between these *Analges* species and their prunellid hosts.

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Appendix

TABLE S1. Estimation of K2P distances between *Analges* COI sequences from prunellid hosts. Prmo—from *Prunella modularis*, Prco—from *P. collaris*, Prhi—from *P. himalayana*. Red—*Analges slovakiensis* sp. nov. vs *A. himalayanus* sp. n., yellow—*A. bidentatus* vs *A. slovakiensis* sp. nov, blue—*A. bidentatus* vs *A. himalayanus* sp. nov, white—intraspecific distances in *A. slovakiensis* sp. nov (left), *A. himalayanus* sp. nov (center), and *A. bidentatus* (right).

	Prco SM3 59	Prco SM3 60	Prco SM3 61	Prco SM3 62	Prco SM3 63	Prco FM2 26	Prhi SM3 64	Prhi SM3 65	Prhi SM3 66	Prhi SM3 67	Prhi SM36 8	Prmo FM7 11	Prmo FM7 14	Prmo FM7 18	Prmo FM71 9	Prmo SM02 6	Prmo SM02 7	Prmo SM02 9
PrcoSM359																		
PrcoSM360	0.003																	
PrcoSM361	0.005	0.005																
PrcoSM362	0.005	0.005	0.003															
PrcoSM363	0.005	0.005	0.003	0.003														
PrcoFM226	0.003	0.003	0.002	0.002	0.002													
PrhiSM364	0.091	0.087	0.093	0.093	0.093	0.091												
PrhiSM365	0.098	0.094	0.096	0.096	0.096	0.094	0.008											
PrhiSM366	0.091	0.087	0.093	0.093	0.093	0.091	0.000	0.008										
PrhiSM367	0.093	0.089	0.091	0.091	0.091	0.089	0.002	0.006	0.002									
PrhiSM368	0.093	0.089	0.091	0.091	0.091	0.089	0.002	0.006	0.002	0.000								
PrmoFM711	0.137	0.134	0.136	0.132	0.136	0.134	0.126	0.124	0.126	0.124	0.124							
PrmoFM714	0.137	0.134	0.135	0.132	0.135	0.134	0.126	0.124	0.126	0.124	0.124	0.003						
PrmoFM718	0.136	0.132	0.134	0.130	0.134	0.132	0.124	0.122	0.124	0.122	0.122	0.002	0.002					
PrmoFM719	0.136	0.132	0.134	0.130	0.134	0.132	0.124	0.122	0.124	0.122	0.122	0.002	0.002	0.000				
PrmoSM026	0.134	0.130	0.132	0.128	0.132	0.130	0.122	0.120	0.122	0.120	0.120	0.003	0.003	0.002	0.002			
PrmoSM027	0.134	0.130	0.132	0.128	0.132	0.130	0.122	0.120	0.122	0.120	0.120	0.003	0.003	0.002	0.002	0.000		
PrmoSM029	0.134	0.130	0.132	0.128	0.132	0.130	0.122	0.120	0.122	0.120	0.120	0.003	0.003	0.002	0.002	0.000	0.000	
PrmoSM158	0.136	0.132	0.134	0.130	0.134	0.132	0.124	0.122	0.124	0.122	0.122	0.002	0.002	0.000	0.000	0.002	0.002	0.002

TABLE S2. Posterior probability (PP) of alternative clustering of *Analges* COI sequences from prunellid hosts. Only clusterings of frequency five or above (of 2001) are shown. Abbreviations for corresponding host species: Prmo—*Prunella modularis*, Prco—*P. collaris*, Prhi—*P. himalayana*.

count	PP	nclusters	Prco FM226	Prco SM362	Prco SM363	Prco SM361	Prco SM359	Prco SM360	Prhi SM366	Prhi SM367	Prhi SM364	Prhi SM368	Prhi SM365	Prmo SM026	Prmo SM027	Prmo SM029	Prmo SM158	Prmo FM711
1081	0.6002	3	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3
114	0.0633	4	2	2	2	2	2	2	3	1	3	3	3	4	4	4	4	4
39	0.0217	4	1	2	2	1	1	1	3	3	3	3	3	4	4	4	4	4
19	0.0105	2	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
17	0.0094	5	2	3	3	2	2	2	4	1	4	4	4	5	5	5	5	5
14	0.0078	4	2	2	2	2	1	2	3	3	3	3	3	4	4	4	4	4
13	0.0072	4	1	1	1	1	1	1	2	2	2	2	2	3	4	4	4	3
12	0.0067	4	1	2	2	2	1	1	4	4	4	4	4	3	3	3	3	3
12	0.0067	4	1	1	1	1	1	1	2	3	2	3	3	4	4	4	4	4
10	0.0056	4	1	2	2	1	2	1	3	3	3	3	3	4	4	4	4	4
10	0.0056	4	1	1	1	1	1	1	2	2	2	3	3	4	4	4	4	4
9	0.0050	4	1	1	1	2	2	1	3	3	3	3	3	4	4	4	4	4
9	0.0050	2	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2
8	0.0044	4	2	2	2	2	2	2	3	3	3	3	3	4	1	4	4	4
8	0.0044	4	2	1	2	2	2	2	3	3	3	3	3	4	4	4	4	4
7	0.0039	4	2	2	2	1	2	2	3	3	3	3	3	4	4	4	4	4
6	0.0033	4	1	1	1	1	1	1	2	2	2	2	2	3	3	4	4	3
6	0.0033	4	2	2	1	2	2	2	3	3	3	3	3	4	4	4	4	4
6	0.0033	4	1	2	2	2	2	1	3	3	3	3	3	4	4	4	4	4
6	0.0033	4	1	1	1	1	1	1	2	2	2	2	2	3	4	3	4	3
6	0.0033	5	2	2	2	2	2	2	3	1	3	3	3	4	5	5	5	4
5	0.0028	4	2	2	2	2	2	2	3	3	3	3	3	4	4	4	4	1
5	0.0028	5	2	2	2	2	2	2	3	1	3	3	3	4	4	5	5	4
5	0.0028	2	1	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1
5	0.0028	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2
5	0.0028	4	1	2	2	2	2	2	3	3	3	3	3	4	4	4	4	4
5	0.0028	5	3	3	3	3	3	3	4	1	4	4	4	5	2	5	5	5
5	0.0028	4	2	2	2	2	2	2	3	3	3	3	3	1	4	4	4	4
.....																		