

A New Feather Mite Species of the Genus *Metanalges* (Acariformes: Analgidae) from the Okinawa Rail, *Hypotaenidia okinawae* (Gruiformes: Rallidae), in Okinawa Island, Japan

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The Okinawa Rail, *Hypotaenidia okinawae* (Yamashina and Mano, 1981), is a flightless bird restricted to the northern part of Okinawa Island, Ryukyu Islands, Japan. A new feather mite species, *Metanalges* (*M.*) *agachi* sp. n. (Analgidae: Megniniinae), is described from the Okinawa Rail. The new species is distinguished from the closest species, *M. (M.) curtus* Gaud and Mouchet, 1959, in having, in males, legs III extending slightly beyond the bases of setae *h2*, and setae *ps1* approximately equidistant from setae *h2* and *h3*, and in females, ventral setae *4a* almost extending to the posterior margin of opisthosoma. This is the first report of feather mites associated with this avian host and the first record of the genus *Metanalges* Trouessart, 1919 in Japan. As the Okinawa Rail is considered to be at high risk of population decline, the new feather mite is thought to be under the risk of extinction.

Key Words: Astigmata, bird, Japan, symbiosis.

Introduction

The avian family Rallidae (Gruiformes) is cosmopolitan and currently includes about 160 species, among which over 30 species are flightless (Gaspar et al. 2020). Among these species, the Okinawa Rail *Hypotaenidia okinawae* (Yamashina and Mano, 1981) is restricted to the northern part of the island of Okinawa in the south of Japan (Hoyo 2020). When the Okinawa Rail was described (Yamashina and Mano 1981), its population was estimated as 1800 individuals, but up to 2005, it has declined to 720 individuals (Ministry of the Environment 2023). Roadkills and introduced predators, such as the Small Indian Mongoose, *Herpestes auro-punctatus* (Hodgson, 1836) (Carnivora: Herpestidae), and the Domestic Cat, *Felis silvestris catus* Linnaeus, 1758 (Carnivora: Felidae), were thought to be the main causes of the population decline (Ozaki et al. 2002; Nagamine 2003). In this island, conservation efforts for the Okinawa Rail, such as the control of cat population, the anthropogenic eradication of mongoose, habitat improvement, and conservation education activities for local residents, caused increasing of the population size (Mizuta and Takagi 2018), resulting in

1500 individuals estimated in 2014 (Ministry of the Environment 2023). In spite of partial restoration of its population, the Okinawa Rail is currently classified as “Critically Endangered (CR)” in the Red List of Japan (Ministry of the Environment 2019), and listed as “Endangered (EN)” on the International Union for Conservation of Nature Red List (BirdLife International 2021).

Feather mites, permanent ectosymbionts of birds, according to the current taxonomic point of view constitute two superfamilies of astigmatan mites, Analgoidea and Pterolichoidea (Acariformes: Astigmata). Most of these mites inhabit various microhabitats in the plumage of their hosts, and feed on preen gland oil and, by accident, on various materials staked to the feathers (algae, bacteria, fungi) (OConnor 1982; Gaud and Atyeo 1996; Doña et al. 2019). Feather mites are highly host specific and most species are monoxenous or stenoxenous (OConnor 1982; Doña et al. 2018). For this reason, some feather mites associated with endangered bird species may become extinct, if the population of bird host strongly declines. For example, the feather mite *Compressalges nipponiae* Dubinin, 1950 (Pterolichoidea: Caudiferidae), a monoxenous parasite of the Crested Ibis, *Nipponia nippon* (Temminck, 1835) (Pelecaniformes:

Threskiornithidae), in the 20th century inhabited the Far East population of this host including Japan (Dubinin 1950, 1956; Kuroki et al. 2020; Waki and Shimano 2020, 2022). At present, *C. nipponiae* is thought to be extinct, because recent investigations have not recovered this feather mite species in the continental Chinese population of the Crested Ibis and the Japanese native population, which has been extinct since 2003 (Waki and Shimano 2020; Kuroki et al. 2020).

Information on feather mites associated with endangered bird species is very important for conservation of both birds and their symbiotic feather mites. Indeed, many species of flightless rails have been extirpated from Pacific islands due to degradation of their natural habitats and the introduction of invasive predators (Savidge 1987; Kirchman 2012). Therefore, the Okinawa Rail is considered to be at high risk of extinction. To investigate feather mites associated with this rail, we examined feathers of this host and discovered a new feather mite species of the genus *Metanalgus* Trouessart, 1919 (Analgoidea: Analgidae). This is also the first record of feather mites from this bird species.

Materials and Methods

Carcasses of dead Okinawa Rails collected between 2008 and 2020 at Kunigami Village, Okinawa, Japan, were used in this study. These specimens were roadkilled birds or individuals that died at a breeding facility of the Ministry of Environment, Japan. After a detailed necropsy, the collected bird specimens were frozen for subsequent investigation. Later on, the frozen carcasses were thawed at room temperature for several hours, and were examined with a naked eye or under a stereomicroscope for the presence of feather mites. When mites were detected, they were picked up with forceps and fixed in 99% ethanol for preservation. In a total,

we examined 33 bird individuals and detected a new feather mite species on five hosts (3–7 mites/host). Six mites were mounted on slides in Hoyer's medium for detailed morphological study. Six mites were used for the molecular study, and four haplotypes of the mitochondrial DNA region, cytochrome *c* oxidase subunit I (COI) gene, were obtained as described below. The COI region and nuclear 28S ribosomal DNA (rDNA) were originally selected as the target genes for DNA markers, and some fixed mites were used for PCR as described by Waki et al. (2023). However, amplification was not successful for obtaining 28S rDNA fragment, possibly due to degradation of samples under poor storage conditions. Sequencing was outsourced to Eurofins Genomics, Inc. (Tokyo); obtained sequences were deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan. The measurements of mite structures were taken using a microscope camera control unit (AdvanCam-U3II, Advan Vision). General morphological terms, leg and idiosomal chaetotaxy follow Gaud and Atyeo (1996), with corrections for coxal setation by Norton (1998). All measurements in the descriptions are in micrometers. The examined mite material is deposited in the Meguro Parasitological Museum (MPM), Tokyo, Japan.

Results

Superfamily **Analgoidea** Trouessart and Mégnin, 1884
Family **Analgidae** Trouessart and Mégnin, 1884
Subfamily **Megniniinae** Gaud and Atyeo, 1982
Genus ***Metanalgus*** Trouessart, 1919

Type species. *Megniniina elongata* Trouessart, 1887, by original designation.

Remarks. The genus *Metanalgus* is restricted in host

Table 1. Host associations and distribution of *Metanalgus* species.

Mite species	Host species	Host family	Locality	Reference
<i>M. (M.) agachi</i> sp. n.	<i>Hypotaenidia okinawae</i> (Yamashina and Mano, 1981)	Rallidae	Japan: Okinawa	Present study
<i>M. (M.) appendiculatus</i> (Haller, 1882) = <i>M. rallorum</i> (Trouessart, 1885)	<i>Rallus aquaticus</i> Linnaeus, 1758*	Rallidae	Europe	Haller (1882), Trouessart (1885), Bonnet (1924a), Gaud (1968), Mironov (1981)
	<i>R. caerulescens</i> Gmelin, 1789	Rallidae	Congo	Gaud (1968)
<i>M. (M.) crexi</i> Mironov, 1981	<i>Crex crex</i> (Linnaeus, 1758)	Rallidae	Russia: European part	Mironov (1981)
<i>M. (M.) curtis</i> Gaud and Mouchet, 1959	<i>Crecopsis erregia</i> (Peters, 1854)	Rallidae	Botswana, Cameroon, Congo,	Gaud and Mouchet (1959), Gaud and Till (1961), Gaud (1968)
<i>M. (M.) elongatus</i> (Trouessart, 1887)	<i>Cabalus lafresnayanus</i> (Verreaux and des Murs, 1860)	Rallidae	New Caledonia	Trouessart (1887, 1919), Bonnet (1924b)
<i>M. (M.) grossus</i> (Berlese, 1898)	<i>Gallinula chloropus</i> (Linnaeus, 1758)	Rallidae	Europe, Congo, South Africa	Berlese (1898), Bonnet (1924a), Gaud (1968), Mironov (1981)
<i>M. (M.) holderi</i> Mironov and Galloway, 2002	<i>Porzana carolina</i> (Linnaeus, 1758)	Rallidae	Canada: Manitoba	Mironov and Galloway (2002)
<i>M. (M.) saracura</i> Berla, 1960	<i>Aramides saracura</i> (Spix, 1825)	Rallidae	Brazil: Espírito Santo	Berla (1960)
<i>M. (A.) calcarata</i> (Haller, 1882)	<i>Porzana porzana</i> (Linnaeus, 1766)	Rallidae	Europe	Haller (1882), Mironov and Malovichko (2023)
<i>M. (A.) eurytarsus</i> Gaud and Mouchet, 1959	<i>Zapornia flavirostra</i> (Swainson, 1837)	Rallidae	Cameroon	Gaud and Mouchet (1959), Gaud (1968)
<i>M. (A.) fortis</i> Gaud, 1968	<i>Z. pusilla</i> (Pallas, 1776)*	Rallidae	Congo, Morocco	Gaud (1968)
	<i>Z. parva</i> (Scopoli, 1769)	Rallidae	Ukraine	Mironov (1981)
<i>M. (A.) porzana</i> Berla, 1960	<i>Mustelirallus albicollis albicollis</i> (Vieillot, 1819)	Rallidae	Brazil: Espírito Santo	Berla (1960)
<i>M. (A.) trigonocolpus</i> Gaud, 1968	<i>Porphyrio porphyrio</i> (Linnaeus, 1758)	Rallidae	Congo	Gaud (1968)

* Type host if a mite species was recorded on several host species.

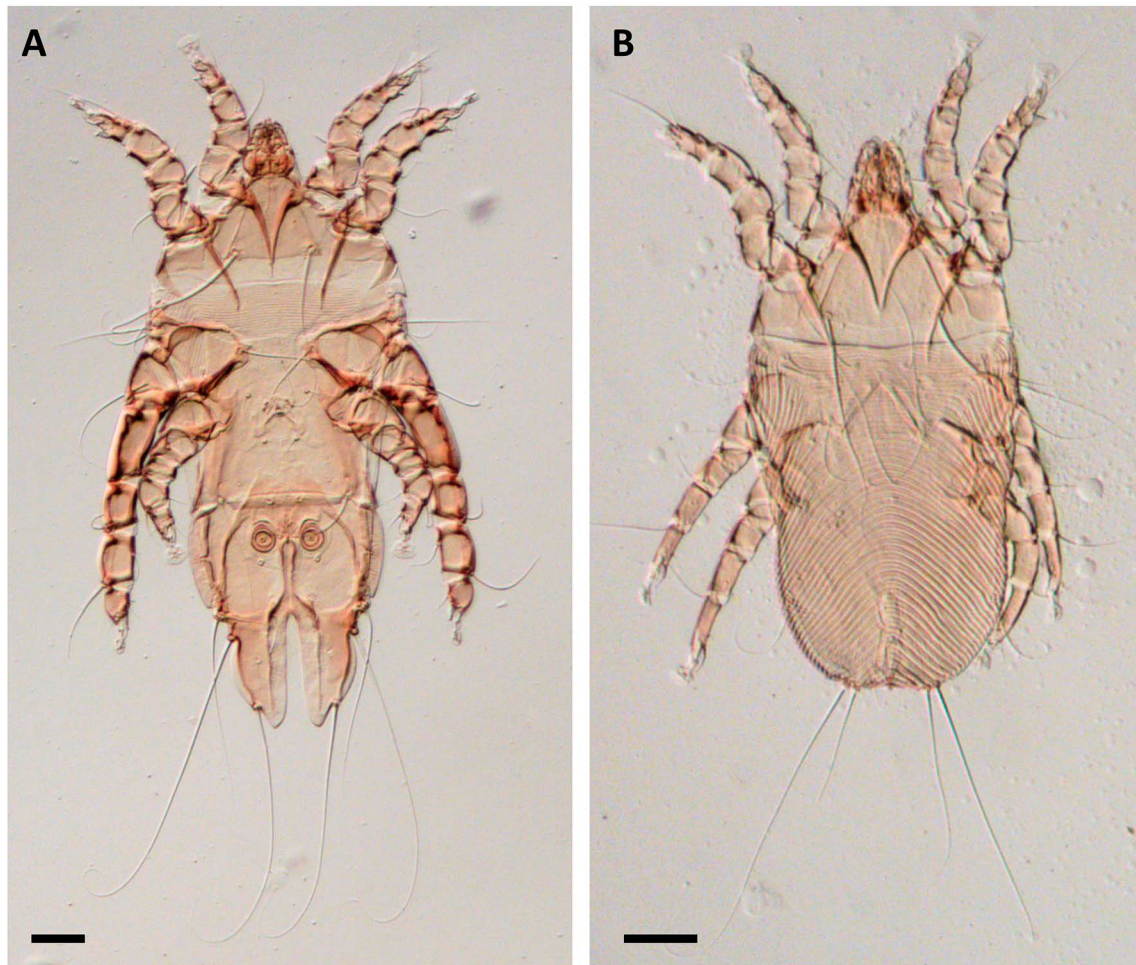


Fig. 1. *Metanalges agachi* sp. n., male, MPM Coll. No. 25251 (A) and female, MPM Coll. No. 25252 (B), ventral views. Scale bars: 50 μ m.

associations to rails (Gruiformes: Rallidae) and, to date, has included 12 species (Table 1) (Trouessart 1919; Bonnet 1924a, 1924b; Berla 1960; Gaud and Mouchet 1959; Gaud and Till 1961; Gaud 1968; Mironov 1981; Mironov and Galloway 2002; Mironov and Malovichko 2023). Representatives of this genus, as for most Analgidae, mainly inhabit the body covert feathers, although some individuals can be found in the basal parts of remiges and rectrices. Gaud and Mouchet (1959) divided the genus into two subgenera, *Metanalges* s. str. and *Agrialges* Gaud and Mouchet 1959, based on the structure of tarsi III in males. In males of the nominate subgenus, tarsus III is roughly cylindrical, with bidentate apical extension and with seta *s* modified in a thick bidentate spine. In males of the subgenus *Agrialges*, tarsus III is cone-like and seta *s* is represented by a blade-like spine with acute apex (Gaud 1968; Mironov 1981). Identification keys to previously known species from rallids of the Old World were provided by Gaud (1968) and Mironov (1981).

Gaud (1968) noted that *Megninia strongylia* Bonnet, 1924 from the Black-sided Robin, *Poecilodryas hypoleuca* (Gray, 1859) (Passeriformes: Petroicidae), could belong to the genus *Metanalges*. This suggestion seems to be erroneous, because the general shape of male opisthosoma, position of setae on opisthosomal lobes and the absence of lateral and interlobar lamellae in *M. strongylia* (Bonnet 1924a: 163, fig.

18) do not match to corresponding character states in the genus *Metanalges*.

In the original description of *Metanalges* (*M.*) *elongatus*, Trouessart (1887) reported this mite from a rallid species, identified as *Ocydromus australis* (Sparrman, 1786) (now *Gallirallus australis*) from New Caledonia, although this bird species is endemic to New Zealand. In a subsequent paper, Trouessart (1919) corrected the host identification for the New Caledonian Rail, *Cabalus lafresnayanus* (Verreaux and des Murs, 1860), an endemic rallid of New Caledonia.

In the original description of *M. (A.) eurytarsus* Gaud and Mouchet, 1959, Gaud and Mouchet (1959) reported the Western Plantain-eater, *Crinifer piscator* (Boddaert, 1783) (Musophagiformes: Musophagidae), as the type host of this mite. In the next paper on feather mites associated with rails, Gaud (1968) indicated that the record of *M. (A.) eurytarsus* on *Cr. piscator* was an error caused by accidental contamination, and the true host of this mite is the Black Crane, *Zapornia flavirostra* (Swainson, 1837).

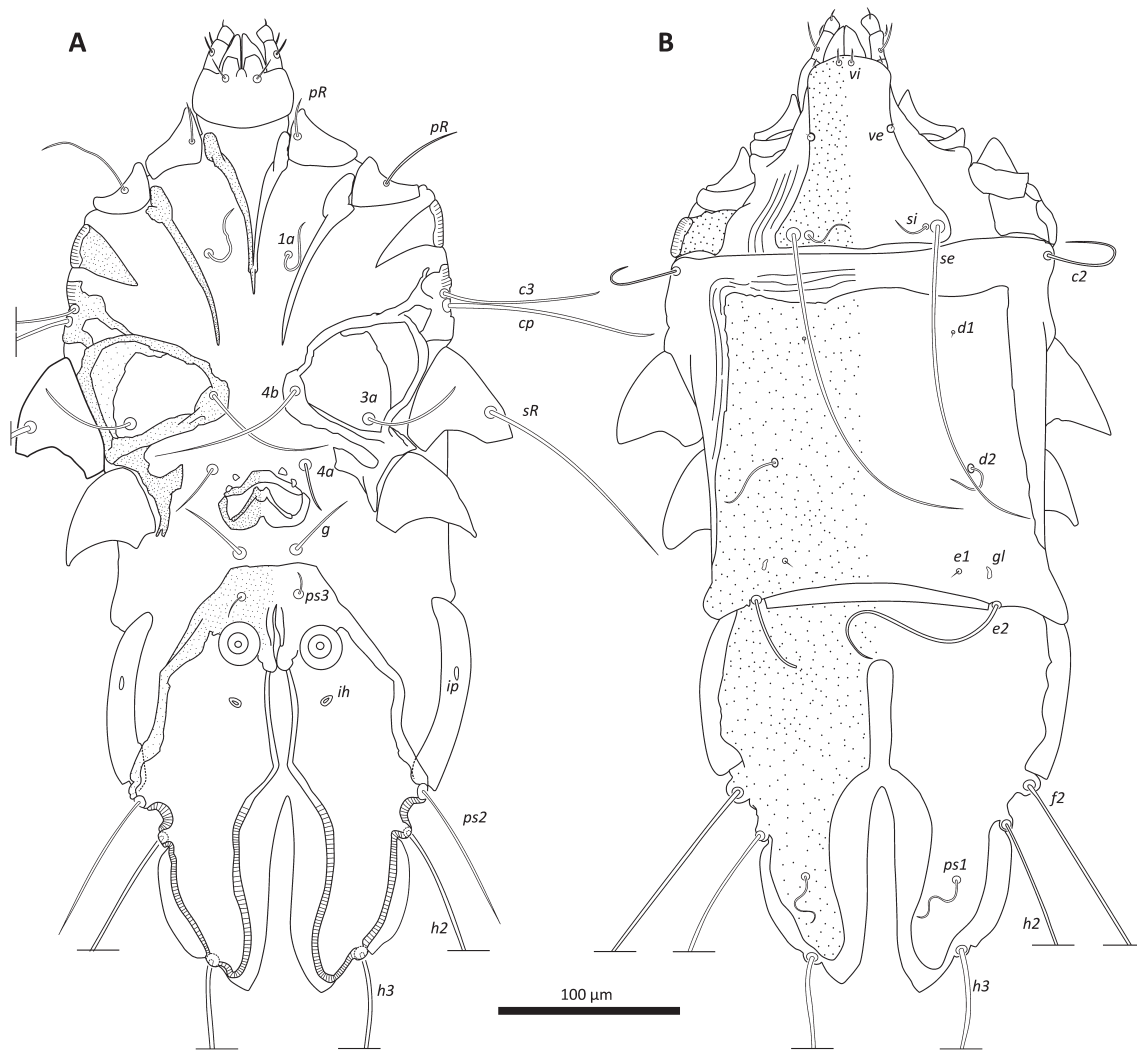


Fig. 2. *Metanalges agachi* sp. n., male, MPM Coll. No. 25250. A, Ventral view; B, dorsal view.

Metanalges (Metanalges) agachi

Waki, Mironov, and Shimano, sp. n.

(Figs 1–5)

[New Japanese name: Yanbaruikuina-umou-dani]

Type materials. Holotype male (MPM Coll. No. 25250) and paratypes, 2 males and 3 females (MPM Coll. Nos 25251–25253), from the Okinawa Rail *Hypotaenidia okinawae* (Yamashina and Mano, 1981) (Gruiformes: Rallidae), Japan, Okinawa Prefecture, Kunigami Village, March–April 2019 (holotype, 2 male and 1 female paratypes), and May 2013 (2 female paratypes), mite coll. T. Waki.

Depository. Meguro Parasitological Museum (MPM), Tokyo, Japan.

Description. *Male* (holotype, range for 2 paratypes in parentheses) (Figs 1A, 2, 3). Total body length including gnathosoma 684 (709–737). Length of subcapitulum including palps 67 (55–74), greatest width of subcapitulum 57 (64–65). Idiosoma length 622 (643–681), greatest width (at level of setae *c3*) 286 (294–306). Prodorsal shield shaped as elongate trapezoid, lateral margins slightly concave, length along midline 97 (94–116), greatest width at poste-

rior margin 98 (107–116). Setae *ve* represented by alveoli. Scapular shields well developed. Length of hysterosoma 513 (525–545). Humeral shields not developed dorsally. Hysteronotal shield covering most of dorsal hysterosoma, split into prohysteronotal and lobar pieces, surface without ornamentation, total length 453 (443–450). Prohysteronotal shield almost quadrangular in shape, length along midline 207 (182–186), width at anterior margin 179 (169–176), width at posterior margin 181 (175–180). Length of lobar shield 270 (260–265). Lateral margins of opisthosoma between levels of setae *e2* and *f2* with narrow opisthosomal membranes with smooth free margin. Opisthosomal lobes strongly elongated, over 3 times longer than wide at midlevel, straight or slightly curved medially, lateral margins from bases of setae *h2* to setae *h3* with narrow lateral membranes, width of opisthosomal lobes at midlength excluding membranes 58 (59–62). Terminal cleft narrow, parallel-sided or narrowly ovate; supranal concavity fused with the cleft, length of terminal cleft 152 (144–147), total length of the cleft including concavity 223 (209–220), greatest width 67 (66). Interlobar membrane occupying entire margin of terminal cleft, extending to lobar apices and forming rounded

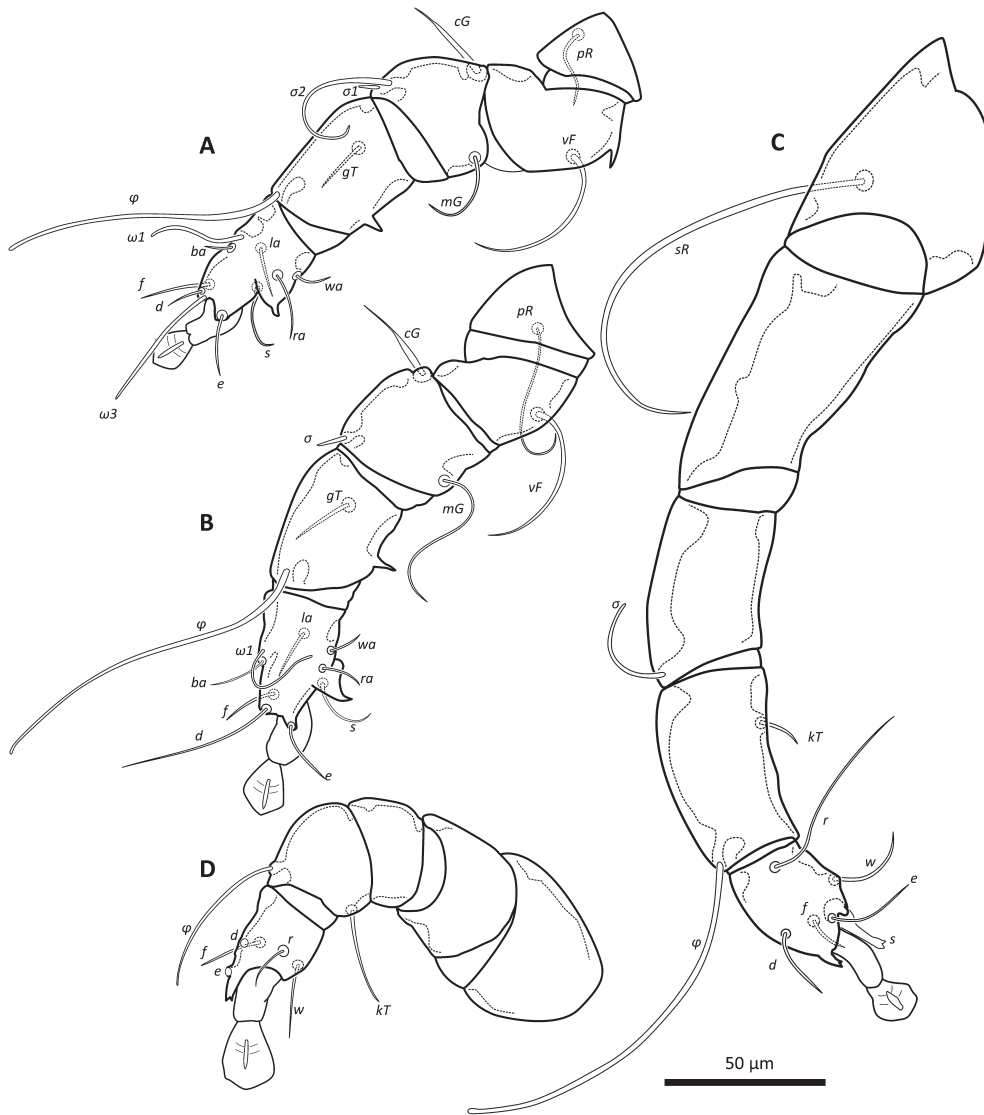


Fig. 3. *Metanalges agachi* sp. n., male, MPM Coll. No. 25251. A–D, Legs I–IV.

terminal extensions extending slightly beyond apices. Incision in interlobar membrane narrow, almost parallel-sided, length from anterior end to margins of terminal extensions 138 (141–142), greatest width 15 (11–19). Setae *f*2, *h*2, and *h*3 represented by macrosetae, setae *ps*2 filiform, about 3/4 the length of opisthosomal lobes. Macrosetae *h*2 situated approximately at level of anterior 1/4 of opisthosomal lobes, macrosetae *h*3 near lobar apices. Setae *ps*1 equidistant from levels of setae *h*2 and *h*3, or slightly closer to setae *h*2. Distances between dorsal setae: *se*–*se* 99 (91–92); *si*–*si* 82 (74); *h*2–*h*2 155 (139–145); *h*3–*h*3 97 (64–91); *c*2–*d*2 153 (151–163); *d*2–*e*2 99 (84–85); *e*2–*h*3 247 (248–260). Lengths of dorsal setae: *vi* 18 (12–13); *si* 44 (37–62); *se* 218 (217–255); *c*2 99 (97–140); *c*3 85 (94–105); *cp* 139 (133–138); *d*2 34 (29–37); *e*2 124 (143–157); *f*2 227 (237–240); *ps*1 48 (53–78); *h*2 412 (442–443); *h*3 366 (358–405).

Epimerites I fused in a V, fused part with thin spiculiform extension. Coxal fields III closed. Genital apparatus situated at levels of trachanters IV. Genital arch 12 (10–16) × 26 (26–34), aedeagus minute, much shorter than arch. Para-

genital apodemes small, fused into a low arch anterolateral to genital apparatus. Anterior pair of genital papillae on soft tegument or on paragenital apodemes, and posterior pair of papillae on paragenital apodemes. Adanal shield roughly trapezoidal, posterior margin with wide median extension partly covering anal opening, lateral parts with band-like oblique adanal apodemes extending to bases of setae *ps*2. Ventral side of opisthosomal lobes not sclerotized. Cupules *ih* well developed. Distances between ventral setae: *4b*–*g* 95 (94–110); *4b*–*4a* 52 (50–54); *4a*–*g* 52 (50–60); *4a*–*ps*3 112 (125–127). Lengths of ventral setae: *1a* 57 (33–54); *3a* 81 (68–79); *4a* 50 (42–51); *4b* 117 (109–124); *g* 49 (56–57); *ps*2 81 (78–90); *ps*3 29 (29–35).

Legs (Fig. 3A–D). Femur I with small acute denticle on lateral margin. Genual solenidion σ 2I slightly longer than genu I. Tarsus III with conical apical extension with bidentate apex, setae *s* large spine-like with narrow bidentate apex. Apical extension of tarsus IV with conical bidentate apex; modified setae *d* and *e* button-like. Lengths of tarsi I–IV: 51 (47–48), 55 (56–60), 66 (58–63) and 39 (45–51).

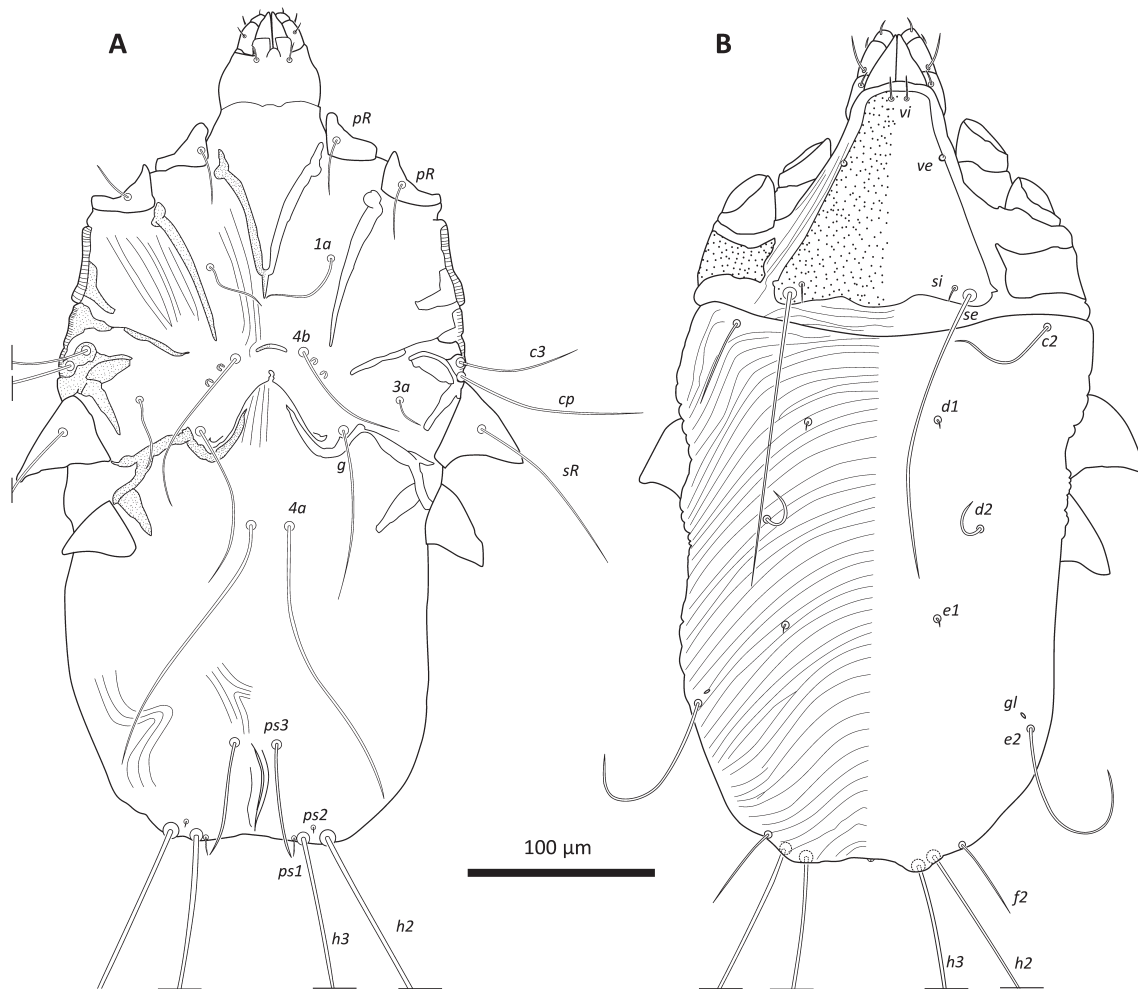


Fig. 4. *Metanalges agachi* sp. n., female, MPM Coll. No. 25252. A, Ventral view; B, dorsal view.

Lengths of solenidia: ω II 48 (52–55); ω 3I 43 (44–49); ω III 41 (45–48); σ 2I 76 (82–83); σ II 16 (13–18); σ III 55 (34–42).

Female (range for 3 paratypes) (Figs 1B, 4, 5). Total body length including gnathosoma 466–554. Gnathosoma as in male, length of subcapitulum including palps 62–67, greatest width 56–70. Length of idiosoma 423–517, greatest width (at level of setae *c3*) 218–255. Prodorsal shield shaped almost as in male, but posterior margin with short median convexity, length along midline 109–118, width at posterior margin 120–125. Setae *ve* represented by alveoli. Scapular shields well developed. Humeral shields not developed dorsally, represented by small triangular plates on ventral side of hysterosoma anterior to epimerites III. Length of hysterosoma 295–369, posterior end widely rounded. Hysteronotal shield absent, entire dorsal surface covered with soft tegument with pronounced and deep striae. Copulatory opening on minute cone-shaped extension on posterior margin of opisthosoma. Distances between dorsal setae: *se-se* 98–104; *si-si* 79–89; *h2-h2* 82–100; *h3-h3* 60–81; *c2-d2* 115–120; *d2-e2* 106–125; *e2-h3* 94–131. Lengths of dorsal setae: *vi* 19–25; *si* 11–19; *se* 170–215; *c2* 51–61; *c3* 65–78; *cp* 122–125; *d2* 28–31; *e2* 121–141; *h2* 335–391; *h3* 217–264; *ps1* 20–31.

Epimerites I shaped as in male. Epigynum shaped as small low bow, 11–17 wide, situated between bases setae

4b. Coxal setae *4a* almost extending to posterior margin of opisthosoma. Setae *ps3* extending beyond posterior margin of opisthosoma. Distances between ventral setae: *1a-4b* 52–56, *4b-g* 37–58; *g-4a* 64–72, *4a-ps3* 133–171. Lengths of ventral setae: *1a* 30–41; *3a* 35–48; *4a* 123–141; *4b* 65–109; *g* 77–120, *ps3* 69–77.

Legs. Setae *s* of tarsi III and IV thick spine-like, with a pair of minute denticles on apex. Lengths of tarsi I–IV: 42–47; 48–53; 52–58; 61–74. Lengths of solenidia: ω II 39–46; ω 3I 41–46; ω III 33–44; σ 2I 71–81; σ II 14–18; σ III 37–41.

Differential diagnosis. *Metanalges agachi* sp. n. belongs to the subgenus *Metanalges* s. str. and is most close to *M. curtus* Gaud and Mouchet, 1959 in sharing the following characters in males: the opisthosomal lobes are narrow and strongly elongated (about 3 times longer than wide at their midlength), the terminal cleft is long with parallel or slightly concave margins the incision in the interlobar membrane is narrow and parallel-sided, the posterior ends of interlobar membrane have short and rounded terminal projections extending beyond the lobar apices. Males of *M. agachi* differ from those of *M. curtus* in the following features: legs III with the pretarsus extend slightly beyond the level of setae *h2*, setae *ps1* are situated at the midlength between levels of setae *h2* and *h3* or slightly closer to *h2*. In males of *M. cur-*

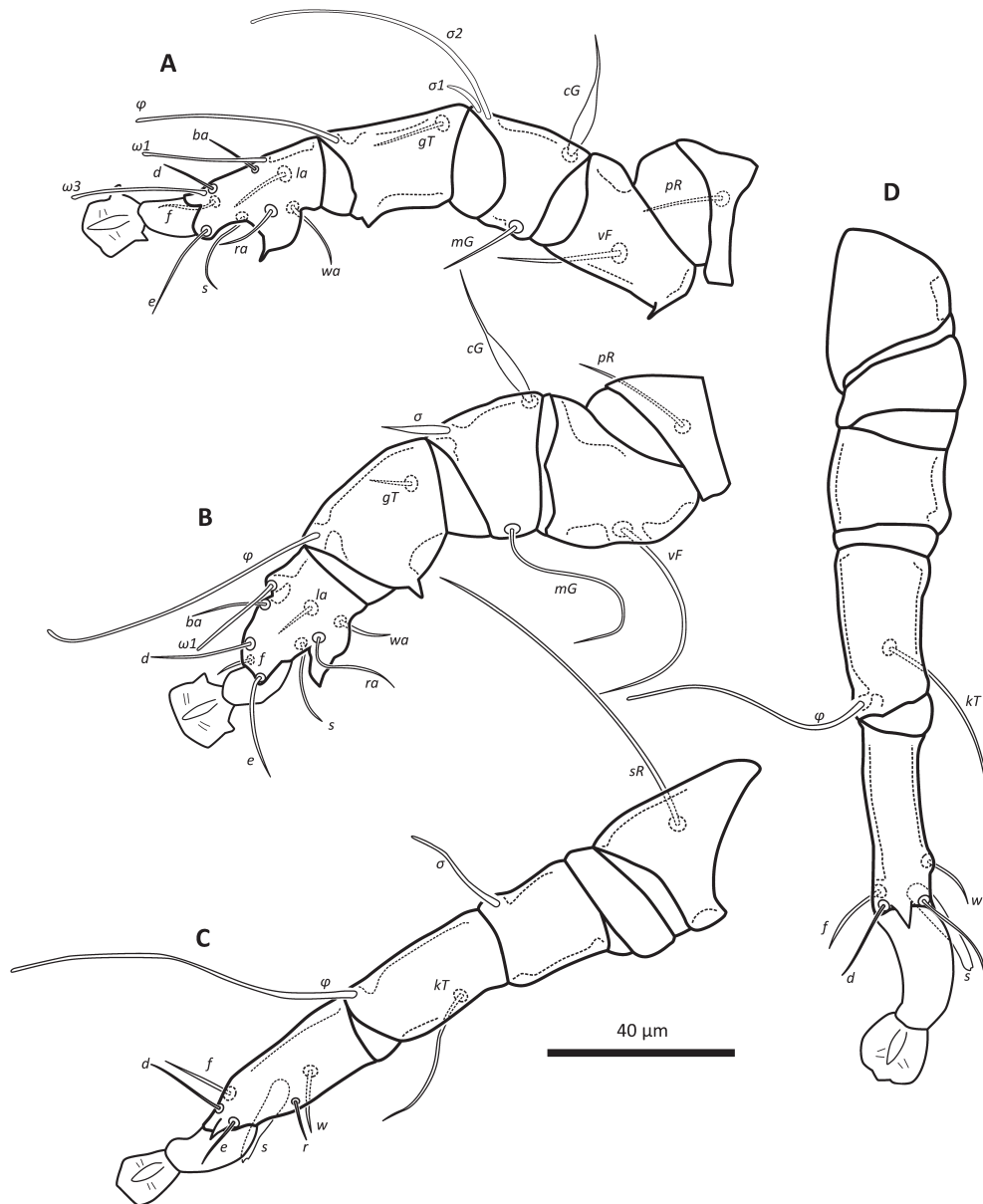


Fig. 5. *Metanalgus agachi* sp. n., female, MPM Coll. No. 25253. A–D, Legs I–IV.

tus, legs III with pretarsus extend almost to the level of setae *h3* situated on the lobar apices, and setae *ps1* are situated almost at the levels of setae *h2*. In the three previously known species of the subgenus *Metanalgus* s. str. having strongly elongated opisthosomal lobes, *M. curtus*, *M. elongatus* and *M. crexi* Mironov, 1981, females are known only in the latter; therefore, females of *M. agachi* can be compared only with that species. In females of *M. agachi*, the posterior margin of the prodorsal shield is slightly convex (vs. straight in *M. crexi*), coxal setae *4a* almost extend to the posterior margin of opisthosoma (vs. not extending to anal opening), and setae *ps3* extend beyond the posterior margin of opisthosoma (vs. not extending).

Etymology. The specific name is a transliteration of the local name of the type host (“Agachi”), and is thus treated as indeclinable. This name, meaning a flustered person, has been used by the local residents of former forested area in the northern part of Okinawa Island (“Yanbaru” region), be-

fore the bird was given a scientific name and Japanese name “Yanbaru-kuina (Rail)”.

DNA markers. INSD accession numbers: LC781786–LC781789 (COI, 637 bp).

Discussion

We described a new feather mite of the genus *Metanalgus* from the Okinawa Rail. This is the first report of feather mites from this avian host. The prevalence of *M. agachi* is not high (15.2%), and the maximum infection intensity of mites detected on host individuals is as low as 7. Some bird specimens used in our study were exposed to ambient temperature until they have been collected and dissected; other specimens after dissection were stored for some time in laboratories at room temperature before they were frozen. Probably for these reasons, a number of mites, while they

were alive, could be lost from the plumage of the examined bird carcasses. Thus, the results of the present study would not reflect the real infection rate of birds in the wild. Prevalence and intensity in the field could be probably much higher than those found in the present study. In addition, we identified four COI haplotypes from six mite individuals that can mean the genetic diversity of *M. agachi* is well preserved although the host population had once declined (Ministry of the Environment 2023). However, the risk of extinction for the new mite species is considered to be high, as the host is still under the risk of decline of the population size (BirdLife International 2021; Ministry of the Environment 2023).

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Authors Contributions

Tsukasa Waki: Resources; Investigation; Writing – original draft. Sergey Mironov: Writing – original draft. Yumiko Nakaya: Resources; Writing – original draft. Takashi Nagamine: Resources; Writing – original draft. Satoshi Shimano: Conceptualization; Supervision; Writing – original draft; Project administration.

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Declarations

Competing interests. The authors declare no conflicts of interest.

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