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# A New Mite of the Winterschmidtiiid Genus *Ensliniella* (Acari: Astigmata) Associated with the Vespid Wasp *Allodynerus mandschuricus* (Insecta: Hymenoptera) from Japan

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A new species of *Ensliniella* is described from deutonymphs from two localities in central Japan as the seventh species of the genus. *Ensliniella asiatica* sp. nov., found in the nests and acarinarium (mite chambers) of the vespid wasp *Allodynerus mandschuricus*, differs from the most similar species, *E. kostylevi*, in having a more reduced, shorter, subconical solenidion  $\phi$  on tibia IV and ensiform setae *e* and *r* on tarsus III. It is readily distinguished from the remaining five known congeners in having a solenidion  $\omega_2$  on tarsus I. This is the second species of *Ensliniella* known from Japan.

**Key words:** taxonomy, wasp-associated mite, Winterschmidtiiidae, Ensliniellinae

## INTRODUCTION

Vitzthum (1925) established the genus *Ensliniella* for a new wasp-associated astigmatid mite from Germany. Later, Zakhvatkin (1941) described a new species from Belarus and Ukraine, and Baker and Cunliffe (1960) reported two new species from Egypt and Morocco. More recently, Klompen and OConnor (1995) described a new species from Turkey and another from Croatia. Cooreman (1942) originally described yet another species, *E. trisetosa*, as a member of *Ensliniella*, but later (1954) established a monotypic genus, *Kennethiella*, for this species. In all, six species of *Ensliniella*, currently assigned to the subfamily Ensliniellinae in the family Winterschmidtiiidae (OConnor, 1982, 1984), are known from the Palaearctic region. Klompen and OConnor (1995) not only documented the morphology and revised the taxonomy of *Ensliniella*, but inferred phylogeny and discussed the evolution of life history traits. Their taxonomic revision, however, was based primarily on material from the European and Mediterranean subregions, and species from other areas have been poorly studied. The only study available for the Manchurian subregion, for instance, is that of Makino and Okabe (2003), who examined *E. parasiatica* Vitzthum, 1925 from central Japan.

Mites of the genus *Ensliniella* are obligatorily associated with mason wasps of the genus *Allodynerus* Blüthgen in the vespid subfamily Eumeninae, and are found on the body or in the nests of the hosts (Enslin, 1922; Vitzthum, 1925). Adults of *Allodynerus* have specialized structures called acarinarium, which are elaborate, deeply invaginated chambers that harbor the deutonymphs of *Ensliniella* (OConnor and Klompen, 1999; Makino and Okabe, 2003). The exist-

ence of such specialized structures as acarinarium suggests that the interactions between *Allodynerus* and *Ensliniella* are close, and this has long intrigued researchers (Enslin, 1922; Vitzthum, 1925; Cooreman, 1942; Benno, 1945; Crèvecoeur, 1945; Cooper, 1955; Giordani Soika, 1987; Klompen *et al.*, 1987; Klompen and OConnor, 1995; OConnor and Klompen, 1999; Makino and Okabe, 2003). Nonetheless, no one has explained why species of *Allodynerus* develop and retain acarinarium (OConnor and Klompen, 1999; Makino and Okabe, 2003). To advance our understanding of this phenomenon, it is necessary not only to evaluate the diversity of *Ensliniella* species, but also to understand their associations with *Allodynerus* in an evolutionary context.

Recently, we obtained deutonymphs of an astigmatid mite associated with an Asian eumenine, *Allodynerus mandschuricus* Blüthgen, 1953, from Saitama and Kyoto Prefectures in central Japan. In a taxonomic paper on *Allodynerus*, Blüthgen (1953) mentioned an *Ensliniella* on *A. mandschuricus* from China but did not identify it to species. After a detailed examination of these mites from Japan, we concluded that they represent an unnamed species of *Ensliniella*, which we describe here.

## MATERIALS AND METHODS

We used trap nests to collect wasps of *Allodynerus mandschuricus* that harbor the new species of *Ensliniella*. At the collection site in Saitama Prefecture, we used a trap nest of bamboo internodes, whereas at the collection site in Kyoto Prefecture, we used a bunch of internodes of the common reed *Phragmites australis* (Cav.) Trin. ex Steud. (Yamane, 1990). We detached the mites carefully from the trap nest or from the body surface of wasps using a minute pin under a dissecting microscope and preserved them in a vial with 99% ethyl alcohol.

Specimens for light microscopy were incubated at 40°C in lactophenol solution mixed with a small amount of Nesbitt's fluid (40 g chloral hydrate, 25 ml distilled water, 2.5 ml concentrated hydrochloric acid) (Krantz, 1978) to clear the body, and were then mounted individually on glass slides in Hoyer's medium (Krantz,

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1978). As in previous studies, specimens were flattened dorsoventrally by gently pressing the cover slips. After the medium dried completely, cover slips were sealed with Glyptal®, a suitable alkylid for ringing (Travis, 1968). Specimens were examined at 1000× magnification under a compound microscope with phase contrast and Nomarski differential-interference contrast optics (Nikon E600), illustrated using a camera lucida, and photographed with a digital camera attached to the microscope. Other specimens were mounted on a stub with double-sided carbon tape and observed and photographed with a scanning electron microscope (Keyence VE-8800) under an acceleration voltage of 1.3 kV.

Morphological characters to be measured were chosen following Klompen and OConnor (1995). The holotype and nine randomly selected paratypes were measured by first tracing characters on paper using a camera lucida, and then digitizing the traces with a scanner and calibrating and measuring them by means of a graphic pen tablet and the freeware program ImageJ version 1.33I (Rasband, 1997–2004; <http://rsb.info.nih.gov/ij>). For terminology, we primarily followed the idiosomal chaetotaxy of Griffiths *et al.* (1990) as modified by Norton (1998) and Okabe and OConnor (2002) and the leg chaetotaxy of Grandjean (1939), and for other characters followed Klompen and OConnor (1995). For comparison, we examined various specimens of the six known species of *Ensliniella* deposited in the Forestry and Forest Products Research Institute, Tsukuba (FFPRI).

The holotype is deposited in the Natural Resources Inventory Center, National Institute for Agro-Environmental Sciences, Tsukuba (NIAES). Paratypes are deposited in NIAES; the Museum of Biological Diversity, Ohio State University Acarology Laboratory, Columbus (OSAL); and the Museum of Zoology, University of Michigan, Ann Arbor (UMMZ). Voucher specimens of the host wasp, *Allodynerus mandshuricus*, are deposited in FFPRI.

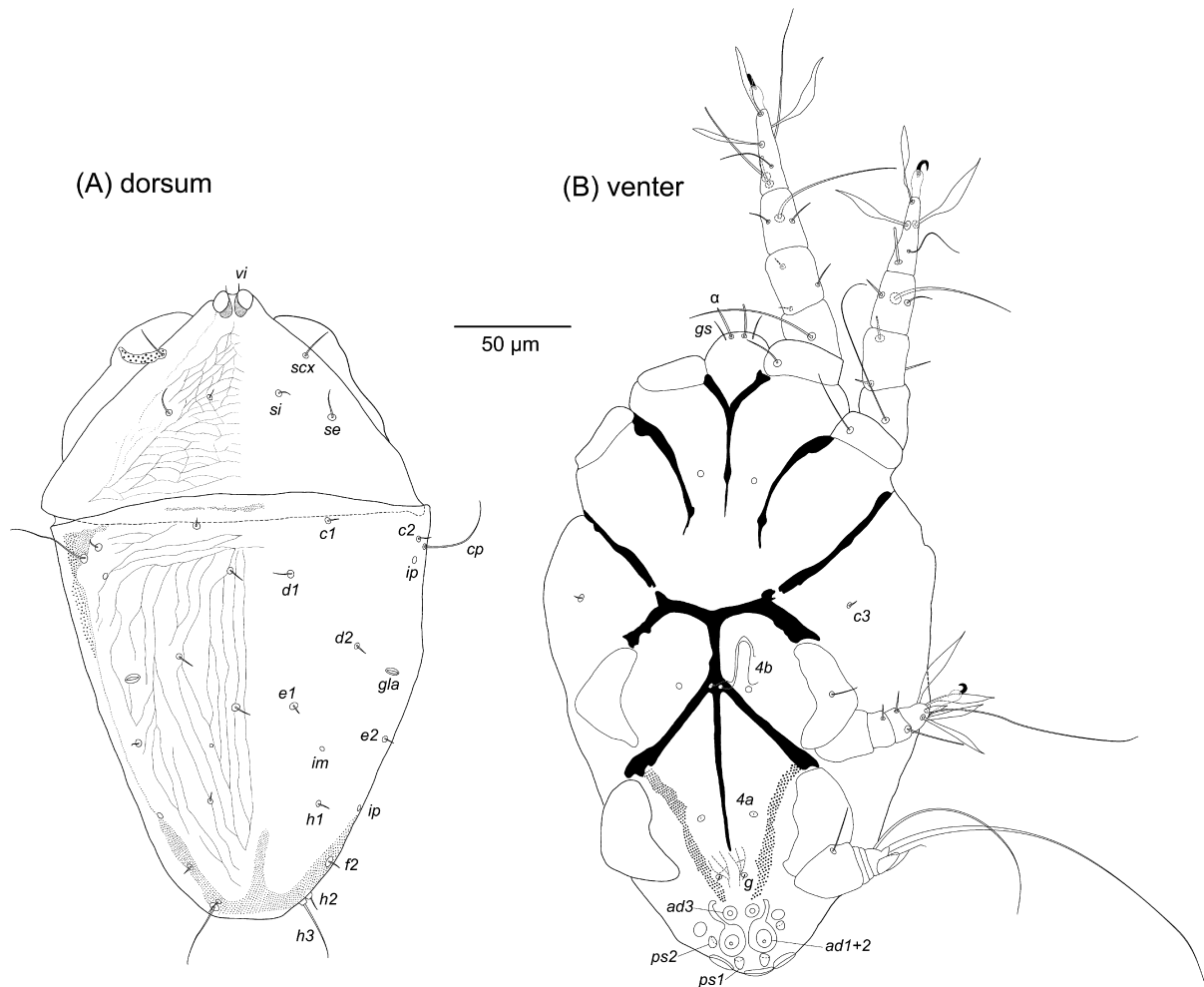
## TAXONOMIC ACCOUNT

### *Ensliniella asiatica* sp. nov.

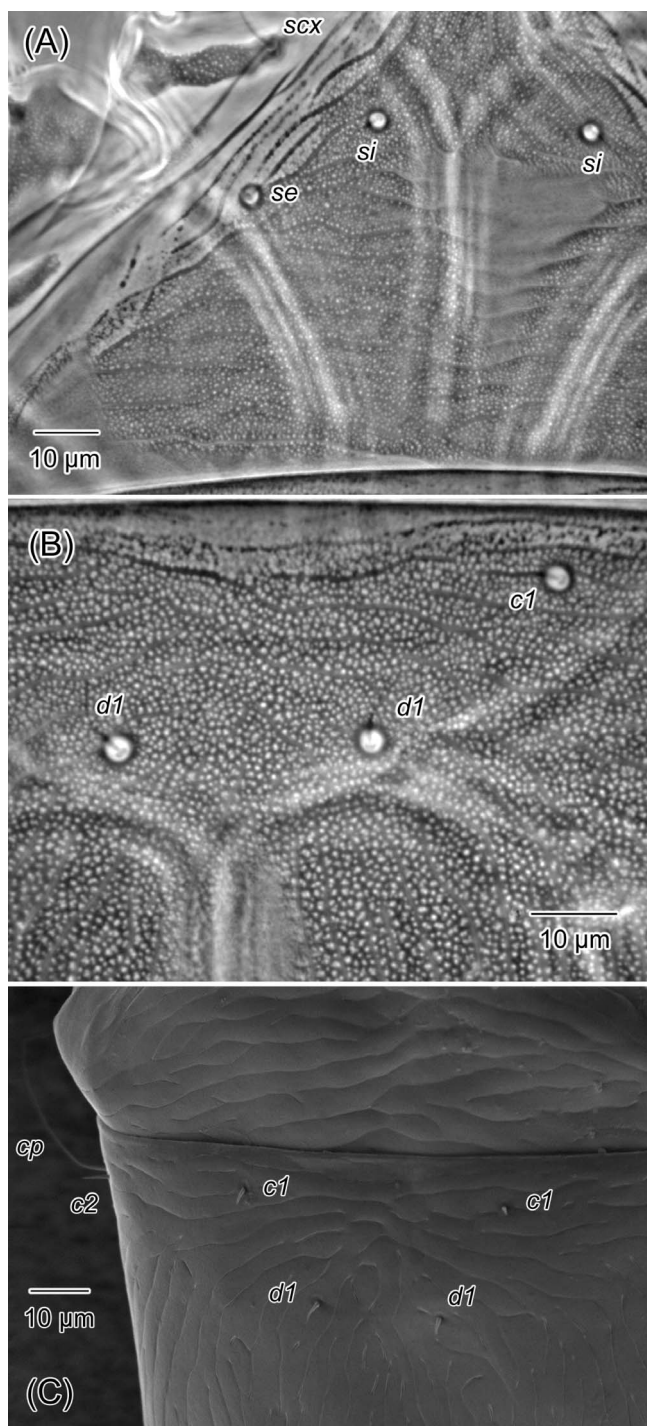
[Japanese name: Ajia-kita-dorobachi-yadori-konadani, new]  
(Figs. 1–4; Table 1)

#### Type series

Holotype (slide specimen): deutonymph (NIAES 440000001); Sakurazawa, Yorii town, Osato District, Saitama Prefecture, central Honshu, Japan; 36°07'49"N, 139°12'28"E; 102 m elevation; collected from nest of vespid wasp *Allodynerus mandshuricus* (collection no. OKBH05-0819-004) on 19 August 2005 by T. Nambu. Seventeen paratypes (slide specimens): deutonymphs (NIAES 440000002–440000018), locality same as holotype, collected from nest and body of *A. mandshuricus* (OKBH05-0819-004). Four paratypes (dry specimens on a stub for



**Fig. 1.** Deutonymph of *Ensliniella asiatica* sp. nov. Holotype (NIAES 440000001). Punctuation on the dorsal shield, which is shown in Fig. 2, is not illustrated here.



**Fig. 2.** Microstructure of dorsal shield of deutonymph of *Ensliniella asiatica* sp. nov., from dorsal view. Paratypes (A, B, NIAES 440000019; C, NIAES 440000039). A, B, phase contrast micrographs; C, scanning electron micrograph.

scanning electron microscope): deutonymphs (NIAES 440000039), locality same as holotype, collected from nest and body surface of *A. mandschuricus* (OKBH05-0819-001-1). Thirteen paratypes (slide specimens): deutonymphs (NIAES 440000019–440000038, OSAL 14396–14400, UMMZ 06-1001-001–005); Naka, Miyama town, Kitakuwata District, Kyoto Prefecture, western Honshu, Japan; 35°18'33"N,

135°38'13"E; 258 m elevation; collected from body of adult female *A. mandschuricus* (OKBH03-1300-058) in July 2002 by T. Endo.

### Etymology

The specific name, *asiatica* (Asian), is a Latin adjective in the nominative case, nomen in supposition, referring to the presumed geographic distribution of the new species (see Discussion).

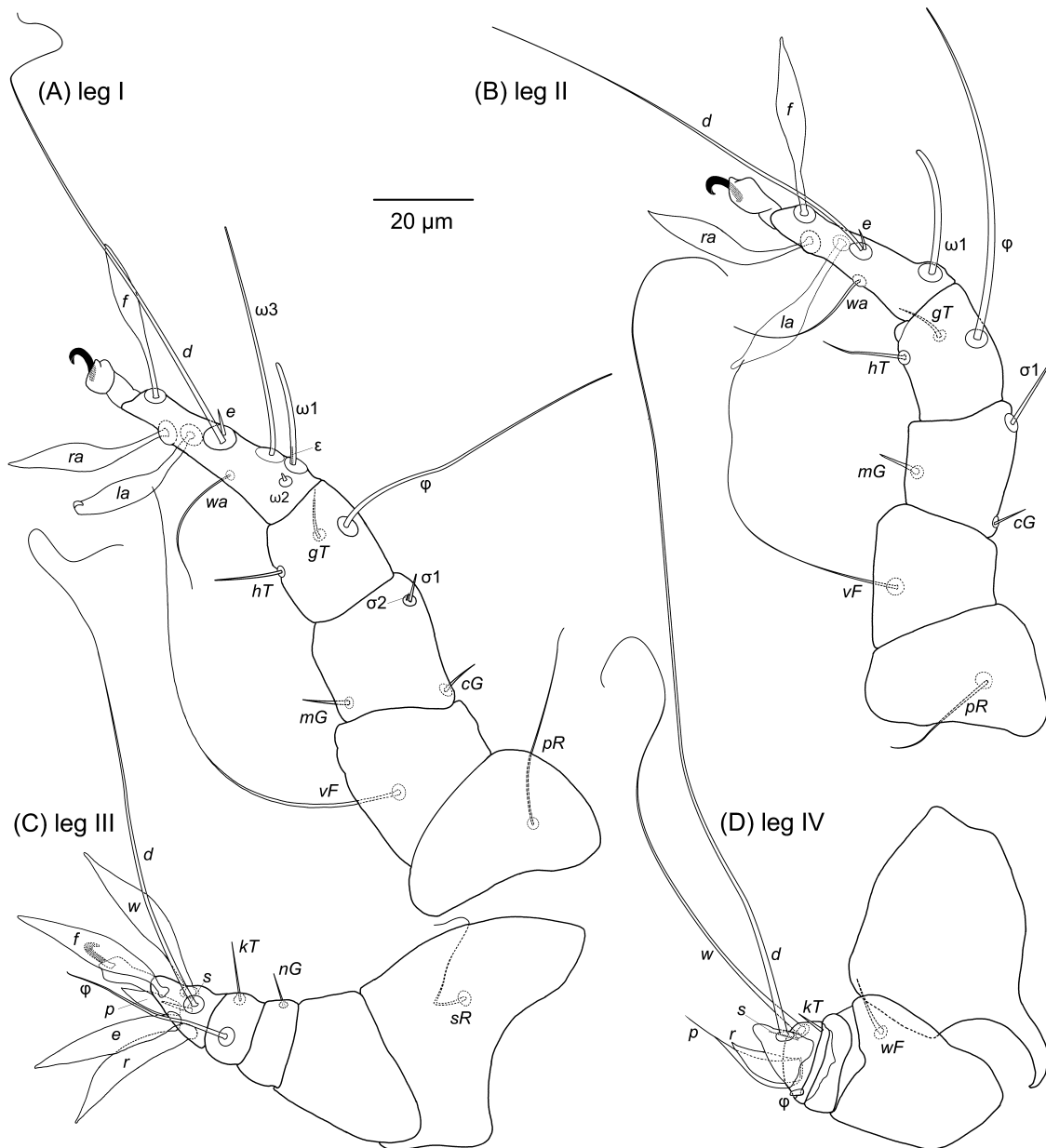
### Description of holotype

**Deutonymph.** Body ovoid, large, depressed dorsoventrally (Fig. 1; see Table 1 for morphometric data); color in life whitish and in preserved material faintly brownish-yellow. Dorsum almost completely covered by propodosomal and opisthosomal shields (Fig. 1A). Sejugal furrow distinct. Propodosomal shield subtriangular, covered thoroughly with shallow, reticulate striae (Figs. 1A; 2A, C). Opisthosomal shield trapezoidal, as broad as long, striated throughout (Figs. 1A; 2B, C); most striae transverse anterior to seta *d1* and oblique or longitudinal posterior to seta *d1* (Figs. 1A, 2C). Area among striae on propodosomal and opisthosomal shields evenly and densely punctate (Fig. 2A, B), except for anterolateral peripheral area of propodosomal shield, which is sparsely punctate or smooth; small, transverse area on propodosoma between seta *scx* and coxa I punctate and sclerotized; punctation composed of minute, mostly polygonal spots. Posterior dorsal apodeme on opisthosomal shield conspicuous; anterior projection short, not reaching level of setae *h1*. Ocelli large, subreniform, situated on anterior edge of propodosoma, with heavily pigmented retina (Fig. 1A); ocelli on both sides situated close to each other. Propodosomal setae *scx*, *se*, *si*, and *vi* simple, filiform, short; setae *se* and *si* inserted on propodosomal shield, setae *scx* and *vi* not inserted on the shield. Opisthosomal setae simple, filiform, short (except for setae *cp* and *h3*, which are long). Cupules *ia*, *im*, and *ip* subcircular, *ia* and *ip* similar in size, *im* smaller; opisthosomal gland *gla* present.

Venter smooth, largely occupied by coxal fields (Fig. 1B). Gnathosomal solenidion  $\alpha$  long, stiff; gnathosomal seta (*gs*) filiform, long, 0.8 times as long as solenidion  $\alpha$  (Table 1). Seta *c3* simple, short, filiform. Attachment organ ovate, with slightly elevated margin (Fig. 1B); median sucker (*ad1+2*) and anterior sucker (*ad3*) subcircular in ventral view, the former much larger than the latter; *ps1* and *ps2* well-developed, subconical, similar in size, posterior and lateral to median sucker, respectively; unpaired and paired circular suckers developed posteroperipherally; posterior coxal apodeme IV and apodeme of attachment organ not fused. Genital papilla 2-segmented; basal segment subcylindrical, apical segment subconical with elongate tip. Seta *g* short, filiform.

Legs mostly punctate, similarly to dorsal shield. Leg segments free. Legs I and II elongate and well-developed, legs III and IV short and stout (Figs. 1B, 3). Leg apodemes distinct; coxal apodemes I fused medially, forming long sternum; anterior coxal apodeme II and coxal apodeme III not fused; posterior coxal apodemes II elongate, almost touching coxal apodeme III. Posterior ventral apodeme weakly flanged, long, extending beyond level of setae *4a*. Coxa I with alveolus of seta *1a*; coxa II glabrous; coxa III with alve-

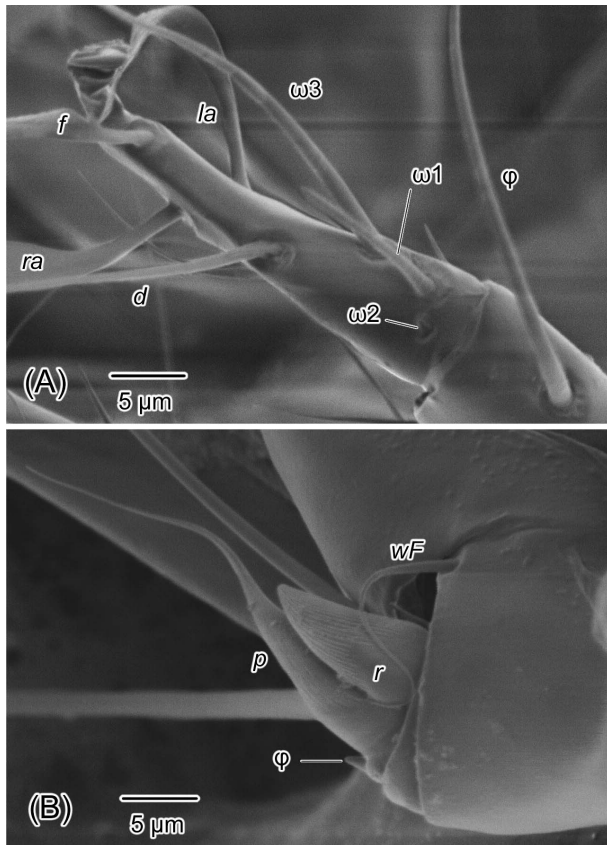




**Fig. 3.** Left legs of deutonymph of *Ensliniella asiatica* sp. nov., from dorsal view. Paratypes (A, C, NIAES 440000019; B, NIAES 440000021; D, NIAES 440000028).

olus of seta *3b*; seta *4b* long, filiform, developed on junction of anterior coxal apodeme IV and posterior ventral apodeme; coxa IV with minute seta *4a*. Trochanters I–IV, especially III and IV, short and stout; trochanters I and II each with filiform seta *pR*; trochanter III with filiform seta *sR*; trochanter IV glabrous. Femora I and II each with long, filiform seta *vF* ventrally; femur IV with short, filiform seta *wF* ventrally; femur III glabrous. Genua I and II well-developed, as long as wide; genu I proximally with short setae *cG* and *mG* and distally with solenidia  $\sigma_1$  and  $\sigma_2$ ; solenidium  $\sigma_1$  spiniform, with subacute tip; solenidium  $\sigma_2$  minute, spiniform; these solenidia situated close to each other, with common, distinct alveolus. Genu II with setae *cG* and *mG*, which are similar in shape, size, position to those on genua I; solenidium  $\sigma_1$  spiniform, its tip subacute, longer and stouter than

solenidium  $\sigma_1$  on genu I; solenidium  $\sigma_2$  absent. Genu III short, compressed, with short, thin seta *nG*; genu IV glabrous, strongly compressed. Tibiae I and II well-developed, slightly longer than wide; setae *gT* and *hT* short, filiform, the former longer than the latter; solenidium  $\phi$  long, stiff, tapering gradually to subacute tip, with distinct alveolus. Tibia III compressed; seta *kT* short, filiform; solenidium  $\phi$  stiff, tapering gradually to subacute tip, shorter than those on tibiae I and II. Tibia IV strongly compressed, with short, thin, filiform seta *kT* and strongly reduced, subconical solenidium  $\phi$ . Tarsus I elongate; solenidium  $\omega_2$  vestigial, papillate, developed proximally, with distinct, subcircular alveolus (Figs. 3A, 4A); solenidium  $\omega_1$  stiff, subisodiametric, with round tip, situated proximally, anterior to solenidium  $\omega_2$ ; famulus  $\epsilon$  spiniform with subacute tip, developed from alveolus of solenidium  $\omega_1$ ;



**Fig. 4.** (A) Right leg I and (B) right leg IV of deutonymph of *Ensliniella asiatica* sp. nov. Paratype (NIAES 440000039). Scanning electron micrographs.

seta *wa* thin, filiform; solenidion  $\omega 3$  stiff, tapering gradually to subacute tip; seta *d* long, filiform, with large, subcircular alveolus; seta *e* short, filiform, developed from alveolus of seta *d*; setae *f*, *la*, *ra* lanceolate, situated distally, similar to one another in size and shape. Tarsus II elongate; solenidia  $\omega 2$  and  $\omega 3$  absent; solenidion  $\omega 1$  stiff, subisodiametric, with round tip, situated proximally; famulus  $\epsilon$  absent; seta *wa* thin, filiform; seta *d* long, filiform, with large, subcircular alveolus; seta *e* short, filiform, developed from alveolus of seta *d*; setae *f*, *la*, *ra* lanceolate, developed distally. Tarsus III normally compressed for the genus, length 1.3 times width (Table 1); seta *d* long, filiform; seta *p* stout, subconical; seta *s* short, elongate apically, swollen basally; setae *f* and *w* lanceolate, setae *e* and *r* ensiform. Tarsus IV subconical; seta *d* extremely long, filiform, developed dorsally, based on subconical projection; seta *w* filiform, long but much shorter than seta *d*; seta *s* short, spiniform; seta *r* a blunt spine; seta *p* stout basally, attenuate distally. Pretarsi I–III terminating in robust, hooked claws; leg IV without pretarsus and claw; tip of tarsus IV pointed.

#### Notes on paratypes

Deutonymphs. Opisthosomal shield as long as or slightly longer than wide (see Table 1 for morphometric data). Anterior projection of posterior dorsal apodeme always short, never reaching level of setae *h1*. Posterior coxal apodemes II always elongate, almost fused to coxal

**Table 1.** Morphometric data of deutonymph *Ensliniella asiatica* sp. nov.

Character	Holotype	Paratypes (N=14)	Mean $\pm$ SEM (N=15)
Idiosoma	280	265–314	294 $\pm$ 4.1
Idiosoma width	178	155–208	189 $\pm$ 3.4
Opisthosomal shield	175	175–198	186 $\pm$ 1.8
Opisthosomal shield width	170	150–195	179 $\pm$ 3.3
Opisthosomal shield length/width ratio	1.0	1.0–1.2	1.0 $\pm$ 0.0
Setae <i>d1-e1</i> distance	57	50–65	58 $\pm$ 1.0
Setae <i>e1-h1</i> distance	42	37–54	44 $\pm$ 1.1
Setae <i>h1-h2</i> distance	39	30–47	43 $\pm$ 1.1
Setae <i>e1-e2</i> distance	41	40–54	47 $\pm$ 0.9
Solenidion $\alpha$	19	17–25	22 $\pm$ 0.5
Gnathosomal seta	15	10–23	15 $\pm$ 0.9
Gnathosomal seta/solenidion $\alpha$ ratio	0.8	0.4–0.9	0.7 $\pm$ 0.0
Distance between ocelli	4	2–4	3 $\pm$ 0.1
Seta <i>vi</i>	5	5–8	7 $\pm$ 0.3
Seta <i>si</i>	6	4–9	6 $\pm$ 0.4
Seta <i>se</i>	12	8–18	12 $\pm$ 0.8
Seta <i>sx</i>	13	11–16	14 $\pm$ 0.3
Seta <i>c1</i>	6	4–9	6 $\pm$ 0.4
Seta <i>c2</i>	6	4–9	7 $\pm$ 0.3
Seta <i>cp</i>	45	32–50	41 $\pm$ 1.4
Seta <i>c3</i>	4	3–5	4 $\pm$ 0.1
Seta <i>d1</i>	10	4–10	7 $\pm$ 0.5
Seta <i>d2</i>	7	4–8	6 $\pm$ 0.4
Seta <i>e1</i>	9	4–10	7 $\pm$ 0.4
Seta <i>e2</i>	6	4–8	5 $\pm$ 0.3
Seta <i>f2</i>	5	3–9	5 $\pm$ 0.3
Seta <i>h1</i>	7	5–11	7 $\pm$ 0.4
Seta <i>h2</i>	8	5–10	7 $\pm$ 0.3
Seta <i>h3</i>	33	21–36	28 $\pm$ 1.1
Seta <i>4b</i>	22	20–32	25 $\pm$ 0.8
Tarsus III	14	11–15	14 $\pm$ 0.3
Tarsus III width	11	9–13	12 $\pm$ 0.2
Tarsus III length/width ratio	1.3	1.1–1.3	1.2 $\pm$ 0.0
Solenidion $\omega 1$ I	16	16–22	20 $\pm$ 0.6
Solenidion $\omega 3$ I	46	37–50	44 $\pm$ 0.7
Solenidion $\phi$ I	56	43–65	59 $\pm$ 2.1
Solenidion $\phi$ III	32	30–39	35 $\pm$ 0.6
Solenidion $\phi$ IV	2	2–3	3 $\pm$ 0.1
Solenidion $\sigma 1$ I	6	5–7	6 $\pm$ 0.1
Solenidion $\sigma 1$ II	11	12–14	12 $\pm$ 0.2
Seta <i>kT</i> III	9	8–13	11 $\pm$ 0.4
Seta <i>kT</i> IV	7	4–8	6 $\pm$ 0.3

Measurement ( $\mu\text{m}$ ) is length unless otherwise indicated. Mean and SEM are calculated with holotype and paratypes together. N: number of specimens measured.

apodemes III. Posterior ventral apodeme long, clearly extending beyond level of setae *4a*; always weakly flanged. Solenidion  $\omega 2$  on tarsus I vestigial. Tarsus III always normally compressed. Solenidion  $\phi$  on tibia IV subconical, strongly reduced.

### Discussion of similar species

Species of *Ensliniella* have six ontogenetic stages: egg, larva, protonymph, deutonymph, tritonymph, and adult (Klompen *et al.*, 1987). Both the deutonymphs and adults have been described for *E. parasitica*, (Vitzthum, 1925), and all stages but tritonymphs have been described for *E. kostylevi*, (Klompen *et al.*, 1987), but the other four species are known only from phoretic deutonymphs (Klompen and OConnor, 1995). Likewise, we were able to collect only deutonymphs of *E. asiatica* sp. nov.; hence, the following discussion is applicable only to this stage.

*Ensliniella* includes six valid species (Klompen and OConnor, 1995): *E. aegyptiana* Baker and Cunliffe, 1960; *E. dignotus* Klompen and OConnor, 1995; *E. floricola* Klompen and OConnor, 1995; *E. koenigi* Baker and Cunliffe, 1960; *E. kostylevi* Zakhvatkin, 1941; and *E. parasitica*. *Ensliniella asiatica* sp. nov. is readily distinguished from all of these except *E. kostylevi* in having solenidion  $\omega_2$  on tarsus I. *Ensliniella asiatica* sp. nov. most resembles *E. kostylevi*, known from the European and Mediterranean subregions of the Palaearctic region, in having solenidion  $\omega_2$  on tarsus I (Figs. 3A, 4A) and elongate posterior coxal apodemes II, which almost reach coxal apodeme III (Fig. 1B). Although Zakhvatkin (1941) did not mention solenidion  $\omega_2$  on tarsus I in the original description of *E. kostylevi*, Klompen *et al.* (1987) found this solenidion on specimens from various localities in Europe (see also Klompen and OConnor, 1995). Moreover, Zakhvatkin (1941) described three lanceolate setae on tarsus III; however, according to Klompen *et al.* (1987), a seta was overlooked in the original description, and the correct number of the setae is four. Unfortunately, Klompen *et al.* (1987) were unable to examine type specimens of *E. kostylevi* in removing these ambiguities, because the type specimens are lost. We nevertheless consider the corrections by Klompen *et al.* (1987) to be appropriate for the following reasons. First, all works other than Zakhvatkin (1941) report four rather than three lanceolate setae on tarsus III. Second, we confirmed that the alveolus on solenidion  $\omega_2$  of tarsus I is present also in all deutonymphs identified as *E. kostylevi* phoretic on *Allodynerus rossii* (Lepelletier, 1841) from Steiermark in Austria. *Ensliniella asiatica* sp. nov. is distinguishable from *E. kostylevi* in having a more reduced, shorter, subconical solenidion  $\phi$  on tibia IV (Figs. 3D, 4B) and ensiform setae *e* and *r* on leg III (Fig. 3C); these setae are lanceolate (their bases are more sharply tapering and slender) in *E. kostylevi* (see Fig. 5f of Klompen *et al.*, 1987).

### DISCUSSION

*Ensliniella asiatica* sp. nov. is known from the type locality in Saitama Prefecture and a locality in Kyoto Prefecture, both in central Japan. Geographic distributions between species of *Allodynerus* and their associated *Ensliniella* correspond reasonably well (Klompen *et al.*, 1987; Klompen and OConnor, 1995). *Allodynerus mandshuricus*, host of *E. asiatica* sp. nov., is known also from northeastern China and South Korea (Blüthgen, 1953; Giordani Soika, 1970; Yamane, 1990). Accordingly, we expect *E. asiatica* sp. nov. to be found in these regions of the Asian continent through further studies. In fact, Blüthgen (1953) mentioned *Ensliniella* associated with *A. mandshuricus* from Harbin in north-

eastern China. Although he did not identify the mites, considering the high host specificity in *Ensliniella*, we suspect that the mites reported by Blüthgen (1953) might be *E. asiatica* sp. nov.

*Ensliniella asiatica* sp. nov. is the second species of the genus from Japan; *E. parasitica* was previously reported from Ibaraki and Nagano Prefectures in central Honshu (Makino and Okabe, 2003). The hosts of these mite species are *Allodynerus mandshuricus* and *A. delphinalis*, respectively. Because these are the only two species of *Allodynerus* known from Japan (Yamane, 1990), we do not expect any new *Ensliniella* from this country.

### ACKNOWLEDGMENTS

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