# ONTOGENETIC INSTARS OF PHYLLHERMANNIA BIMACULATA HAMMER, 1979 (ACARI, ORIBATIDA, HERMANNIIDAE)

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The morphology of juvenile instars (larva, proto-, deuto- and tritonymph) of the Oriental oribatid mite species *Phyllhermannia bimaculata* Hammer, 1979 (Oribatida, Hermanniidae) is studied, based on arboreal material from southern Vietnam. The SEM micrographs and morphological traits summarized for adults of *P. bimaculata* are presented. Juveniles of *P. bimaculata* are compared to those of other known species of the genus.

Key words: oribatid mites, ontogeny, juvenile instar, morphology, supplementary description, Vietnam.

## INTRODUCTION

The oribatid mite genus *Phyllhermannia* (Acari, Oribatida, Hermanniidae) was proposed by Berlese (1916) as subgenus of *Hermannia* Nicolet, 1855, with *Hermannia phyllophora* Michael, 1908 as type species. According to SubíAs (2004, online version 2022), *Phyllhermannia* comprises 85 species, which are distributed in the Pantropical, Subtropical and Sub-Antarctic regions.

In the course of taxonomic identification of materials collected from the bark of different trees in Cat Tien National Park (southern Vietnam) during a Russian–Vietnamese expedition in 2021, we found all the ontogenetic instars of *Phyllhermannia bimaculata* Hammer, 1979. This species was described by HAMMER (1979) from Java. Later, LUXTON (1991) presented a supplementary description of the ventral side of the body. So far, *P. bimaculata* was registered besides Java only in Vietnam (ERMILOV & ANICHKIN 2014, CORPUZ-RAROS & ERMILOV 2020).

The morphology of juvenile instars in *Phyllhermannia* is known (partially or completely) for six species (*P. bandabanda* Colloff, 2011, *P. falklandica* Balogh, 1988, *P. gladiata* Aoki, 1965, *P. goldengatensis* Ermilov, Hugo-Coetzee, A. Khaustov et V. Khaustov, 2020, *P. lemannae* Colloff, 2011, *P. sauli* Colloff 2011). The revised juvenile diagnosis for *Phyllhermannia* was presented by ERMILOV *et al.* (2012), with a subsequent update by ERMILOV and KHAUSTOV (2015) and BAYARTOGTOKH *et al.* (2021).

The main goal of our paper is to describe and illustrate all juvenile instars of *P. bimaculata*, and to compare them to those of the other *Phyllhermannia* species. Additionally, summarized morphological traits for adult *P. bimaculata* are provided.

#### MATERIAL AND METHODS

Specimens – Samples of bark (including specimens of *P. bimaculata*) were collected by V. M. SALAVATULIN and A. A. KUDRIN (20.06.2021–04.07.2021) via climbing trees (using spikes and other special equipment) and removing outer bark from different trees in Cat Tien National Park, Dong Nai Biosphere Reserve, Dong Nai Province, southern Vietnam.

Localities: five adults, three larvae, two proto- and one tritonymph: sample 1-3, 11°25′40″N, 107°25′34″E, *Haldina cardifolia*, height: 25 m; three adults, five larvae, one proto- and two deutonymphs: sample 2-1, 11°25′40″N, 107°25′34″E, *Ochrocarpos siamensis*, height: 0.5 m; three adults, one larva, one proto-, one deuto- and one tritonymph: sample 8-1, 11°25′45″N, 107°25′38″E, *Haldina cardifolia*, height: 0.5 m; one adult, three larvae, three proto-, three deuto- and one tritonymph: sample 8-3, 11°25′45″N, 107°25′38″E, *Haldina cardifolia*, height: 0.5 m; one adult, three larvae, three proto-, three deuto- and one tritonymph: sample 8-3, 11°25′45″N, 107°25′38″E, *Haldina cardifolia*, height: 0.5 m; two adults, four larvae, one proto-, three deuto- and one tritonymph: sample 13-3, 11°26′30″N, 107°25′56″E, *Dipterocarpus alatus*, height: 25.5 m.

Specimens of *P. bimaculata* were subsequently extracted by high-pressure flushing and further heptane flotation in laboratory conditions. Detailed descriptions of arboreal acarofauna collection and extraction techniques are presented in SALAVATULIN (2019).

All ontogenetic instars are preserved in 70% ethanol solution with a drop of glycerol and deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

The identification of juveniles belonging to *P. bimaculata* is based on the following reasons: adults were of the appropriate size to have juvenile instars with such dimensions; only this species of *Phyllhermannia* was registered when studying concrete samples.

Observation and documentation – Specimens were mounted in lactic acid on temporary cavity slides for measurement and illustration. Body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the notogaster. Gastronotic width refers to the maximum width of the gastronotum in dorsal view. Lengths of body setae were measured in lateral aspect. All body measurements are presented in micrometers. Formulas for leg setation are given in parentheses according to the sequence trochanterfemur-genu-tibia-tarsus (famulus included). Formulas for leg solenidia are given in square brackets according to the sequence genu-tibia-tarsus. Drawings were made with a camera lucida using a Leica transmission light microscope "Leica DM 2500". Images were obtained with an AxioCam ICc3 camera using a Carl Zeiss transmission light microscope "Axio Lab.A1". For SEM microscopy alcohol preserved mites were dusted with gold and scanned with the aid of a TESCAN Mira3 LMU SEM microscope.

Terminology – Morphological terminology used in this paper follows that of F. GRANDJEAN: see TRAVÉ and VACHON (1975) for references, NORTON (1977) for leg setal nomenclature, and NORTON and BEHAN-PELLETIER (2009), for overview.

Abbreviations – *Prodorsum*: *ro*, *le*, *in*, *bs*, *exd* = rostral, lamellar, interlamellar, bothridial, and dorsal exobothridial seta, respectively; *exv* = vestige of the second (ventral) exobothridial seta; *pmr* = prodorsal median ridge. *Gastronotic region*: *c*, *d*, *e*, *f*, *h*, *p* = setae; *ia*, *im*, *ih*, *ips* = cupules; *gla* = opisthonotal gland opening; δ*t* = longitudinal ecdysial cleavage line (line of dehiscence); *nplr* = gastronotic posterolateral region. *Gnathosoma*: *a*, *m*, *h* = subcapitular setae; *or* = adoral seta; *ep* = postpalpal seta; *d*, *l*, *v*, *sup*, *cm*, *acm*, *ul*, *su*, *vt*, *lt* = palp setae;  $\omega$  = palp solenidion; *cha*, *chb* = cheliceral setae; *Tg* = Trägårdh's organ. *Epimeral and lateral po-dosomal regions*: 1*a*-1*c*, 2*a*, 3*a*-3*c*, 4*a*-4*d* = epimeral setae; *Cl* = Claparède's organ. *Anogenital region*: *ag*, *an*, *ad* = aggenital, anal and adanal seta, respectively; *iad* = adanal cupule. *Legs*:  $\omega$ ,  $\varphi$ ,  $\sigma$  = solenidia;  $\varepsilon$  = famulus; *d*, *l*, *v*, *bv*, *ev*, *ft*, *tc*, *p*, *u*, *a*, *s*, *pv*, *pl* = setae. *Instars*: La = larva, Pn = protonymph, Dn = deutonymph, Tn = tritonymph, Ad = adult.

#### TAXONOMY

Family Hermanniidae Genus *Phyllhermannia* Berlese, 1916 Type species: *Hermannia phyllophora* Michael, 1908

### Phyllhermannia bimaculata Hammer, 1979 (Figs 1–8)

Main diagnostic traits of adult - (Figs 1A-D; 2A-C; based on our data and data from HAMMER (1979) and LUXTON (1991)). Body length: 540-700. Body color brown, covered by thick layer of gel-like cerotegument, partially forming tubercles on dorsal side and foveate-reticulate ornamentation on epimeres I-III and leg segments; cuticle densely porose; epimeral border IV with some tubercles. Rostrum rounded. Dorsal part of prodorsum with two longitudinal, arch-like (concave in median part) ridges; interbothridial part of prodorsum with two oval, convex structures having one posterior tubercle; lateral longitudinal ridge present, extending basally into lateral (anterior) tubercle (opposite posterior lateral tubercle absent). Rostral and lamellar setae short, setiform, barbed; interlamellar seta medium-sized, broadly phylliform, barbed; bothridial seta long, bacilliform proximally and gradually dilating in distal half to well visible, flattened head having microscales; dorsal exobothridial seta spiniform; bs > in > ro = le > exd. Anterodorsal part of notogaster with median longitudinal and one pair of lateral semicircular band-like furrows resembling the shape of two large cells. Notogastral setae medium-sized, broadly phylliform, barbed. Subcapitular setae *a* and *h* setiform, roughened, *m* minute, simple (*m*, absent);  $a > \bar{h} > m$ ; adoral seta *or*<sub>1</sub> dilated and truncate distally, or, bacilliform. Palp with setation  $0-1-1-3-9(+\omega)$ . Chelicera with two setiform, barbed setae; cha > chb. Epimeral setal formula: 3-1-3(4)-5(6); lateral setae of epimere III medium-sized, other setae short, setiform, all barbed. Genital, aggenital, adanal, and anal setae setiform, roughened or barbed. Adanal lyrifissure diagonal. Formulas of leg setation and solenidia (see Table 1): I (1-6-5-5-20) [1-2-1], II (1-6-5-5-15) [1-1-1], III (2-3-4-4-14) [1-1-0], IV (1–3–4–4–14) [0–1–0]; *tc*' elongate phylliform on tarsi III, IV.

Description of juvenile instars (Figs 2D; 3A, B; 4A–E; 5A, B; 6A, B; 7A–D; 8A–D) – *Measurements*. Total length of larva: 270–285 (n = 16), protonymph: 330–345 (n = 8), deutonymph: 345–375 (n = 9), tritonymph: 435–510 (n = 4). Total width of larva: 120–135 (n = 16), protonymph: 150–173 (n = 8), deutonymph: 165–180 (n = 9), tritonymph: 225–240 (n = 4).

*Integument*. Body cuticle colorless (larva and protonymph) to light brownish (deutoand tritonymph). Cuticle porose. Prodorsum with rare ridges; two of them well developed, diagonal, convergent, connected or separated medially. Interlamellar (between bothridium and prodorsal median ridge) and posterolateral gastronotic regions, and lateral parts of epimeres slightly tuberculate-reticulate in nymphal instars (versus indistinct in larva). Gastronotic and anogenital regions folded. Genital valves longitudinally striate. Subcapitular mentum and leg segments partially foveate-reticulate in nymphal instars.

*Prodorsum*. Triangular, about 2/3 length of gastronotic region. Rostrum rounded. Rostral seta (La: 5; Pn, Dn: 9–11; Tn: 15) setiform, erect, slightly barbed, divergent, located on tubercle. Lamellar seta (La: 5; Pn, Dn: 7–9; Tn: 9–11) similar in morphology to rostral setae in larva and protonymph versus slightly thickened or indistinctly phylliform in deuto-and tritonymph. Interlamellar seta (La: 5–7; Pn: 9–11; Dn: 15–22; Tn: 19–26) broadly phylliform,



**Fig. 1.** *Phyllhermannia bimaculata* Hammer, 1979, adult, SEM micrographs: A, B = dorsal view; C = ventral view; D = ventrolateral view

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barbed. Bothridial seta (La: 60–64; Pn: 71–75; Dn: 82–86; Tn: 90–94) bacilliform proximally and gradually dilating in distal half to well visible, flattened head having microscales. Dorsal exobothridial seta (La, Pn, Dn: 7; Tn: 7–9) spiniform, smooth, located very close to bothridium. Ventral exobothridial seta represented by alveolus.

*Gastronotic region*. Prodorsum and gastronotum separated by folds. Posterior part rounded. Longitudinal ecdysial cleavage line well visible (transverse line not observed). Larva with 13 pairs of setae;  $h_3$  vestigial,  $h_2$  (13–15) setiform, erect, roughened, other setae (7) broadly phylliform, barbed. Nymphal instars with 16 pairs (Pn:  $p_1$ ,  $p_2$ : 7–9, others: 9–11; Dn:  $p_1$ ,  $p_2$ : 9–11, others: 15–22; Tn:  $p_1$ ,  $p_2$ : 11, others: 19–26) of broadly phylliform, barbed setae (except  $p_2$  and  $p_3$  setiform, roughened in protonymph). Cupules *ia* and *im* and opisthonotal gland opening (near seta  $f_2$ ) visible, cupule *ip* not observed.

*Gnathosoma*. Subcapitulum size: La:  $52-56 \times 52-56$ ; Pn:  $71 \times 64$ ; Dn:  $90 \times 79$ ; Tn:  $97 \times 86$ . Subcapitular setae *a* and *h* (La: *a*: 15-19, *h*: 9-11; Pn: *a*: 19, *h*: 11; Dn, Tn: *a*: 22-26, *h*: 15) setiform, roughened; *m* vestigial. Two pairs of adoral setae present; adoral seta or<sub>1</sub> (La: 7-9; Pn: 9; Dn, Tn: 11) dilated and truncate distally, *or*<sub>2</sub> (La: 7-9; Pn: 9; Dn, Tn: 11) setiform. Palp



**Fig. 2.** *Phyllhermannia bimaculata* Hammer, 1979, adult (A–C) and protonymph (D), SEM micrographs: A = dorsoanterior view; B = right lateral view; C = notogastral seta  $e_1$  and notogastral cerotegument; D = dorsal view

(La: 30; Pn: 37; Dn: 45; Tn: 52) setation:  $0-1-1-3-9(+\omega)$ . Postpalpal seta (length: La: 15–19; Pn, Dn, Tn: 15) setiform, blunt-ended, erect, roughened. Chelicera (length: La: 64; Pn: 79; Dn: 101; Tn: 112) with two setiform, barbed setae (*cha*: La: 15–19; Pn: 22; Dn: 30; Tn: 34) distinctly barbed; *chb*: La: 9–11; Pn: 13; Dn: 17–19; Tn: 19–22) barbed.

*Epimeral and lateral podosomal regions*. Setal formulas for epimeres: La: 3–1–2 (third seta of first epimere forms protective scale over respective Claparède's organ); Pn: 3–1–2–1; Dn: 3–1–2(sometimes 3)–3; Tn: 3–1–3–4. Epimeral setae (La, Pn: 7; Dn, Tn: 9–11) setiform, erect, roughened but setae 1a, 2a and 3a distally bifurcate in larval instar.

Anogenital region. Ontogeny of genital, aggenital, adanal, and anal setal formulas, larva to tritonymph, 0–1–4–7 (Pn: 11; Dn: one pair: 19–26, others: 7–11; Tn: one pair: 26–33, others: 9–11), 0–0–1–2 (Dn, Tn: 9–11), 0–0–3–3 (Dn, Tn: 9–11), 0–0–0–2 (Tn: 9–11), respectively. All setae setiform, roughened to barbed. Cupules *ih*, *ips*, *iad* appearing in normal ontogenetic pattern; becoming inconspicuous when shifted away from anal valves.

*Legs*. Claw of each leg strong, slightly barbed on dorsal side. Femora I–IV and trochanters III, IV with poorly visible ventroparaxial porose area. Formulas of leg setation and solenidia (see Table 1 for designations): La: I (0–2–3–4–16) [1–1–1], II (0–2–3–4–13) [1–1–1], III (0–2–2–3–12) [1–1–0]; Pn: I (0–4–4–5–16) [1–1–1], II (0–4–4–4–13) [1–1–1], III (1–2–2–3– 12) [1–1–0], IV (0–0–1–0–7) [0–0–0]; Dn: I (1–5–4–5–16) [1–2–1], II (1–5–4–5–13) [1–1–1], III (2–3–2–3–12) [1–1–0], IV (1–2–2–3–12) [0–1–0]; Tn: I (1–5–5–5–18) [1–2–1], II (1–5–5–5–13) [1–1–1], III (2–3–3–4–12) [1–1–0], IV (1–3–3–4–12) [0–1–0]. Tibiae I–IV and genua I–III with seta *d* coupled with respective solenidion; *it*", *it*' absent on all tarsi; *tc*' elongate phylliform on tarsi III, IV; *ft*' absent on tarsus III.



Fig. 3. *Phyllhermannia bimaculata* Hammer, 1979, deutonymph, microscope images: A = dorsal view; B = right lateral view

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## Ontogenetic transformations

The juvenile instars colourless to light-brown; adult of brown or dark brown colour. In juvenile instars, cuticle of gastronotum and anogenital region folded; in adult, cuticle without heavy ornamentation or sculpturing but covered by thick layer of gel-like cerotegument, partially forming tubercles



**Fig. 4.** *Phyllhermannia bimaculata* Hammer, 1979, larva (A–C) and protonymph (D, E): A, D = dorsal view; B, E = ventral view (gnathosoma and legs except trochanters not shown); C = right lateral view (gnathosoma and legs except trochanters not shown). Scale bars: 50  $\mu$ m

on dorsal side of the body and foveate-reticulate pattern on epimeres I-III. Rostrum rounded. With six pairs of prodorsal setae: rostral and lamellar setae setiform (except le slightly thickened or indistinctly phylliform in deuto-and tritonymph); interlamellar seta phylliform; bothridial seta long, bacilliform proximally and gradually dilating in distal half to well visible, flattened head, having microscales; dorsal exobothridial seta spiniform; ventral exobothridial seta represented by alveolus; ro and le shortest, bs longest. Bothridium small, cup-like, with smoothly rounded opening. In juvenile instars, dorsal side of gastronotum with longitudinal ecdysial cleavage line (transverse line absent). All gastronotic setae broadly phylliform (except vestigial  $h_2$  in larva, setiform  $h_2$  in larva, and  $p_2$ ,  $p_3$  in protonymph); larva with 13 pairs, nymphal instars and adult with 16 pairs of gastronotic setae (in protonymph setae of p-series newly appeared). Subcapitulum with three pairs of subcapitular setae (*a*, *h* setiform; *m* vestigial;  $m_2$  not appear during ontogeny) and two pairs of adoral setae  $(or_1 dilated and truncated distally, or_2 setiform)$ . Palp femur with one seta in all instars. Larva with six pairs of epimeral setae (1a, 1b, 1c, 2a, 3a, 3b), seta 4a



**Fig. 5.** *Phyllhermannia bimaculata* Hammer, 1979, deutonymph: A = dorsal view; B = ventral view (gnathosoma and legs except trochanters not shown). Scale bar 50 μm

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newly appeared in protonymph, while 4b, 4c added in deutonymph, setae 3c and 4d developed in tritonymph, other setae added in adult. Number of anogenital setae gradually increasing from larva to adult: larva without anogenital setae; one pairs of genital setae added in protonymph (without aggenital and anoadanal setae); three pairs of genital, one pair of aggenital and three pairs of adanal setae appeared in deutonymph; three pairs of genital, one pair of aggenital and two pairs of anal setae added in tritonymph; two pairs of genital setae appeared in adult. Paraproctal valves without setae. Leg setation increasing from instar to instar as shown in Table 1.

#### REMARKS

The morphology of juvenile instars in *Phyllhermannia* was studied for six species: 1) *P. bandabanda* (only tritonymph is known; see Colloff 2011), 2) *P.* 



**Fig. 6.** *Phyllhermannia bimaculata* Hammer, 1979, tritonymph: A = dorsal view; B = ventral view (gnathosoma and legs except trochanters not shown). Scale bar 50 μm

	Trochanter	Femur	Genu	Tibia	Tarsus
Leg I					
La	-	d, bv″	(l), dσ	(l), v', $d\varphi_1$	(ft), (tc), (p), (u), (a), s, (pv), (pl), ε, ω
Pn	_	l", v"	v''	v''	-
Dn	v'	ľ	_	$\phi_2$	-
Tn	_	_	v'	-	$(v_1)$
Ad	-	v'	_	_	$(v_2)$
Leg II					
La	-	d, bv″	(l), dσ	(l), v′, dφ	$(ft), (tc), (p), (u), (a), s, (pv), \omega$
Pn	-	l", v"	v''	-	_
Dn	v'	l′	_	v''	_
Tn	-	_	v'	-	_
Ad	_	v'	_	_	(v)
Leg III					
La	-	d, ev′	l', dσ	l', v', dφ	ft", (tc), (p), (u), (a), s, (pv)
Pn	v'	_	_	-	_
Dn	l'	l'	_	_	_
Tn	-	_	v'	v''	_
Ad	-	_	v''	_	(v)
Leg IV					
Pn	-	_	d	_	ft", (p), (u), (pv)
Dn	v'	d, ev′	ľ	l', v', dφ	( <i>tc</i> ), ( <i>a</i> ), <i>s</i>
Tn	_	ľ	v'	v''	_
Ad	_	_	71″	_	(7))

Table 1. Development of leg setation of Phyllhermannia bimaculata Hammer, 1979

Note: Roman letters refer to normal setae; Greek letters refer to solenidia (except  $\varepsilon$  — famulus);  $d\varphi$  and  $d\sigma$  – seta and solenidion coupled. Single quotation mark (') designates seta on the anterior and double quotation mark (") seta on the posterior side of a given leg segment; parentheses refer to a pair of setae. Setae are listed only for the stage in which they first appear.

*falklandica* (all instars described; see ERMILOV & KHAUSTOV 2015), 3) *P. gladiata* (nymphs described; see ERMILOV *et al.* 2012), 4) *P. goldengatensis* (all instars described; see ERMILOV *et al.* 2020), 5) *P. lemannae* (all instars described; see Colloff 2011), and 6) *P. sauli* (all instars described; see Colloff 2011). A comparison between juveniles of *P. bimaculata* and above listed species is given below.

1) Tritonymph of *P. bimaculata* can be distinguished from that of *P. bandabanda* by the presence of bothridial seta with developed head (versus seti-

form), broadly (versus narrowly) phylliform dorsal gastronotic setae, localization of gastronotic seta  $c_3$  to  $c_2$  (posterolateral versus posteromedial), three pairs (versus two) of setae on epimere III, and short (comparatively long) epimeral setae 4c, 4d.

2) Larva and nymphs of *P. bimaculata* can be distinguished from that of *P. falklandica* by the presence of long (versus comparatively short) bothridial seta with elongate narrow (versus broadly oval) head, phylliform (versus setiform) interlamellar and dorsal gastronotic setae, and setation of legs (see Table 1 versus Table 2 in ERMILOV & KHAUSTOV 2015).

3) Nymphs of *P. bimaculata* can be distinguished from that of *P. glad-iata* by the presence of bothridial seta with developed head (versus setiform), broadly phylliform (versus narrowly phylliform) dorsal gastronotic setae, short (some comparatively long) epimeral setae on epimere III, and setation of legs (see Table 1 versus Table 3 in ERMILOV *et al.* 2012).

4) Larva and nymphs of *P. bimaculata* can be distinguished from that of *P. goldengatensis* by the presence of broadly (versus narrowly) phylliform interlamellar and dorsal gastronotic setae and setation of legs (see Table 1 versus Table 1 in ERMILOV *et al.* 2020).

5) Larva and nymphs of *P. bimaculata* can be distinguished from that of *P. le-mannae* by the presence of bothridial seta with developed head (versus setiform), broader (versus narrower) phylliform dorsal gastronotic setae (in nymphs), and short (some comparatively long) epimeral setae on epimere III, IV.

6) Larva and nymphs of *P. bimaculata* can be distinguished from that of *P. sauli* by the presence of bothridial seta with developed head (versus in-



Fig. 7. Phyllhermannia bimaculata Hammer, 1979, gnathosoma (dissected and pressed) of larva: A = subcapitulum, ventral view; B = left lip with adoral setae; C = palp, right, antiaxial view; D = chelicera, right, antiaxial. Scale bars 10 μm

distinct), broader (versus narrower) phylliform dorsal gastronotic setae (in nymphs), and short (some comparatively long) epimeral setae on epimere IV in deuto- and tritonymph.



**Fig. 8.** *Phyllhermannia bimaculata* Hammer, 1979, legs of larva (A–C) and protonymph (D): A = leg I, left, paraxial view; B = leg II, right, antiaxial view; C = leg III, left, antiaxial view; D = leg IV, left, antiaxial view. Scale bars 20 μm

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