Sucking lice ~~in~~ of bandicoot rats with ~~first~~ the description of *Hoplopleura malabarica* nymphs.

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Abstract

Background. Studies of insect biodiversity and parasitism are often based on imaginal stages, as immature stages are poorly known and often cannot be identified to species level. However, sucking lice (Anoplura) are stationary parasites with single-host life cycles, making it possible to track the occurrence of all life stages ~~within~~ on the host, including both imagines and nymphs, which, as hematophagous parasites, impact the host. Only the complete identification of all life stages provides a full picture of parasitism, including infestation levels, parasite topography ~~within~~ on the host, and host specificity.

Methodology. The description of the nymphal stages of *Hoplopleura malabarica* is based on specimens of sucking lice from the greater bandicoot rat *Bandicota indica* from Southeast Asia, specifically from the Vientiane area of Lao PDR, which were subjected to morphometric analysis and scanning microscopy.

Results. This study presents the first description of the nymphal stages of *Hoplopleura malabarica*, an oligoxenous parasite of rodents of the genus *Bandicota*. In addition, a global checklist of Anoplura parasitizing rodents of the genus *Bandicota* was compiled.

Conclusions. The detection of different life stages of lice within the host confirms that they reproduce and develop on a given host species, fully utilizing its resources.

Introduction

Analyses of insect biodiversity, including parasitic insects, are typically based on imaginal stages. Many species have been described solely from adults, sometimes from only one sex. Immature stages are often poorly known for this reason cannot be identified to species level. In some cases, they inhabit different environments, requiring distinct methodologies for study. However, Anoplura are stationary parasites with single-host life cycles, making it possible to track the occurrence of all life stages within the host. This is particularly important because all active stages are hematophagous parasites, meaning they all can impact the health status of the host. Identification based only on imaginal stages is therefore incomplete and does not provide a full picture of parasitism, including aspects from the classical measures of parasitism to parasite ecology.

Many Anoplura species lack descriptions of their nymphal stages, making it extremely difficult or impossible to properly analyze infection levels, parasite topography within the host, and host specificity. The presence of different life stages within a host confirms that lice of a given species reproduce and develop on that host, fully utilizing its resources. In contrast, the mere detection of adult lice—especially occasional ones—may indicate only an attempt to colonize a new host rather than a successful parasitic relationship. To obtain a comprehensive understanding of lice parasitism and properly analyze the parasite–host system, including data on the range of host specificity of the parasite, but also the analysis of the groups composition of different lice species within a host, it is necessary to recognize and describe immature stages. An interesting subject for such studies is *Hoplopleura malabarica* (Kim, 1968), an oligoxenous parasite found exclusively on rodents of the genus *Bandicota*, including *B. bengalensis* (Gray, 1835), *B. savilei* (Thomas, 1916), and *B. indica* (Bechstein, 1800) (Durden & Musser, 1994). Knowledge of and the ability to identify immature stages will allow for future studies on the complete parasite–host relationships of *H. malabarica* and its potential hosts, as well as the determination of possible host preferences, distinguishing between primary and auxiliary hosts. The previously undescribed nymphal stages of *H. malabarica* can be characterized in detail on the basis of morphological features visible on examination using light and scanning microscopy. In addition, the developmental stages of this louse differ morphologically in such a way that they can be uniquely identified.

The aim of the current study was to make for a first time a detailed description of all nymphal stages of *Hoplopleura malabarica* which is typical and probably specific parasite of *Bandicota indica*. Identification of all stages of lice is necessary to determine the total level of infestation (the host's parasite load), as well as to know the life cycle of lice. In addition, data on the global checklist of Anoplura parasitizing rodents from the genus *Bandicota* was prepared.

Materials & Methods

Global checklist of Anoplura parasiting rodents from the genus *Bandicota*

The world checklist of *Hoplopleura* species observed in rodents of the genus *Bandicota* is based on publications from 1923 to 2014 (Ferris, 1923; Werneck, 1954; Johnson, 1959; Durden & Musser, 1994; Cardozo-de-Almeida et al., 1999; Dong et al., 2014), no posterior literature to 2014 is found. The scientific names, common names, geographical range and systematics were based following the Mammal Diversity Database (2023).

Material of sucking lice and host

The study material comprised 12 specimens of the greater bandicoot rat (*Bandicota indica*, Rodentia) from the collections of the Laboratory of Parasitology, Faculty of Pharmacy, University of Barcelona (Spain). The specimens originated from Southeast Asia, specifically from the Vientiane province of Lao PDR.

Bandicota indica is not a protected species and is not listed under CITES. The IUCN Red List (International Union for Conservation of Nature) classifies the greater bandicoot rat as *Least Concern* (LC). Rodents were handled in accordance with the guidelines of the American Society of Mammalogists and the European Union legislation (Directive 86/609/EEC). The trapping campaign was validated by national health authorities as part of a rodent-borne disease survey. Ethical approval for investing rodents for ectoparasitism was granted by the National Ethics Committee for Health Research (NECHR), Ministry of Health of Lao PDR, number 039/2016/NECHR.

The skin of the entire rodents preserved in ethanol were surveyed for the presence of sucking lice under the binocular, all detected individuals were collected with the aid of tweezers and stored in 70% ethyl alcohol. For the morphometrical analyses, individuals were placed in polyvinyl-lactophenol solution (Kadulski & Izdebska, 2006) and posteriorly examined under a light microscope (Olympus CX 40). All measurements in this work are given in millimeters (mm). Sucking lice individuals intended for analysis with scanning electron microscopy were subjected to a series of alcohols (80–100%) and then dried (mix of ethyl alcohol and hexamethyldisilazane - HMDS) in proportions: 1:3, 1:1, and 3:1. Finally, the specimens were transferred to pure HMDS and placed in an incubator for 24 h (37°C) (Murtey et. al., 2016). Next specimens were stuck to double-sided copper tape (by Mierzejewski Materiały Samoprzylepne) and positioned on the table of Field Emission Scanning Electron Microscope JSM—7800F (manufacturer JEOL; stocked in Department of Materials Engineering and Bonding—Faculty of Mechanical Engineering, Gdansk University of Technology, Gdansk, Poland).

Hoplopleura malabarica was the only louse species observed in *Bandicota indica*, eliminating any possibility of misidentification of nymphs.

Only the skins of rodents were submitted for study, and the Anoplura extracted from them, successively deposited.

Three species of *Anoplura* were recorded in all known species of *Bandicota* (one species from the genus *Hoplopleura* and two from *Polyplax*). The distribution of sucking lice is restricted to Asian territories and is closely associated with their hosts. The exception is *Polyplax spinulosa*, a cosmopolitan parasite (Table 1).

Description of nymphal stages

The lice material included 29 specimens of *Hoplopleura malabarica*, consisting of five males, 15 females, two first-instar nymphs, two second-instar nymphs, and five third-instar nymphs. These lice were collected from five of the 12 examined specimens of *B. indica* (Table 1).
Nymph body measurements are provided in Table 3 (in mm).

Nymph I (Fig. 1)

Head

Ventral side—No gular plate. Four AHS (two on each side). Two OS. Four VMHS (two on each side). Two additional bristles near the site of the future VPHS.
Dorsal side—No CS. Four DAHS, evenly spaced in a row. Two PAS on each side. AS and PCHS very faint. OSHS and ISHS present, spaced apart by the length of the bristles. ACHS is present, positioned close together. MMHS is closer to AMHS than PMHS. PDHS is thicker than DPTS, reaching the first thoracic segment. ADHS present.

Thorax

Ventral side—Evenly covered with V-shaped scales; no additional bristles.
Dorsal side—DPTS extends to the beginning of the abdomen. DPtS, DMsS, and DMtS present.

Abdomen

Shaped like an inverted water droplet. Visible undulation where pleural plates will develop. Segmentation is not visible. MAS, AnS, and AcS absent.

Nymph II (Fig. 2)

Head

Ventral side—Four AHS (two on each side). Two OS. Two VMHS on each side. VPHS present.
Dorsal side—Four DAHS (two on each side). PAS, PCHS, ACHS, and AS are visible. ISHS and OSHS are present. All MHS evenly spaced. PDHS extends to the end of the first thoracic segment. ADHS short, positioned above PDHS, extending to its beginning.

Thorax

Ventral side—Evenly covered with V-shaped scales.
Dorsal side—DPTS extends to the first abdominal segment. DMsS present next to the mesothoracic spiracle. DPtS and DMtS are present. The surface is mostly smooth, with sparse, wide U-shaped scales, particularly in the anterior part of the first segment.

Abdomen

Inverted water droplet shape, as in Nymph I, but more tapered at the end (sometimes flat-topped). Segmentation is not visible. The entire abdomen is evenly covered with U-shaped

196 scales, with rare V-shaped scales at the beginning and end of the abdomen. MAS, AnS, and AcS
197 absent.
198
199 Nymph III (Fig. 3)
200 Head
201 Ventral side—Very convex scales present around the antennae, mouth opening, and middle of
202 the head, though sparsely distributed. Four AHS. Four VMHS on each side. Two OS bristles.
203 VPHS measuring ¼ to 1/5 of head length. Numerous smaller (central and lower) and larger
204 (lateral) nodular formations at the site of the future gular plate (GP), some with fine bristles,
205 forming a rhomboid shape with a rounded lower part or an arrow-like pattern.
206 Dorsal side—Haustellum raised higher than the angles of the labrum. Four AHS (two on each
207 side). Two CS. Four evenly spaced DAHS. Two PAS on each side. PCHS, ACHS, and AS
208 present. ISHS and OSHS are spaced 1–1.5 bristle lengths apart. AMHS, MMHS, and PMHS
209 evenly spaced apart. Postanntenal angles are sharp and angular. PDHS extends almost to the
210 femur of the second pair of legs. ADHS present, positioned above PDHS.
211 Thorax
212 Ventral side—Evenly covered with V-shaped scales, with some U-shaped scales interspersed.
213 No additional bristles.
214 Dorsal side—DPTS extends to the beginning of the abdomen. DPtS, DMsS (twice as long as the
215 others), and DMtS present.
216 Abdomen
217 Barrel-shaped, rectangular from a dorsal view, tapering only at the very bottom. Segmentation is
218 not visible. The entire abdomen is evenly covered with U-shaped scales, transitioning to V-
219 shaped scales in the middle. MAS, AnS, and AcS absent.
220

221 Discussion

222 According our findings and the literature review, *Hoplopleura malabarica* appears to be a highly
223 specific parasite, observed exclusively in rodents of the genus *Bandicota*. It is likely
224 monoxenous to this genus, which is represented worldwide by only three rodent species
225 (Mammal Diversity Database, 2023).
226 Among species of *Hoplopleura* parasitizing *Bandicota indica*, *H. malabarica* is the only reported
227 species, minimizing the possibility of misidentification. In contrast, two species of the genus
228 *Polyplax*—*P. asiatica* and *P. spinulosa*—have been observed in the greater bandicoot rat. The
229 morphology of immature stages of *Hoplopleura* and *Polyplax* differ significantly, facilitating
230 accurate identification.
231 Considering all *Hoplopleura* species observed in neighboring countries of Lao PDR within the
232 Indomalayan realm, the following species have been recorded (Johnson, 1959, 1964; Durden &
233 Musser, 1994; Kazim et al., 2022):
234 • *H. captiosa* Johnson, 1960 on *Mus caroli* Bonhote, 1902;
235 • *H. diaphora* Johnson, 1964 on *Berylmys bowersi* (Anderson, 1879);

- *H. dissicula* Johnson, 1964 on *Leopoldamys sabanus* (Thomas, 1887), *Maxomys whiteheadi* (Thomas, 1894), *Niviventer cremoriventer* (Miller, 1900), *Rattus argentiventer* (Robinson and Kloss, 1916), *R. baluensis* (Thomas, 1894), *R, rattus* (Linnaeus, 1758), *Sundamys muelleri* (Jentink, 1879) and *S. infraluteus* (Thomas, 1888);
- *H. kitti* Kim, 1968 on *Berylmys bowersi*;
- *H. malaysiana* Ferris, 1921 on *Leopoldamys sabanus* and *Sundamys muelleri*;
- *H. pacifica* Ewing, 1924 on *Rattus argentiventer*, *R. exulans* (Peale, 1848), *R. norvegicus* (Berkenhout, 1769), *R. rattus* and *R. tiomanicus* (Miller, 1900);
- *H. pectinata* (Cummings, 1913) on *Maxomys alticola* (Thomas, 1888), *M. rajah* (Thomas, 1894), *M. surifer* (Miller, 1900), *M. whiteheadi*, *Niviventer cremoriventer*, *N. niviventer* (Hodgson, 1836), *N. rapit* (Bonhote, 1903);
- *H. rajah* Johnson, 1972 on *Maxomys surifer*;
- *H. sicata* Johnson, 1964 on *Niviventer cremoriventer*.

The described first-instar nymphs of *Hoplopleura malabarica* can be morphologically differentiated from previous species reported in the Indomalayan realm. The presence of MAS in *H. captiosa*, *H. diaphora*, *H. dissicula*, *H. kitti*, *H. pacifica*, *H. pectinata*, and *H. sicata*. differs of *H. malabarica* that lacks of this structure. Third-instar nymphs of *H. malabarica* do not have tergal plaques, which are present in *H. pacifica*, *H. pectinata*, *H. sicata*, and *H. rajah* nymphs. The same applies to second-instar nymphs of *H. dissicula* and *H. sicata*. Additionally, MAS are present in all nymphal instars of *H. diaphora* but are absent in *H. malabarica*. Second-instar and third-instar nymphs of *H. kitti* have AnS and some dorso-central abdominal setae (DCAS), which are not found in *H. malabarica*. Second-instar *H. pacifica* also has AnS. No descriptions of *H. malaysiana* nymphs are available (Kim, 1966, 1968; Johnson, 1972).

In their work, Adhikary and Ghosh (1994) describe the nymphs of *H. malabarica*, but the taxonomical identity have to be taken with caution, as is based on mass material, as many as 56 species of lice from a large area (India) are described. However, the description of *H. malabarica* and provided diagrams, indicate that the nymphs they examined do not belong to the above species. The description is quite laconic, while the diagrams lack elements important in identification (no scale, lack of most bristles, MAS bristles in questionable quantity).

As a conclusion, our study contributes to a better understanding of the genus *Hoplopleura*, providing morphological data that can be used to differentiate between the known species of this group of sucking lice.

Conclusions

Using scanning microscopy ~~methods~~, the ~~world's~~ first descriptions of the nymphal stages of the *Hoplopleura malabarica* lice have been ~~created~~ preserved. The analysis of occurrence shows that it is a ~~very~~ rare parasite, whose range of occurrence is limited to southern Asia (India, Sri Lanka, Thailand). *H. malabarica* is also a specific species of sucking lice that parasitizes only bandicoot rats (*Bandicota indica* - greater Bandicoot Rat, *Bandicota bengalensis* - lesser Bandicoot Rat,

1 **Table 1 *Hoplopleura* species parasitizing rodents of the genus *Bandicota* with their known geographic distributions.**

Host	Host distribution	Anoplura species	Parasite distribution	Author
Bandicota indica (Bechstein, 1800) greater Bandicoot Rat	Bangladesh, Cambodia, China, India, Lao PDR, Myanmar, Nepal, Sri Lanka, Thailand, Vietnam and introduced in Malaysia	*Hoplopleura malabarica*	India, Sri Lanka, Thailand	Werneck, 1954; Johnson, 1959; Durden & Musser, 1994; Cardozo-de-Almeida, et al. 1999
		Polyplax asiatica	China, Egypt, India, Iran, Myanmar, Pakistan, Taiwan, Tajikistan, Thailand	Ferris, 1923; Durden & Musser, 1994; Dong et al., 2014
Bandicota bengalensis (Gray, 1835) lesser Bandicoot Rat	Bangladesh, India, Myanmar, Pakistan, Sri Lanka and introduced in Indonesia and Malaysia	*Hoplopleura malabarica*	India, Sri Lanka, Thailand	Durden & Musser, 1994
		Polyplax spinulosa	cosmopolitan	Durden & Musser, 1994
		Polyplax asiatica	China, Egypt, India, Iran, Myanmar, Pakistan, Taiwan, Tajikistan, Thailand	Durden & Musser, 1994
Bandicota savilei Thomas, 1916 Savile's Bandicoot Rat	restricted to Cambodia, Lao PDR, Myanmar, Thailand, Vietnam	*Hoplopleura malabarica*	India, Sri Lanka, Thailand	Johnson, 1959; Durden & Musser, 1994

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Table 1 Details of examined rodent ~~materials~~ and ~~collection~~ numbers of ~~found~~ *Hoplopleura malabarica* from Vientiane province in Lao PDR.

Bandicota indica collection numbers and sex	Date of collection	Location		*Hoplopleura malabarica* collection numbers
		District	GPS data	
L0499 ♂	12.05.2015	Phonehong	18°17'01.0"N 102°30'45.6"E	
L0507 ♀	13.05.2015	Phonehong	18°18'30.7"N 102°30'52.2"E	4 Females (UGDIZPMBindAHHmal1f; UGDIZPMBindAHHmal2f; UGDIZPMBindAHHmal3f; UGDIZPMBindAHHmal4f)
L0516 ♂	13.05.15	Thulakom	18°18'30.7"N 102°30'52.2"E	1 Male (UGDIZPMBindAHHmal1m)
L0517 ♀	13.05.15	Thulakom	18°18'30.7"N 102°30'52.2"E	
L0518 ♀	13.05.15	Thulakom	18°18'30.7"N 102°30'52.2"E	
L0519 ♂	13.05.15	Thulakom	18°18'30.7"N 102°30'52.2"E	
L0522 ♂	14.05.2015	Phonehong	18°21'21.0"N 102°27'21.2"E	1 Female (UGDIZPMBindAHHmal5f); 2 Nymphs third instar (UGDIZPMBindAHHmal2N3; UGDIZPMBindAHHmal3N3)
L0527 ♂	14.05.2015	Thulakom	18°18'30.7"N 102°30'52.2"E	
L0528 ♀	14.05.2015	Thulakom	18°18'30.7"N 102°30'52.2"E	2 Females (UGDIZPMBindAHHmal14f; UGDIZPMBindAHHmal15f).
L0529 ♂	15.05.2015	Phonehong	18°21'21.0"N 102°27'21.2"E	
L0553 ♀	17.05.2015	Thulakom	18°17'31.2"N 102°33'35.5"E	
L0556 ♂	18.05.201	Thulakom	18°17'31.2"N	2 Nymphs first instar (UGDIZPMBindAHHmal1N1;

Table 1. Measurements of *Hoplopleura malabarica* nymphal body.

		nymphs first instar			nymphs second instar			nymphs third instar		
		average	max	min	average	max	min	average	max	min
length	head	0,20	0,21	0,18	0,16	0,16	0,15	0,16	0,18	0,15
	thorax	0,13	0,14	0,11	0,17	0,17	0,16	0,15	0,16	0,12
	abdomen	0,56	0,60	0,51	0,49	0,55	0,42	0,71	0,74	0,69
	total	0,88	0,95	0,80	0,81	0,86	0,75	1,02	1,06	0,98
width	head	0,17	0,18	0,15	0,16	0,17	0,14	0,16	0,17	0,15
	thorax	0,31	0,31	0,30	0,28	0,29	0,26	0,32	0,36	0,27
	abdomen	0,50	0,52	0,48	0,42	0,48	0,36	0,66	0,76	0,56