Journal of Natural History
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713192031

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Online Publication Date: 01 June 1994
To cite this Article: Katakura, H., Nakano, S., Hosogai, T. and Kahono, S. (1994) 'Female internal reproductive organs, modes of sperm transfer, and phylogeny of Asian Epilachninae (Coleoptera: Coccinellidae)', Journal of Natural History, 28:3, 577 - 583
To link to this article: DOI: 10.1080/00222939400770261
URL: http://dx.doi.org/10.1080/00222939400770261

JOURNAL OF NATURAL HISTORY, 1994, **28**, 577–583

Female internal reproductive organs, modes of sperm transfer, and phylogeny of Asian Epilachninae (Coleoptera: Coccinellidae)

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(*Accepted 21 June 1993*)

A total of 22 Indonesian and Japanese species of epilachnine ladybird beetles were divided into the following three groups on the basis of the conditions of bursa copulatrix and spermatheca, and modes of sperm transfer. Group 1, '*Henosepilachna*', being characterized by a spermatheca derived from the lateral side of a well developed bursa copulatrix and formation of a 'spermatophore' prior to ejaculation; group 2, *Epilachna admirabilis* species-group and *E. chapini* group, by a spermatheca derived from terminal end of a well developed bursa copulatrix and lack of spermatophore formation; and group 3, *E. flavicollis* group and *E. fallax* group, by a reduced bursa copulatrix with or without a terminally positioned spermatheca and lack of spermatophore formation. It was suggested that groups 2 and 3 are phylogenetically more closely related to each other than they are to group 1.

KEYWORDS: Reproductive organ, female, bursa copulatrix, spermatheca, sperm transfer, Epilachninae, Indonesia, Japan, supraspecific classification, phylogeny.

Introduction

Generic classification of Asian and African species of ladybird beetles belonging to the subfamily Epilachninae (Coleoptera, Coccinellidae) is controversial. Until recently, the majority of these species were assigned to two large genera, *Epilachna* Dejean (*sens.* Li and Cook, 1961) and *Henosepilachna* Li. The former is chiefly characterized by toothless tarsal claws and the sixth abdominal sternite of females being not split, whereas the latter by toothed tarsal claws and divided sixth sternite. However, Iablokoff-Khnzorian (1980) and Richards (1983) synonymized *Henosepilachna* with *Epilachna*, regarding the characters that were said to separate these two groups as not reliable. Some agreed with their synonymy (Katakura *et al.*, 1988) while others still treated them as two separate genera (Fürsch, 1990, 1991). In order to establish a satisfactory classification system, it seems necessary to introduce new characters into the taxonomic practice of epilachnines.

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0022–2933/94 $10·00

Table 1. A list of epilachnine ladybird beetles treated in the present study.

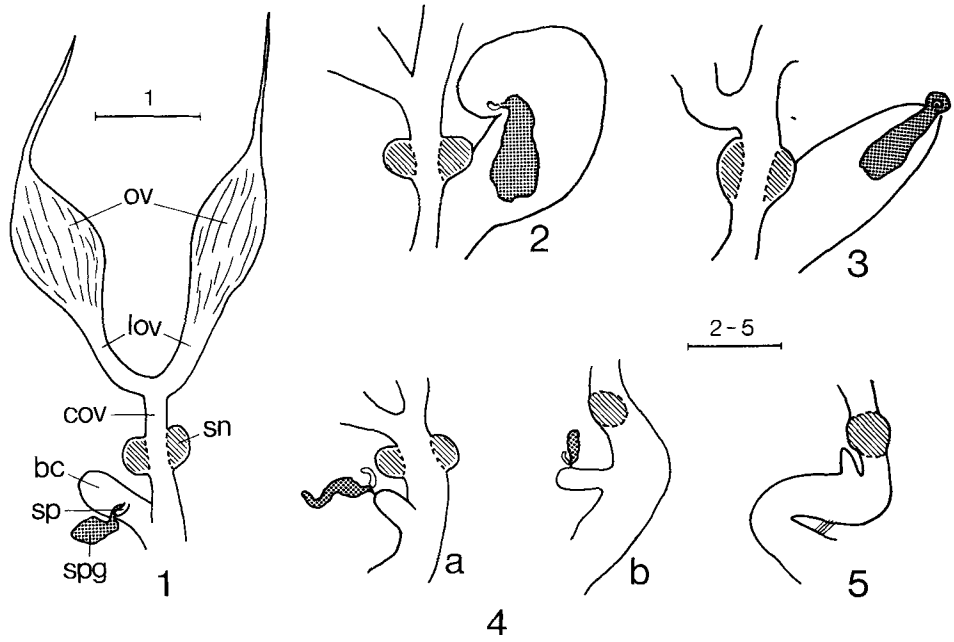
	Species	Species group	Source of materials
1	*E. vigintioctomaculata* Motschulsky	'*Henosepilachna*'	Japan
2	*E. vigintioctopunctata* (Fabricius)	'*Henosepilachna*'	Java, Sumatra
3	*E. dodecastigma* (Wiedemann)	'*Henosepilachna*'	Java, Sumatra
4	*E. septima* Dieke	'*Henosepilachna*'	Java, Sumatra
5	*E. bifasciata* (Fabricius)	'*Henosepilachna*'	Java
6	*E. sumatrensis* Fürsch	'*Henosepilachna*'	Sumatra
7	*E. pytho* Mulsant	'*Henosepilachna*'	Sumatra
8	*E. boisduvali* Mulsant	'*Henosepilachna*'	Japan
9	*E.* sp. 3	'*Henosepilachna*'	Java, Sumatra
10	*E.* sp. 4	'*Henosepilachna*'	Sumatra
11	*E.* sp. 5	'*Henosepilachna*'	Java
12	*E. enneasticta* Mulsant	'*Henosepilachna*'	Java, Sumatra
13	*E. admirabilis* Crotch	*admirabilis* group	Japan
14	*E.* sp. H (aff. *grayi*)	*admirabilis* group	Java, Sumatra
15	*E.* sp. F	*admirabilis* group	Java
16	*E. decipiens* Crotch	*chapini* group	Java
17	*E.* sp. G	*chapini* group	Java
18	*E. orthofasciata* Dieke	*flavicollis* group	Java, Sumatra
19	*E.* sp. K	*flavicollis* group	Java
20	*E. gedeensis* Dieke	*fallax* group	Java
21	*E. incauta* Mulsant	*fallax* group	Java, Sumatra
22	*E.* sp. C	unsettled	Sumatra

In the present paper, we will show that female internal reproductive organs and modes of sperm transfer provide valuable information for the supraspecific classification of epilachnine beetles. We will first describe three types of female reproductive systems recognized in certain Indonesian and Japanese epilachnines, together with the mode of sperm transfer confirmed or suggested for each type. Then we will present possible phylogenetic relationships between several groups of Asian epilachnines on the basis of these findings.

Materials and methods

A total of 22 species are treated in the present paper (Table 1). Beetles were fixed in Kahle's solution (formalin 6, 95% ethanol 17, acetic acid 2, water 28) or FAA solution (formalin 20, 95% ethanol 80, acetic acid 10) and preserved in 70% ethanol. These fixed beetles were later dissected and their internal reproductive organs examined. Live females of some species were also dissected for examination. In addition, mating duration and sperm transfer were examined in the laboratory for several representative species. Observations were made under a binocular microscope with 6–50× magnifications. Drawings were made under the binocular microscope with the aid of a drawing apparatus.

Since our taxonomic study of the Indonesian species is still in progress, about half of them, possibly including some new species, are referred to by species-specific code numbers or code letters, which will be used in our papers until their exact taxonomic status is determined. Brief diagnoses of these Indonesian species, including figures of pronotal and elytral spot patterns and genitalia of both sexes, have been provided by Katakura *et al.* (unpublished data) and are available upon request to HK. All voucher specimens are now deposited in the Zoological Museum, Faculty of Science, Hokkaido University, and a part of them will eventually be deposited in Bogor



FIGS 1–5. Female internal reproductive systems of various *Epilachna* species. 1, *E. viginti-octomaculata* (*'Henosepilachna'*; redrawn from Katakura, 1981); 2, *E. enneasticta* (*'Henosepilachna'*); 3, *E.* sp. G (*E. chapini* group); 4a, *E. incauta*; 4b, *E. gedeensis* (*E. fallax* group); 5, *E.* sp. K (*E. flavicollis* group). 1–3, ventral view; 4a, dorsal view; 4b, 5, lateral view (dorsal side left). Ov, ovary; lov, lateral oviduct; cov, common oviduct; sn, seminal node; bc, bursa copulatrix; sp, spermatheca; spg, spermathecal gland. Bar scale = 1 mm.

Zoological Museum. Moreover, to avoid causing taxonomic problems by yielding new combinations, all the species are tentatively placed in *Epilachna* (*sens.* Richards, 1983) in the present paper. But the species belonging to *Henosepilachna* (*sens.* Li and Cook, 1961) are indicated as *'Henosepilachna'* if necessary. The species other than *'Henosepilachna'* (= *Epilachna sens.* Li and Cook) are further assigned to the species groups defined by Dieke (1947) on the basis of genitalic and other morphological characters.

Results

Female internal reproductive system

The female internal reproductive system of epilachnine beetles consists of a pair of ovaries, a pair of lateral oviducts, a common oviduct with lateral swelling (henceforth 'seminal node'), and a bursa copulatrix from which a vestigial spermatheca and a spermathecal gland are derived (Ehara, 1952; Katakura, 1981; Fig. 1). We found the following three types that differ in the size of bursa copulatrix and position of spermatheca (Figs 1–5).

Type 1 (Figs 1 and 2). The bursa copulatrix is columnar or somewhat spherical in shape, and the spermatheca and the spermathecal gland are derived from the lateral side of the bursa.

All the species of *'Henosepilachna'* so far studied by us have this type of female reproductive system (Table 1). The bursa copulatrix of *E. enneasticta*, belonging to

the *enneasticta* group within '*Henosepilachna*' (Dieke, 1947), slightly differ from those of other '*Henosepilachna*' species in its large size and folded apical portion (Fig. 2).

Type 2 (Fig. 3). Very similar to type 1, but the bursa copulatrix is larger, somewhat elongate in shape, and often tapering apically. The spermatheca is situated at the terminal end of the bursa.

This type is found in the members of the *Epilachna admirabilis* group and the *E. chapini* group, and also in *Epilachna* sp. C whose taxonomic position is as yet unsettled.

Type 3 (Figs 4 and 5). The female reproductive system virtually lacks functional bursa copulatrix. The bursa copulatrix is reduced to a small process attached to the common oviduct. The spermatheca and spermathecal gland are absent or very vestigial. The following two subtypes are further discriminated.

Type 3a (Fig. 4). Bursa copulatrix is small. Spermatheca and spermathecal gland present. Common oviduct below the seminal node is thick and straight. Found in the members of the *E. fallax* group.

Type 3b (Fig. 5). Bursa copulatrix is very small and vestigial. Spermatheca and spermathecal gland appear to be absent. Common oviduct below the seminal node is curved in a 'U' shape, the base and median part being connected by musculature. Found in the members of *E. flavicollis* group.

Mode of sperm transfer

The above three types also differ in the mode of sperm transfer between sexes as shown below, though evidence is as yet limited.

Type 1. Sperm transfer of this type was examined in detail for the Japanese species, *E. vigintioctomaculata* (Katakura, 1985). At copulation, the male first makes a gelatinous 'spermatophore' within the bursa copulatrix, and then ejaculates sperm into the bursa. The sperm then move to the seminal node via the common oviduct and eventually they are preserved there. The gelatinous 'spermatophore' seemed to be digested later within the bursa. Copulation usually lasts for about 1 to 2 hours in *E. vigintioctomaculata*, of which first 45 min or more were spent in making spermatophore. Mating durations so far examined by us for *E.* sp. 3, *E. vigintioctopunctata*, *E. dodecastigma*, *E. septima*, *E. boisduvali* and *E. enneasticta* were usually between 40 min and 2·5 h, though the durations seemed different according to the species or physiological condition of the females concerned.

Type 2. Sperm transfer of this type was studied only for Japanese *E. admirabilis*. In this species, no gelatinous 'spermatophore' is made prior to ejaculation. Sperm are directly emitted in the bursa and then move to the seminal node. Copulation lasts about 30 min ($n = 6$, 27–32 min). Additional observations were made on matings of two field caught females of Javanese *E.* sp. H under laboratory conditions. Their mating durations were very short, being 6 and 10 min, respectively. Like *E. admirabilis*, the two females had sperm but lacked spermatophores in their bursa copulatrix. However, it is uncertain whether the observed matings were successful or not, since the preserved sperm might have come from earlier matings made before we collected the females.

Type 3. Copulation and subsequent sperm transfer of this type were studied with field collected adults of *E. incauta* (type 3a) and *E.* sp. K (type 3b). Sperm were directly ejaculated in the common oviduct and were eventually preserved in the

Table 2. Three groups of east Asian epilachnines discriminated on the basis of female internal reproductive systems and modes of sperm transfer. Supposed ancestral ([a]) and derived ([d]) conditions of bursa copulatrix, position of spermatheca and spermatophore formation are also given.

	Group 1	Group 2	Group 3
Species group involved†	'*Henosepilachna*'	*admirabilis* group *chapini* group *Epilachna* sp. C	*flavicollis* group *fallax* group
Internal reproductive system of females	Type 1	Type 2	Type 3
Bursa copulatrix	Functional[a]	Functional[a]	Reduced[d]
Position of spermatheca	Lateral[d]	Terminal[a]	Terminal[a]
Spermatophore formation	+ [a]	− [d]	− [d]

† Species group of *Epilachna* sp. C is not yet determined.

seminal node. No spermatophore was detected. Copulation lasted a very long time, about 10 h in *E. incauta* (n = 1), and from *c.* 3–7 h in *E.* sp. K (n = 7).

Discussion

Dobzhansky (1924, 1926) was the first to use the female internal reproductive organs for the classification of coccinellids. He noticed that the female internal reproductive system of epilachnines is unique among coccinellids in having a vestigial spermatheca and a pair of lateral swellings at the common oviduct. The lateral swellings were later proved to be a specialized sperm storage organ of female epilachnines; their vestigial spermatheca do not function as the sperm reservoir (Ali, 1979; Katakura, 1981). Katakura (1981) pointed out that the specialized sperm reservoir and reduced functionless spermatheca are derived characters shared by all the members of the subfamily Epilachninae. The present study indicates that female internal reproductive organs and associated characters are also useful for the supraspecific classification of epilachnines.

As shown above, Indonesian and Japanese epilachnines are divided into three groups on the basis of female internal reproductive systems and modes of sperm transfer (Table 2): group 1, '*Henosepilachna*'; group 2, *E. admirabilis* group, *E. chapini* group and *Epilachna* sp. C; group 3, *E. flavicollis* group and *E. fallax* group. We also summarize in Table 2 possible ancestral and derived conditions of the three types of female reproductive systems and sperm transfer, assuming that spermatophore formation, possession of distinct (functional) bursa copulatrix, and terminally positioned spermatheca are ancestral. These assumptions are based on the fact that the first condition is met in some higher coccinellids (*Chilocorus* [Fisher, 1959], *Harmonia* [Obata and Hidaka, 1987]), and the latter two in most groups of higher coccinellids other than epilachnines (Dobzhansky, 1926).

A possible cladogram constructed from Table 2 (Fig. 6) shows that groups 2 and 3 (= *Epilachna sens.* Li and Cook, 1961) are more closely related phylogenetically than they are to group 1 (= '*Henosepilachna*'). Although the suggested phylogenetic relationship must be tested by further critical investigations, we believe that in any future studies dealing with the taxonomy and phylogeny of epilachnines close attention should be paid to the morphology of female reproductive organs and associated characters.

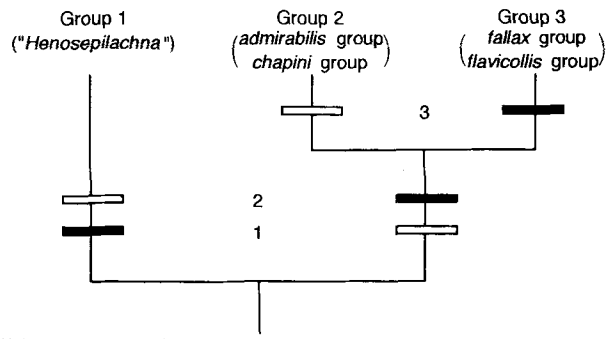


FIG. 6. A possible phylogenetic relationship between the three groups of east Asian epilachnines discriminated by the difference in the female internal reproductive organs and modes of sperm transfer (constructed from Table 1). *Epilachna* sp. C, whose taxonomic position is not yet settled, is excluded. 1, position of spermatheca; 2, spermatophore formation; 3, bursa copulatrix. Open symbol, ancestral condition; solid symbol, derived condition.

Acknowledgements

This study is an outcome of a cooperative study by Japanese and Indonesian entomologists on evolutionary biology and ecology of Indonesian epilachnines. We thank Dr K. Nakamura (Kanazawa University), Dr S. Wiryoatmodjo (Director of the Center for Research and Development in Biology, Indonesian Institute of Science [LIPI]) and Dr J. Kamil (Rector of Andalas University, Padang) who make our joint study possible. We also thank Dr K. Nakamura (Kanazawa University), Mr M. Amir, Mrs W. A. Noerdjito (Bogor Zoological Museum), Dr I. Abbas (Andalas University, Padang) and all other members of our joint study for their kind help in various ways. S.N. wishes to express his thanks to Professor Emeritus S. Kawamura (Kyoto University), Dr A. Baker (Andalas University) and INPEX Foundation for their generous support during his stay in Indonesia. This study was supported in part by the Grant in Aid for Overseas Scientific Survey from Ministry of Education, Science and Culture of Japan (No. 02041033).

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