

A NEW MARINE TRICLAD ECTOPARASITIC ON MALAYSIAN AND INDONESIAN HORSESHOE CRABS (PLATYHELMINTHES, TURBELLARIA, TRICLADIDA)

by

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SUMMARY

A new species of marine triclad, *Ectoplana undata* n. sp., ectoparasitic on the horseshoe crab *Tachypleus gigas*, is described. Cocoons of triclads were found on *T. gigas* as well as on the horseshoe crab *Carcinoscorpius rotundicauda*; since from the latter species no triclads were collected, it remains unknown whether these cocoons are those of *E. undata* or of another species. The genus *Ectoplana*, though poorly defined, is here retained, for stated reasons, pending a revision of the Procerodidae.

RÉSUMÉ

On décrit une nouvelle espèce de Triclades marins (*Ectoplana undata* n. sp.), ectoparasite du Xyphosoure *Tachypleus gigas*. Des cocons de Triclades ont été trouvés aussi bien sur *T. gigas* que sur le Xyphosoure *Carcinoscorpius rotundicauda*; puisqu'on n'a pas trouvé des Triclades sur cette dernière espèce, la question de savoir si ces cocons sont ceux de *E. undata* ou bien d'une autre espèce, reste sans réponse. Le genre *Ectoplana* est retenu, en dépit du fait qu'il est imparfaitement défini, en attendant une révision des Procerodidae; les raisons de cette initiative sont expliquées.

INTRODUCTION

Marine triclads are known to live ectoparasitically on the American Horseshoe Crab *Limulus polyphemus* (Linnaeus, 1758) and the Chinese Horseshoe Crab *Tachypleus tridentatus* (Leach, 1819). These planarians live on the gills and legs of the crabs and profit from the food particles left over from the meals of the latter.

From *L. polyphemus* no less than four species of triclads have been described (cf. Wilhelmi, 1909). All these belong to the family Bdellouridae, which also contains free-living members. A different form of *Bdelloura candida*

or, perhaps, even a fifth species, has been found on an American Horseshoe Crab from North Carolina (Sluys & Ball, unpublished manuscript). Ijima & Kaburaki (1916) described the marine triclad *Procerodes limuli* which had been obtained from specimens of the Chinese Horseshoe Crab. One year later, Kaburaki (1917) transferred the species to the genus *Ectoplana* which is considered to belong to the Procerodidae and to constitute the only member of the subfamily Ectoplaninae (cf. Bresslau, 1933). Some years later Kaburaki (1922) provided a more detailed description of *Ectoplana limuli*.

In this paper a new species of triclad is described, of which the specimens were obtained from a species of horseshoe crab other than the two already mentioned above. In assessing the generic status of this species of triclad it will be necessary to examine critically the defining characters of *Ectoplana*, as described in the literature. Planarian cocoons which were found on specimens of still another species of horseshoe crab from Malaysian and Indonesian regions will also be described.

MATERIAL AND METHODS

The Zoological Museum in Amsterdam (ZMA) houses a small alcohol collection of horseshoe crabs in which three species are represented, viz. *Limulus polyphemus*, *Carcinoscorpius rotundicauda* (Latreille, 1817), and *Tachypleus gigas* (Müller, 1785). Specimens of the last two species were searched for marine triclads and/or their cocoons. These horseshoe crabs were

either collected and preserved immediately, or shipped to the Aquarium of Artis Zoo in Amsterdam (NAM), where they lived for several years before they died and were deposited in the collection of the museum. Horseshoe crab specimens from which cocoons and/or planarians were collected, are listed below with their sample locality, date of collection, and collector.

ZMA Xi.1007, *Tachypleus gigas* (mistakenly identified as *Carcinoscorpius rotundicauda*), Singapore, date of collection unknown, died in Aquarium NAM 7.09.1965: one triclad and cocoons.

ZMA no collection number, *T. gigas*, Belawan, Deli, Sumatra, 1931, leg. Capt. G. J. Terwiel, died in aquarium at unknown date: two triclads and cocoons.

ZMA no collection number, *T. gigas*, Belawan, Deli, Sumatra, 1931, leg. Capt. G. J. Terwiel, died in Aquarium NAM at unknown date: cocoons.

ZMA *ibid.*: cocoons.

ZMA *ibid.*: cocoons.

ZMA Xi.1004, 2 specimens of *T. gigas*, Java Sea, 1925, leg. Capt. G. J. Terwiel: cocoons.

ZMA Xi.1002, *T. gigas*, Java Sea at Pekalongan, Java, 1927: cocoons.

ZMA Xi.1001, *T. gigas*, east coast of Sumatra, no further information: cocoons.

ZMA Xi.1003, *Carcinoscorpius rotundicauda*, Sabang, Sumatra, no date of collection, leg. G. Herman: cocoons (there is a town Sabang on Celebes as well as on a small island N. of Sumatra, but according to Van Benthem Jutting (1939) G. Herman was stationed on Sabang, Sumatra).

ZMA Xi.1006, 3 specimens of *C. rotundicauda*, Singapore, died in Aquarium NAM in October 1964: cocoons from the gills of all three crabs.

SYSTEMATIC SECTION

TRICLADIDA MARICOLA

Genus *Ectoplana* Kaburaki, 1917

Ectoplana undata n. sp.

Holotype: ZMA V.Pl.603: sagittal sections on 2 slides, stained in Mallory-Heidenhain; specimen collected from *Tachypleus gigas* from Singapore (ZMA Xi.1007).

Paratypes: ZMA V.Pl.604.1: sagittal sections on 2 slides, stained in Mallory-Heidenhain; specimen collected from *T. gigas* from Deli, Sumatra; ZMA V.Pl.604.2: sagittal sections on 1 slide, stained in Mallory-Heidenhain; animal collected from same specimen of *T. gigas* as paratype ZMA V.Pl.604.1.

All sections were made at intervals of 8 μ m.

Etymology.— The specific epithet *undata* (from the Latin *unda*, wave) refers to the wavy course of the distal portion of the male atrium.

External features (fig. 1).— The holotype measured about 2.5 mm in length and 1.25 mm in diameter. The body is elongate-oval-shaped, whereas front and hind end are rounded. The small eyes lie at a considerable distance from the front end and are close together. Both paratypes were very much shrunken, due to contraction, and of semi-circular shape. The animals are white, due to lack of pigment; ovaries, testicular follicles, intestinal rami, part of the copulatory apparatus and ventral nerve cords are visible through the body wall.

Epidermis and dermal musculature.— Dorsal and ventral epithelium measure about 4.8 μ m in thickness; both are packed with rhabdites. Ventral and dorsal body surface are covered with numerous, well-developed cilia which have about the same height as the epidermal cells. The basement membrane is well developed and conspicuous. Dorsally and ventrally it is about 2.4 μ m in diameter. The dermal musculature is well developed. Immediately beneath the basement membrane there is a layer of circular muscles, consisting of two rows of fibres. Interiorly of this layer there is a layer of longitudinal muscles which measures dorsally about 7.2 μ m in diameter and ventrally about 12 μ m.

Alimentary system.— In the holotype the pharynx measures between one-fifth and one-sixth of the body-length. It is constructed as follows: outer ciliated epithelium of about 2.4 μ m, outer longitudinal muscle layer of 2.5 μ m, outer circular muscle layer of 4.8 μ m, parenchymatous zone of 49 μ m, inner longitudinal muscle layer of 4.8 μ m, inner circular muscle layer of 24 μ m, inner ciliated epithelium of 2.4 μ m.

The anterior ramus of the intestine does not extend in front of the eyes, but terminates behind the brain. It gives off about 6 or 7 pairs of lateral diverticula. Both posterior rami are

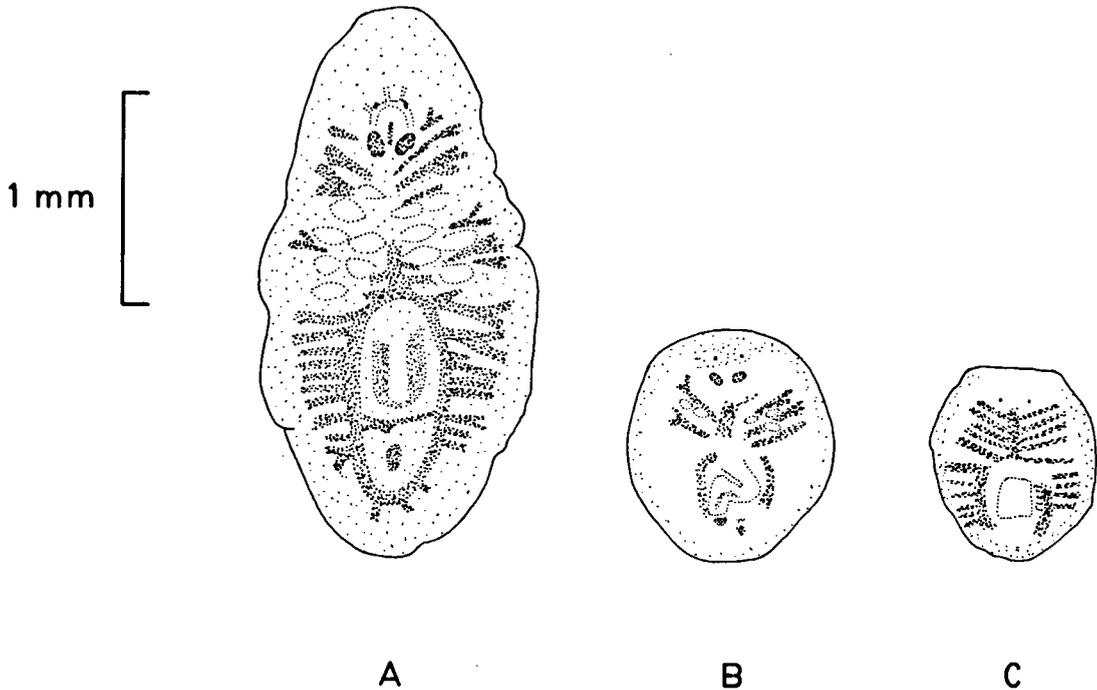


Fig. 1. Dorsal view of preserved specimens of *Ectoplana undata* n. sp. A: holotype, B: paratype ZMA V.Pl.604.1, C paratype ZMA V.Pl.604.2.

confluent at the hind end of the body. This was difficult to observe in the holotype, whereas it could not be seen in the paratypes. Commissures between the posterior rami may be present. The mouth opening is situated at the hind end of the pharyngeal pocket.

Male reproductive system (figs. 2, 3).— The testicular follicles are few in number: about 6-8 on either side of the body. They occur more or less in pairs between the intestinal branches (see fig. 1A). In dorsal view the follicles are oval-shaped, whereas in sagittal sections they appear as semi-circular or oval-shaped bodies. The follicles are large and occupy most of the space between dorsal and ventral body surface. They extend from behind the ovaries up to the root of the pharynx.

In the pharyngeal region the vasa deferentia enlarge to form false seminal vesicles. At first these run laterally of the ventral nerve cords, but at the level of the mouth opening they turn medially as well as dorsally and narrow before penetrating the penis bulb. Just after entering

the bulb, the vasa deferentia open into a large, rounded seminal vesicle. From the distal surface of this vesicle a narrow ejaculatory duct arises, which opens into the blunt tip of the penis papilla. The seminal vesicle is lined with a cuboidal, nucleate epithelium and is surrounded by a layer of circular muscle fibres.

The penis consists of a small bulb and a very short and blunt papilla which has a disposition more or less parallel to the body surface. The nature of the lining epithelium of the papilla could not be discerned and neither nuclei nor subepithelial muscles were seen. A fine granular secretion could be discerned in the papilla, but the exact location of the glandular cells remained obscure. The musculature of the penis bulb is only weakly developed.

The penis papilla projects into a rather spacious dorsal portion of the male atrium which narrows considerably into a narrow distal part that opens into the common atrium. This narrow distal portion of the male atrium is rather long and has a more or less undulated, wavy course. In paratype ZMA V.Pl.604.1 the

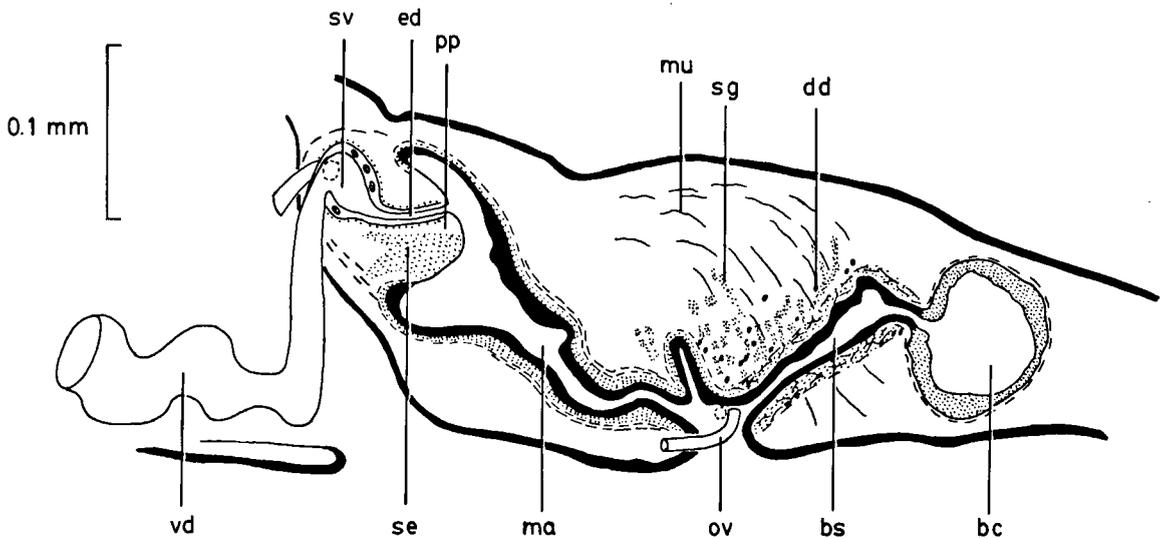


Fig. 2. Sagittal reconstruction of the copulatory apparatus of the holotype. Abbreviations: bc: copulatory bursa, bs: bursal canal, dd: densely staining dots, ed: ejaculatory duct, ma: male atrium, mu: muscle fibres, ov: oviduct, pp: penis papilla, se: secretion, sg: shell glands, sv: seminal vesicle, vd: vas deferens.

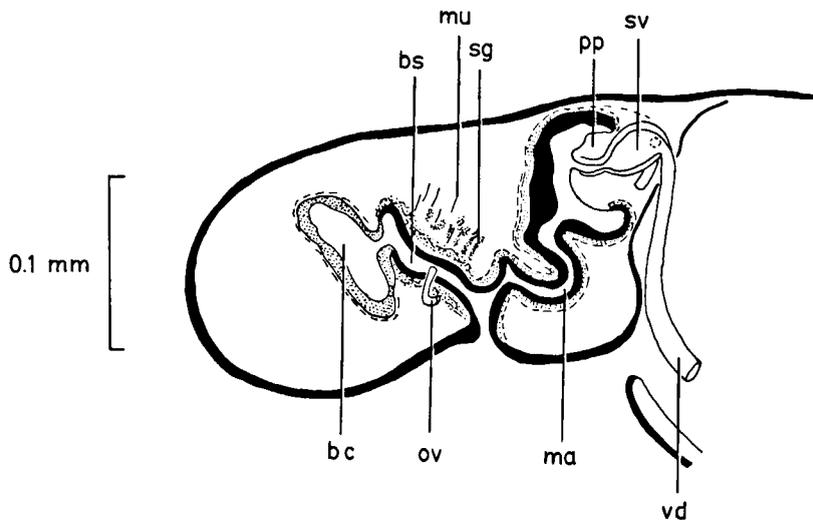


Fig. 3. Sagittal reconstruction of the copulatory apparatus of paratype ZMA V.Pl.604.1. Abbreviations as in fig. 2.

male atrium shows a very pronounced bend (fig. 3), whereas in the holotype and paratype ZMA V.Pl.604.2 the bending is much smoother. The lining epithelium of this narrow distal portion of the male atrium shows no nuclei. In this respect it differs from the proximal, more spacious, part of the male atrium which shows a nucleate epithelium. In the holotype and paratype ZMA V.Pl.604.1 the

very distal part of the male atrium shows a narrow, dorsally directed fold. In paratype ZMA V.Pl.604.2 this fold was not as conspicuous as in the two other type-specimens. The male atrium is surrounded by a layer of circular muscles, consisting of several rows of muscle fibres, with entally to this layer, a zone of longitudinal muscles, also consisting of several rows of fibres. The thickness of both layers

diminishes on the proximal portion of the atrium.

Female reproductive system (figs. 2, 3).— The paired, oval-shaped ovaries are situated directly behind the brain. In the holotype their size is about $101 \times 72 \mu\text{m}$, and in the paratypes ZMA V.Pl.604.1 and ZMA V.Pl.604.2 $74.5 \times 48 \mu\text{m}$ and $31 \times 17 \mu\text{m}$, respectively. In none of the type-specimens I was able to discover a trace of oviducts in the anterior part of the body. Therefore, I am unable to describe in what way the oviducts arise from the ovaries. At the level of the gonopore the oviducts bend medially and open separately into the distal part of the bursal canal. In the holotype the oviducts open into the very distal part of the bursal canal, very close to the opening of the canal into the common atrium. In the paratypes the openings of the oviducts are located somewhat more dorsally. Shortly before opening into the female copulatory apparatus, the oviducts receive the openings of shell glands.

The vitellaria are only moderately developed. They may extend from dorsal to ventral body surface, and occur from behind the ovaries up to the level of the copulatory apparatus.

The female copulatory apparatus consists of a rounded, oval- or sac-shaped bursa and an obliquely oriented bursal canal. The latter arises from the anterior surface of the bursa and close to this point it shows a small, dorsally directed extension. Hereafter, the bursal canal runs obliquely towards the ventral body surface and opens into the common atrium. The bursa is lined with a nucleated and vacuolated epithelium, consisting of cuboidal cells. It is surrounded by a layer of muscle fibres.

The epithelium of the bursal canal is densely stained because it receives the openings of shell glands that surround it. This lining epithelium did not show any nuclei. The musculature of the bursal canal is well developed, but of a type that is difficult to interpret. It does not consist of regularly arranged rows of circular and longitudinal muscle fibres, but shows a more irregular arrangement. The major part consists of more or less circularly running muscles, in-

terspersed with longitudinally or irregularly running fibres. There are also fibres which from the bursal canal run into the anterodorsal portion of the parenchyma surrounding the canal.

Apart from the orange-red staining shell glands, the bursal canal is also surrounded by deep blue staining dots. It could not be decided whether these dots correspond with the insunk nuclei of the epithelium or with unicellular glands that open into the bursal canal, or even with nuclei of muscle fibres.

Eye.—The eyes consist of a single-celled pigment cup; the number of retinal cells could not be determined with certainty. There is no lens to the eye.

Cocoons (fig. 4).— Many cocoons were found attached to the gill leaves of the horseshoe crab specimens from which the type-specimens of *Ectoplana undata* were collected. An intact cocoon was obtained only from the crab on which the holotype was found. This cocoon was yellowish brown, whereas the ruptured cocoons were brown. The ruptured cocoons from the horseshoe crab from Deli, on which the paratypes of *Ectoplana undata* were found, showed a yellowish brown coloration. The dimensions of the cocoons may be derived from the figures. The cocoons consist of an egg-shaped receptacle with a short pedicel. It was impossible to determine the exact circumference, and thus the size, of the end plate to which the pedicel is attached.

COCOONS FROM OTHER HORSESHOE CRAB SPECIMENS (fig. 4)

Apart from the horseshoe crabs from which the type-specimens of *Ectoplana undata* were obtained, cocoons were also found attached to the gill leaves of other specimens of *Tachypleus gigas*. Cocoons were found on *T. gigas* from Sumatra's east coast (fig. 4C), Java Sea at Pekalongan (fig. 4D), and on another specimen from Deli (fig. 4E). The size and shape of these cocoons can be derived from the figures.

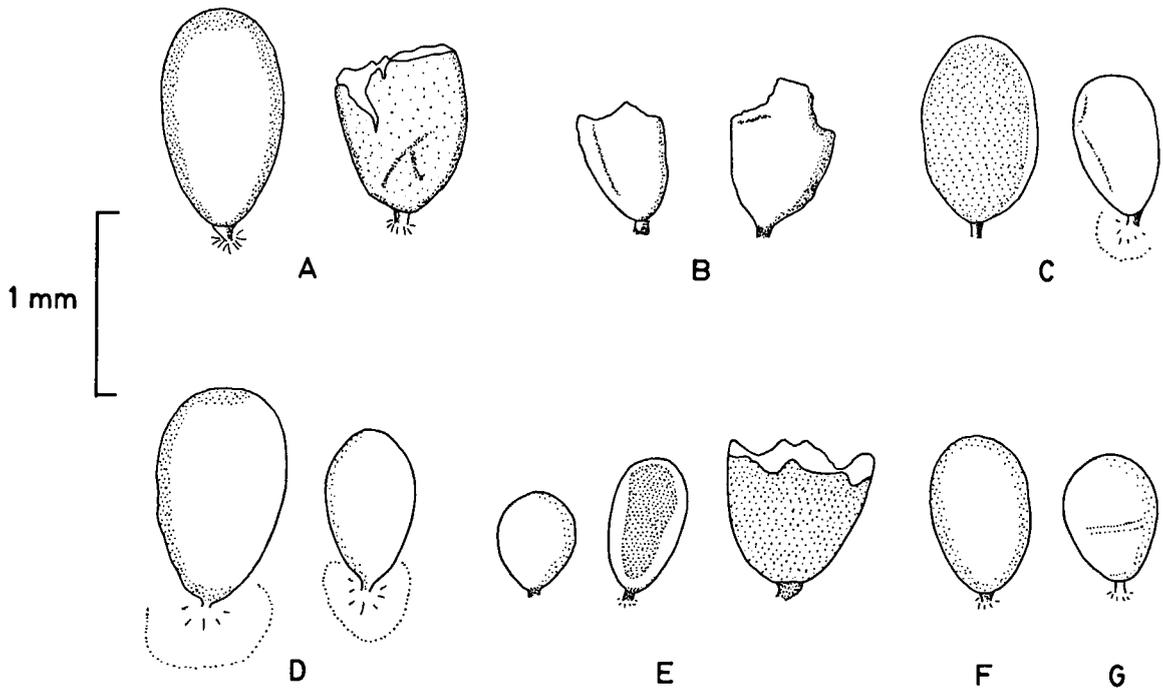


Fig. 4. Cocoons obtained from various horseshoe crabs. A: *Tachypleus gigas* from which holotype of *Ectoplana undata* n. sp. was collected, B: *T. gigas* from which paratypes were collected, C: *T. gigas*, Sumatra's east coast, D: *T. gigas*, Java Sea at Pekalongan, E: *T. gigas*, Deli, F: *Carcinoscorpius rotundicauda*, Singapore, G: *C. rotundicauda*, Sabang.

Interestingly, cocoons were found attached also to the gills of the horseshoe crab *Carcinoscorpius rotundicauda*. Cocoons were obtained from specimens collected at Singapore and Sabang (figs. 4F, G).

DISCUSSION

Wilhelmi (1909) was the first to mention the presence of marine triclads on Asian horseshoe crabs. On preserved crabs from Singapore, Wilhelmi found triclads which he thought to be bdellourids, although the poor state of preservation did not allow him a specific determination. Since Ijima & Kaburaki published their description of *Ectoplana limuli* only in 1916, Wilhelmi's conclusion about the taxonomic status of his specimens is not surprising, especially when one considers the external resemblance between *Ectoplana undata* and some specimens of *Bdelloura*.

Apart from living on a different host species, *Ectoplana undata* differs in several respects from

E. limuli. In *E. undata* the number of testes is small as compared with *E. limuli*, the former having 6-8 and the latter about 18 testicular follicles on either side of the body. The male copulatory apparatus also differs between both species. In *E. limuli* the penis papilla is large and conical, whereas in *E. undata* it is small and stubby. In *E. undata* the vasa deferentia open into a rounded seminal vesicle, but in *E. limuli* they unite first into a short common duct which subsequently expands slightly in diameter and thereafter narrows and opens into the tip of the penis papilla. With respect to the male copulatory apparatus another important difference between both species concerns the male atrium. In *E. limuli* there is only a small and typical atrium which differs considerably from the atrium of *E. undata* as described above. The female copulatory apparatus also shows important differences between the two species. In *E. limuli* the bursal canal has, for its greater part, a dorsoventral disposition, whereas in *E. undata* it runs obliquely. Common to both species is the

anterodorsal enlargement of the bursal canal, although this is more developed in *E. limuli*. The bursal canal of *E. limuli* shows a number of laterally directed folds which exhibit bilateral symmetry. In *E. undata* the bursal canal does not show lateral outbulgings. In *E. limuli* the oviducts open into the dorsal portion of the bursal canal, whereas in *E. undata* they open into the ventral part of the latter. In both species the shell glands open into the bursal canal, close to the openings of the oviducts. This implies that in *E. limuli* the dorsal portion of the bursal canal receives the openings of shell glands, whereas in *E. undata* the glands open into the ventral portion of the canal.

A number of structural similarities between *E. limuli* and *E. undata* may be considered indicative of the close genealogical relationship between the two species, although, of course, a definite hypothesis can only be formed after a comprehensive analysis of the entire group of marine triclads. Both species show (1) a penis papilla which is lined with an infranucleate epithelium, (2) oviducts which receive the openings of shell glands just before opening into the bursal canal, (3) an insunk epithelium lining the bursal canal, (4) an unusual, and difficult to interpret type of musculature around the bursal canal. With respect to the last-mentioned feature Kaburaki (1922) also found the situation difficult to interpret for his description is not unequivocal. In *E. limuli* Kaburaki described the musculature of the bursal canal to be composed "...for the most part... of three sets of fibres, viz. internal circular, *middle longitudinal* and outermost circular, of which the *middle circular* layer is developed to a considerable degree" (emphasis mine). This inconsistent description should be checked on new material of *E. limuli*, for the type-specimens are lost, and compared with the situation found in *E. undata*. Kaburaki's description of *E. limuli* and my observations on *E. undata* agree to the extent that in both species the major part of the musculature around the bursal canal consists of circular muscles. With respect to the absence of nuclei in the epithelium lining the bursal canal and the distal portion of the male atrium in *E.*

undata, I cannot rule out the possibility that small nuclei were obscured by the staining of these epithelia. Moreover, I could not find conclusive evidence for nuclei outside these epithelia. This agrees very well with the situation which Kaburaki (1922) described for the bursal canal of *E. limuli*. According to this investigator the bursal canal of *E. limuli* "...is surrounded by numerous pyriform cells which perhaps represent partly the insunken parts of the lining epithelium and partly unicellular glands". Concerning the epithelium lining the male atrium of *E. limuli*, Kaburaki (1922: 38) wrote that "...it shows no nuclei, which are apparently displaced just beneath the muscular layer". In *E. undata* only the narrow, distal part of the male atrium shows an insunk epithelium.

The species that is described as new in the present paper, is assigned to the genus *Ectoplana* although this is at the moment poorly defined. When Kaburaki (1917) transferred the species *Procerodes limuli* to the newly erected genus *Ectoplana*, he did not provide a definition which distinguished the genus from other Procerodidae. Kaburaki (1917) considered the presence of lateral outbulgings in the bursal canal and the fact that the oviducts open directly into the canal, to be sufficient to maintain the separate generic status of *E. limuli*. In his extended description Kaburaki (1922) came to the conclusion that the bursal canal of *E. limuli* (which he called vestibulum) is homologous with the bursal canal (which he called vagina) of *Stummeria trigonocephala*. This implies that the defining character of the genus *Ectoplana*, viz. "Oviducts opening separately into the extremely wide, dorsally prolonged vestibulum" (Kaburaki, 1922: 46), does not differentiate this genus from, for example, *Stummeria*. The latter genus was characterized by Kaburaki (1922: 46) by its "Oviducts opening separately into the vagina". Moreover, there are other Procerodidae known to date, in which the oviducts open separately into the bursal canal, viz. *Miroplana trifasciata* Kato, 1931, *Tryssosoma jennya* Ball, 1977, *Sabussowia wilhelmi* Ball, 1973, and *S. dioica* (Claparède, 1863). Thus, it is clear that the genus *Ectoplana* needs redefinition or

should be abolished. Of course, a proper decision on the subject cannot be reached without a careful analysis of the Procerodidae as a whole, for this taxon "...is the one most badly in need of revision" (Ball, 1977: 25). For the moment I can indicate only some of the features which made me decide to retain the genus *Ectoplana* and to assign the newly described species to this genus. In short, these are the four characteristics listed above, which were considered to be indicative of a close genealogical relationship between *E. limuli* and *E. undata*.

As can be seen in fig. 4, some differences in size could be observed between cocoons obtained from different specimens of *Tachypleus gigas* as well as between cocoons obtained from the same specimen of horseshoe crab. Because

no triclads were collected from specimens of *Carcinoscorpius rotundicauda*, it remains unknown whether the cocoons attached to this horseshoe crab came also from specimens of *E. undata* or belong to a different species of triclad.

The type-specimens of *E. undata* were obtained from *T. gigas* collected from widely separated areas, whereas cocoons were found on members of *T. gigas* from still other areas. From this it may be concluded that, most likely, the distribution of *E. undata* overlaps with the entire range of *T. gigas* (fig. 5). *Carcinoscorpius rotundicauda* has a somewhat smaller range, which for an important part overlaps with that of *T. gigas*, but also extends more westward (fig. 5). The ranges of *Tachypleus tridentatus* and *T. gigas* overlap along the coast of Vietnam,

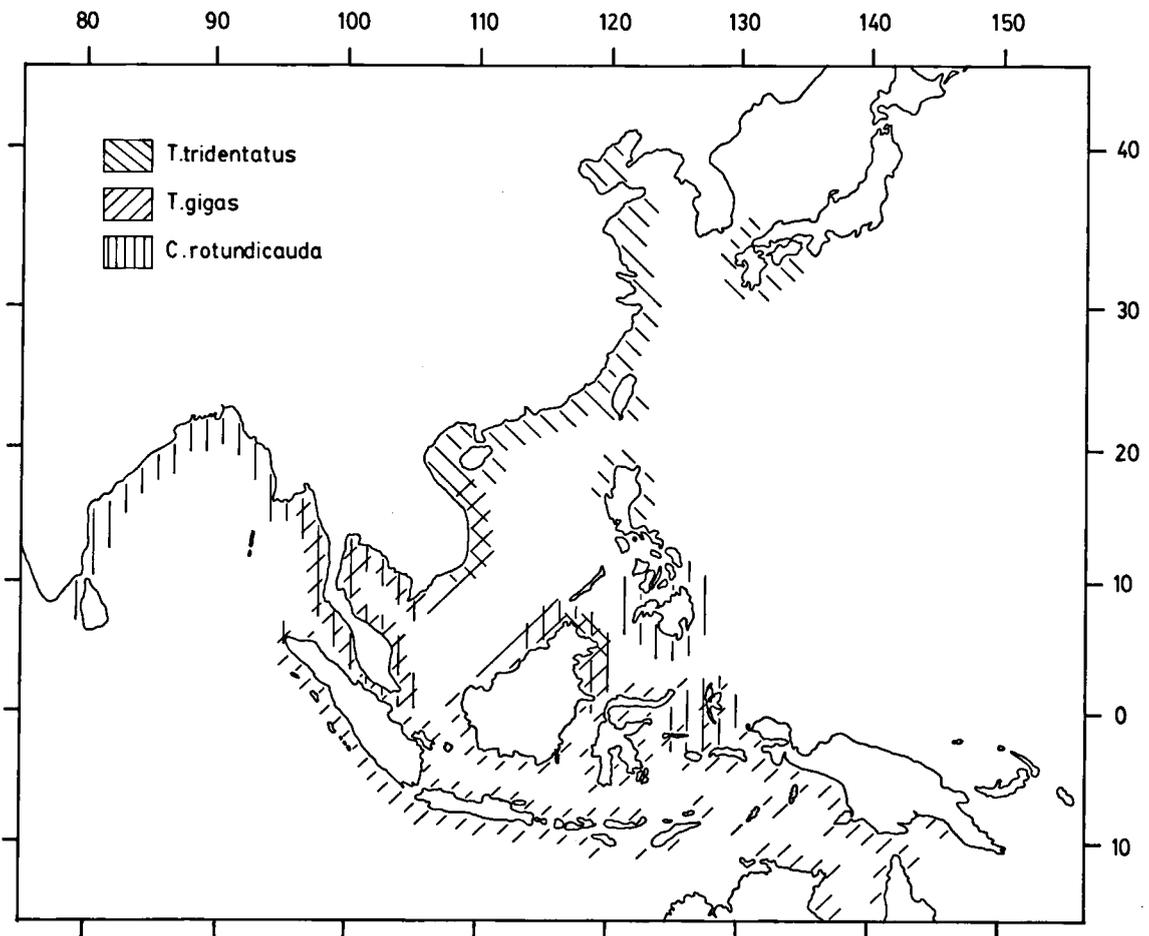


Fig. 5. Distribution of the Asian horseshoe crabs *Tachypleus tridentatus*, *T. gigas* and *Carcinoscorpius rotundicauda* (after Pocock, 1902; Smedley, 1929; Waterman, 1958; present material).

whereas those of *T. gigas* and *C. rotundicauda* overlap along the coast of the Malay Peninsula. All three Asian horseshoe crabs occur in northern Borneo (fig. 5). It is not unreasonable to assume that each of these three, partly sympatric, species of horseshoe crab has its own ectoparasitic species of marine triclad. Only further collecting efforts on preserved or living crabs may provide the answer.

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