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A NEW SPECIES OF *CYLINDROBULLA* FROM PHUKET, THAILAND, WITH A DISCUSSION OF THE SYSTEMATIC AFFILIATION OF THE GENUS.

by

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ABSTRACT

A new species of the rare opisthobranch genus *Cylindrobulla* is described from Phuket. It is a small species having a thin, white, fragile, cylindrical shell of a length of less than 1 cm. The spire is deeply sunken. There is a deep sutural slit lined by a steep keel. The outer lip of the shell is curved and overlapping for slightly over half of its length. The plicate gill forms a band all around the body whorl. It is attached to the mantle fold over its entire length. The radular teeth are small, squarish and resemble the central tooth of some Diaphanidae. The radula forms an ascending limb, a descending limb, and there is a heap of discarded teeth at the posterior end of the descending limb. This resembles the arrangement in the Ascoglossa (= Sacoglossa). The systematic position of the genus is discussed.

I. INTRODUCTION

The genus *Cylindrobulla* was originally described by Fischer (1856). The very brief description of *C. beauii* was based on 2 specimens from Guadeloupe in the Caribbean. In the same year Jeffreys described *Cylichna fragilis* from the Mediterranean. This species was later transferred to the genus *Cylindrobulla* (Jeffreys, 1882). Since then 10 more species of *Cylindrobulla* have been described (Table 1). These will be discussed below.

In 1972 Marcus introduced the genus *Ascobulla* with type species *Cylindrobulla* (Marcus & Marcus, 1970). This species has ascoglossan radular teeth whereas *C. beauii* has a uniseriate

Table 1 List of species of *Cylindrobulla* previously described.

C. beauii Fischer, 1856
C. fragilis (Jeffreys, 1856)
C. fischeri Adams & Angas, 1864
C. pusilla G. & H. Nevill, 1869
C. sculpta G. & H. Nevill, 1869
C. souverbiei (Montrouzier, 1874)
C. turtoni Bartsch, 1915
C. systremma Melvill, 1918
C. japonica Hamatani, 1969
C. ulla Marcus & Marcus, 1970
C. californica Hamatani, 1971
C. xishaensis Lin, 1978

radula with numerous small teeth resembling the central tooth of some Diaphanidae (Marcus & Marcus, 1970). Marcus (1972) also transferred *C. japonica* Hamatani, 1969 and *C. californica* Hamatani, 1971 to *Ascobulla*. However, both genera were retained in the cephalaspidean family Cylindrobullidae. The internal anatomy of most of the species of *Cylindrobulla* is unknown.

The systematic affiliations of *Cylindrobulla* and *Ascobulla* have been debated for a long time. Pruvot-Pol (1954) placed *Cylindrobulla* in the family Akeridae whereas Boettger (1954) claimed that they belong to the Diaphanidae. Marcus & Marcus (1956) created a new family Cylindrobullidae, with affiliations to the Diaphanidae, Akeridae, and Ascoglossa. Thus the Cylindrobullidae was given a position near the base of the Cephalaspidea (Marcus & Marcus, 1956). Later the Cylindrobullidae has been placed at the base of the Ascoglossa (= Sacoglossa) (Kay, 1968; Marcus, 1982).

In the present study a new species of *Cylindrobulla, C. phuketi* n.sp., is described. A generic reassignment of the previously described species is presented, and the systematic affiliations of the genera *Cylindrobulla* and *Ascobulla* are discussed.

II. MATERIALS AND METHODS

Eight specimens were collected intertidally at Naiyang Beach, Phuket, on 25 Nov. 1986. They emerged from clumps of the green siphonalean alga *Halimeda* sp. brought into the laboratory. The animals were kept alive in the laboratory for about a week. Then they were preserved in 4% neutral formaldehyde and transferred to 80% ethanol. The animals were examined by fine dissection. They were lightly stained with acetocarmine. Radulae were examined by placing the buccal mass in 40% NaOH until the tissues dissolved. Then the radula was rinsed in distilled water and transferred to abs. ethanol. One radula was mounted on a SEM stub and examined with a JEOL JSM-840 scanning electron microscope. Another radula was mounted on a slide with Turtox CMC-9AF mounting medium and examined light microscopically.

III. DESCRIPTION

Holotype: Shell length 3.5 mm, maximum width 1.9 mm; width at apical area 0.8 mm. The holotype is deposited at PMBC. A paratype is deposited at the Zoological Museum, Copenhagen (ZMUC).

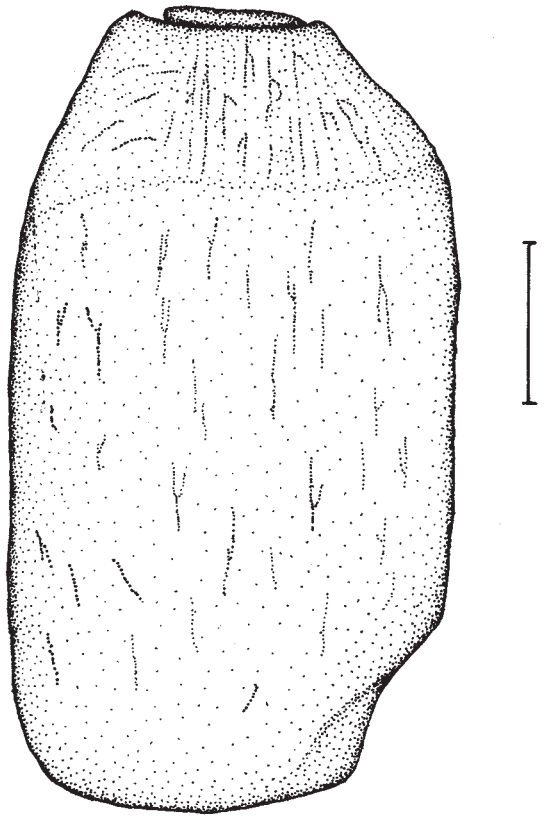

Fig. 1. *Cylindrobulla phuketi* n.sp. Dorsal view of shell. Scale line is 1 mm.

External features: The shell appears creamy white, but is actually transparent. It is thin and fragile with numerous hairfine cracks which tend to obscure the pattern of fine growth lines. The shape is basically cylindrical (Fig. 1). It has a deep sutural slit. The outer lip is overlapping for most of its length, but the anterior opening is rather wide and almost half as long as the total length of the shell (Fig. 2). The margin of the inner lip is thickened. The spirally coiled apex of the shell has a rather sharp keel and is deeply sunken into the body whorl (Fig. 3A). The protoconch (Fig. 3B) could only be seen after breaking the shell. It was attached to the bottom of the apical "funnel". The shell has a rather tough periostracum. Shell measurements for 6 specimens are shown in Table 2.

Cylindrobulla phuketi has a distinct cephalic shield composed of two very mobile lobes, which can be flattened out laterally, stretched out backwards so they resemble rhinophores, or contracted into low bulges on the head. The cephalic shield is separated from the foot by a deep groove. The eyes are sometimes visible from the dorsal side, rather far anteriorly and almost median. Usually the eyes are only visible laterally, under the lobes of the cephalic shield. In living crawling animals, the foot extends further posteriorly than the lobes of the cephalic shield. There is a very narrow neck region connecting the head-foot region and the visceral mass. In a specimen relaxed in $MgCl_2$, the penial opening was seen in the groove between the cephalic shield and the foot on the

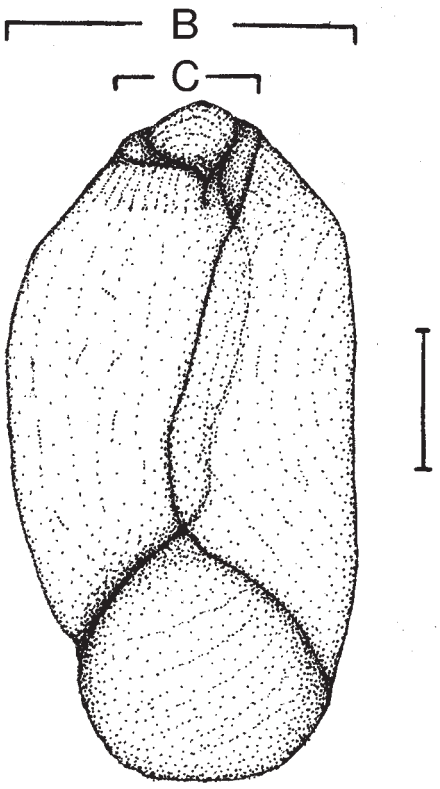

Fig. 2. *Cylindrobulla phuketi*. Ventral view of shell. B and C refer to measurements given in Table 2. Scale line is 1 mm.

right side, behind the eye (Fig. 4). A Hancock's organ could not be distinguished.

Two bands of white granular tissue are visible through the shell on either side of a greyish-brown band, corresponding to the gill (Fig. 5). In front of the anterior granular band a large white triangular structure is seen. This is part of the female reproductive system, the "mucus gland". This massive structure occludes the mantle cavity completely anteriorly, leaving only a narrow space in front of it. The mantle-fold is very thin except along the anterior edge, where it is attached to the shell. The opening to the mantle cavity is on the right side. It is functionally divided by an adhesive ridge posteriorly, probably creating separate inhalent and exhalent openings. The posterior opening extends posteriorly to the sutural slit. The mantle cavity is closed off anteriorly by another adhesive band winding to the left from the mediodorsal edge of the mantle. A very strong adductor muscle winds from the anterior right corner of the mantle opening and spreads out ventrally on the left side. Where this muscle penetrates the floor of the mantle cavity is a small papilla. This is the female genital papilla.

The mantle cavity is large. The gill forms a wide folded band extending almost all the way around the animal (Fig. 5). It is attached to the mantle roof throughout its length. The heart is located anteriorly and to the right of the mediodorsal line. The auricle is a thin-walled triangular structure. The ventricle is anterior to,

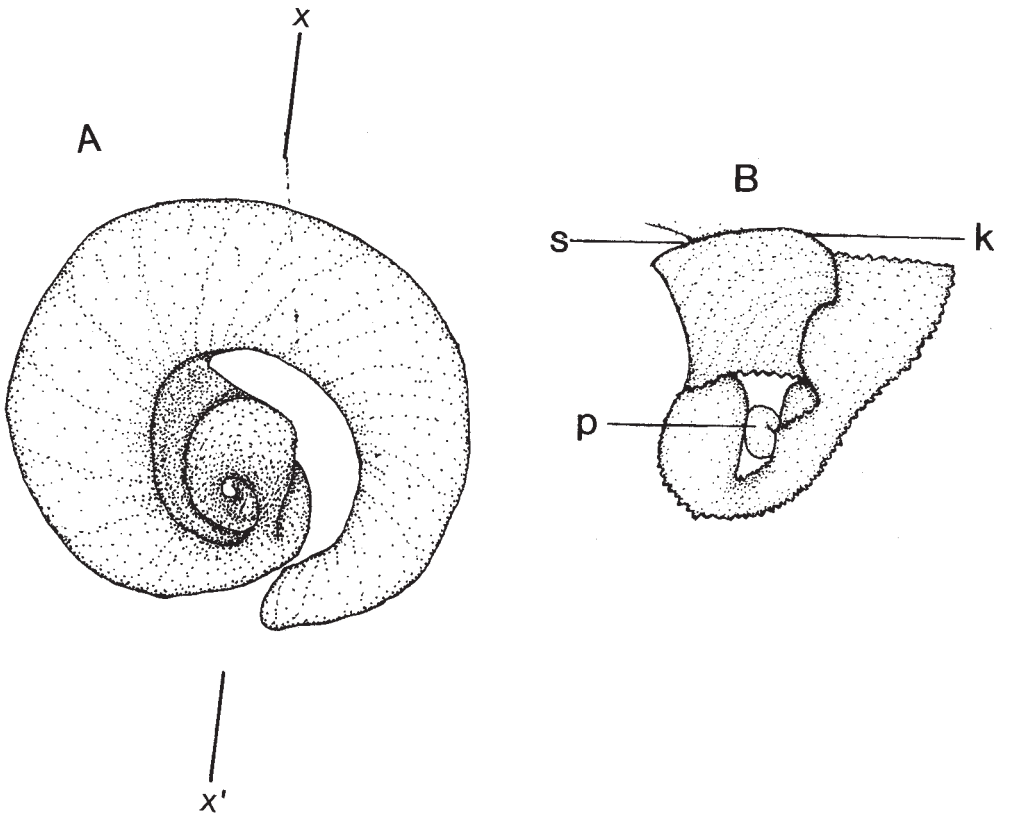

Fig. 3. *Cylindrobulla phuketi*. A-apical area of shell. The line x-x' indicates the approximate axis along which the shell was broken to reveal protoconch, as shown in B. Legend: k-apical keel; p-protoconch; s-sutural slit.

and slightly to the left of the auricle. The aorta descends along the right margin of the mucus gland, but could not be traced through the diaphragm separating the head-foot region from the visceral mass. Posterior to the gill a band of white granular tissue is located. A similar band is seen along the anterior border of the gill, to the left of the heart. This is probably kidney tissue. This band widens ventrally behind the gill, near the inner lip of the shell. Extending over the inner shell lip is a rather wide tissue fold, the infrapallial lobe (Fig. 5B, C). This has a rather thick margin formed by the adhesive ridge, and apparently it contains branches of the digestive gland. The adhesive ridge continues as a ciliated band on the floor of the mantle cavity, but a distinct pallial caecum is not formed.

Internal anatomy: The pharynx is very small and narrow (Fig. 6). The cephalic ganglia are almost as long as the pharynx and positioned on top of the posterior part of the pharynx. The radula is uniseriate. It consists of a long ascending limb originating from a distinct radular sac forming a "bump" posteriorly on the pharynx, ventrally to the esophagus. There is an equally long descending limb of the radula, and a small heap of discarded teeth at the posterior end of the descending limb. This heap does not form a regular ascus as in ascoglossans. The number of

Table 2

Measurements of shell dimensions of *Cylindrobulla phuketi*. All measurements in mm.

	shell length A	max. width B	width of apical area C	A/B	B/C
	3.04	1.67	0.652	1.82	2.56
	2.97	1.74	0.58	1.71	3.0
	2.25	1.16	0.352	1.94	3.3
	2.39	1.3	0.507	1.84	2.56
	1.16	0.616	0.217	1.88	2.84
	4.64	3.12	0.87	1.49	3.59
mean	2.74	1.6	0.53	1.78	2.97
s.d.	1.05	0.77	0.209	0.148	0.373

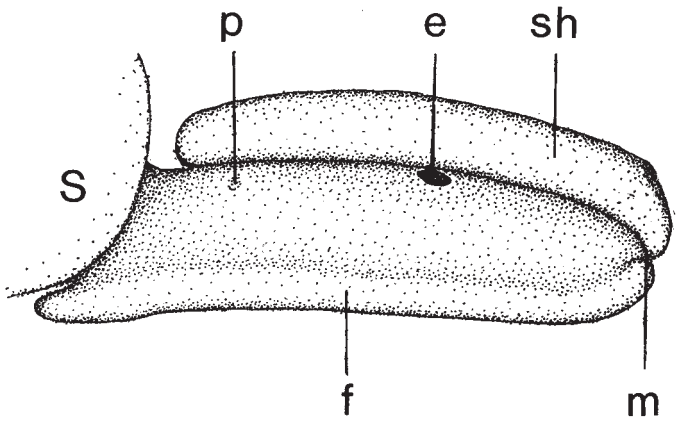

Fig. 4. *Cylindrobulla phuketi*. Lateral view of cephalic region. legend: e-eye; f-foot; m-mouth; p-penial opening; s-shell; sh-cephalic shield.

teeth in one radula was 41 in the ascending limb, 44 in the descending limb, and at least 20 in the posterior heap. The radular teeth are short and wide, almost squarish (approx, 12μm long and approx, 11um wide), resembling the central radular teeth of some Diaphanidae. The number of denticles on each tooth is rather variable (Fig. 7). There is a small esophageal pouch just in front of the diaphragm separating the head-foot region from the visceral mass. The connection of the esophagus through this diaphragm broke in all the specimens dissected, so the connection to the stomach could not be traced. Nor could an intestine or an anus be found. The digestive gland is a rather solid mass of thin tubules.

The reproductive system consists of a number of hermaphrodite follicles located centrally in the visceral mass. The follicles are connected by thin ducts. There is a bean-shaped ampulla just posterior to the female mucus gland. Unfortunately the thin ducts leading to and from this ampulla were very fragile in the preserved specimens, and it could not be determined where the vas deferens separated from the oviduct. There is a rather large spermatheca in

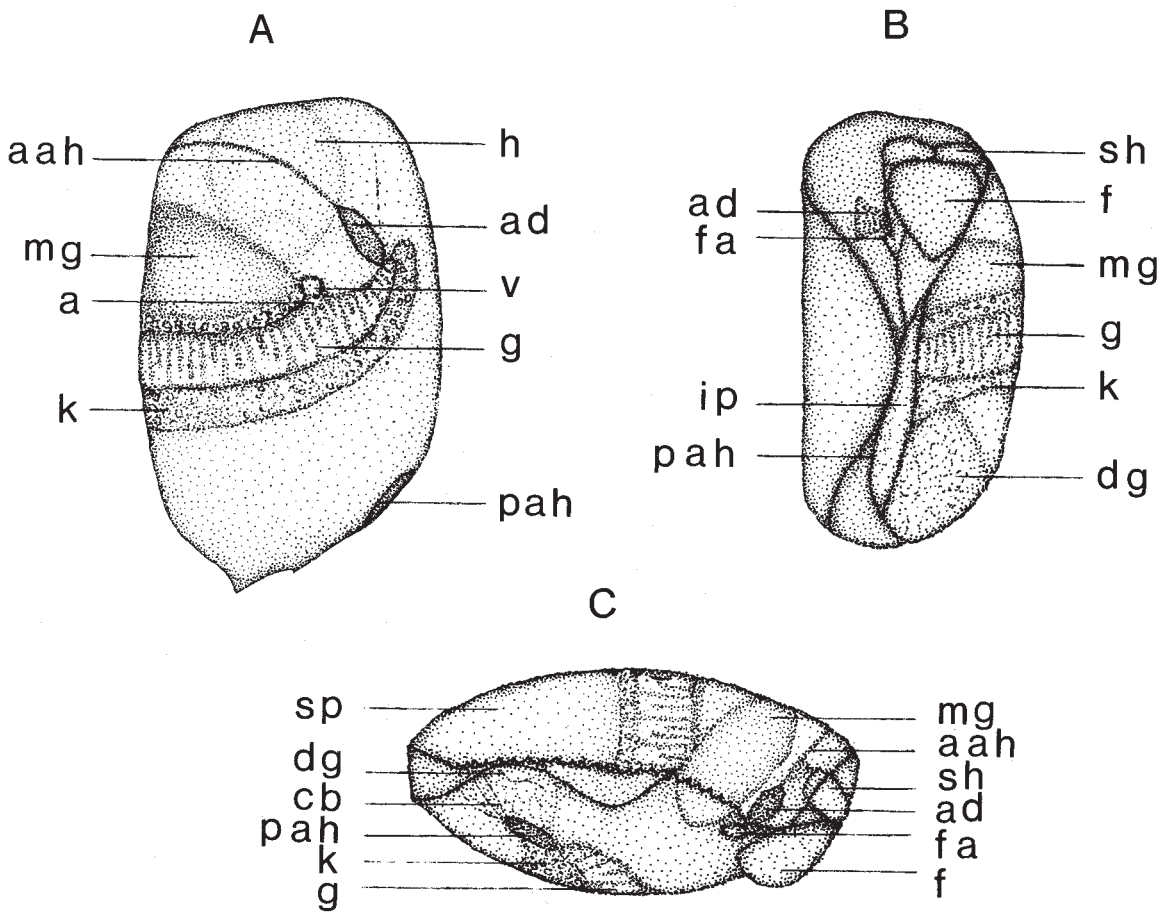

Fig. 5. *Cylindrobulla phuketi*. Mantle organs after removal of the shell. A-dorsal view; B-ventral view; C-lateral view (with part of mantle fold cut off). Legend: a-auricle; aah-anterior adhesive ridge; ad-adductor muscle; cb-ciliary band; dg-digestive gland ductules; f-foot; fa-female genital opening; g-gill; h-head; ip-infrapallial lobe; k-kidney; mg-mucus gland of female reproductive system; pah-posterior adhesive ridge; sh-cephalic shield; sp-suprapallial lobe; v-ventricle.

front of the mucus gland. It appears to connect to a point near the female gonopore. There is also a smaller yellowish spherical structure just in front of the hermaphrodite ampulla. This is most likely a spermatocyst, but unfortunately its connections to the remainder of the reproductive system could not be traced. The penis is rather large, and located inside the cephalic hemocoel. It does not have a cuticular style, but the tip is composed of several lobes. The vas deferens is embedded in the lateral cephalic body musculature, but its connection through the diaphragm could not be traced.

As mentioned the nerve-ring is postpharyngeal, but the visceral chain could not be followed, because the diaphragm was very difficult to dissect.

Observations on live animals: C. phuketi moves in a caterpillar like fashion. It extends the head-foot region slowly by ciliary movements on the pedal sole, then suddenly pulling up the vis-

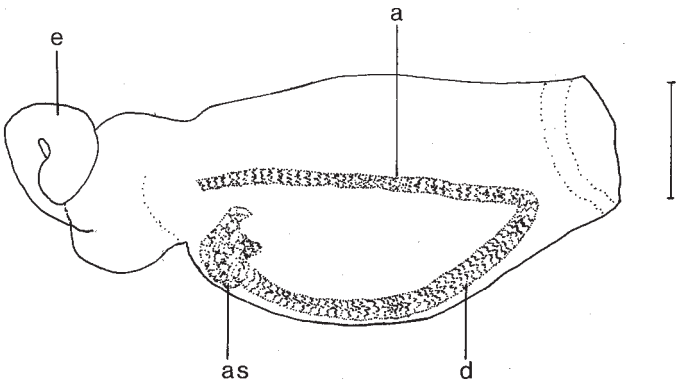

Fig. 6. *Cylindrobulla phuketi*. Camera lucida drawing of pharynx. Scale line is 0.1 mm. Legend: a-ascending limb of radula; d-descending limb of radula; as-"ascus"; e-esophagus.

ceral mass by contracting the columella-muscle. The animals usually hide on the underside of *Halimeda* segments. Hence feeding could not be observed, but it seems very likely that this species feeds on *Halimeda*. After several days in the laboratory the animals crawled out of the water and would have died of desiccation, if they had not been forced back into the water. After the animals were returned to the water, they exuded a viscous mucus from the mantle cavity. This mucus was filled with multiciliated protozoans. The natural habitat of *C. phuketi* had a swift tidal current, and probably the stagnant water of the laboratory containers caused the infestation of the mantle cavity with these microorganisms.

IV. DISCUSSION

C. phuketi resembles *C. xishaensis* (Lin, 1978) rather closely. The shell dimensions are within the same ranges, and also, the number of radular teeth is similar. However, there are a number of differences. The visceral mass of *C. xishaensis* was described as orange (Lin, 1978), whereas that of *C. phuketi* is white to greyish-brown. Judging from the figures of *C. xishaensis*, its sutural slit is deeper and the apical keel narrower than in *C. phuketi*, and also the innermost whorl is larger, or less deeply sunken in the former species. If the scale line in the figure of a radular tooth of *C. xishaensis* is correct, the teeth of this species are more than 10 times as big as those of *C. phuketi*. However, this could have been a misprint, so that the scale line should have read 0.02 mm instead of 0.2 mm, in which case the teeth are only slightly larger than those of *C. phuketi*. In *C. xishaensis* as well as in *C. beauii* the width of the teeth is greater than the length. In *C. phuketi* the length is about equal to the width. In *C. xishaensis* the lateral denticles are shorter and the central tip more prominent than in *C. phuketi*. *C. beauii* is larger than *C. phuketi* and also relatively wider. The shell has a relatively longer apical keel (about 1/2 whorl more), whereas the sutural slit appears about the same relative length. Also, the number of lateral denticles on the radular teeth is larger in *C. beauii* than in *C. phuketi*. Finally the cerebral ganglia of the former species are smaller relative to the pharynx than in the latter species.

Externally the genus *Cylindrobulla* appears indistinguishable from *Ascobulla*, which has distinctly ascoglossan radular teeth. Comparisons of drawings of the shells of several species of *Cylindrobulla* and *Ascobulla* indicate that there may be a slight generic difference in the shape of the outer shell lip; it appears to be straight in *Ascobulla* and slightly rounded in *Cylindrobulla*.

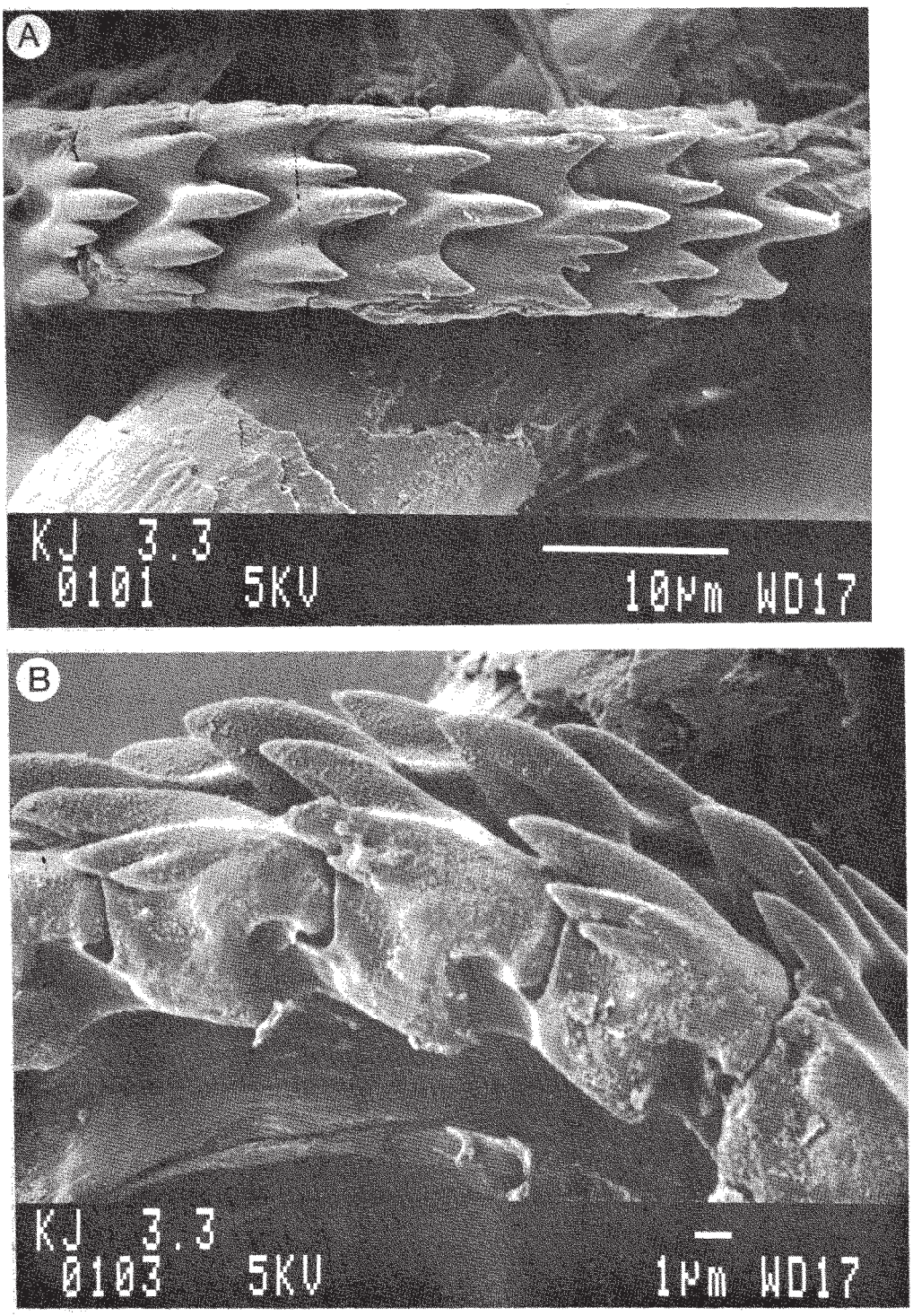

Fig. 7. *Cylindrobulla phuketi*. SEW-photos of radular teeth. A-dorsal aspect; B-lateral aspect.

The species of the genus *Ascobulla* have always been found associated with the siphonalean green algal genus *Caulerpa*, whereas a specific algal association has not been described for the species definitely ascribed to the genus *Cylindrobulla*. *C. xishaensis* was collected from *Caulerpa* (Lin, 1978), and *C. phuketi* from *Halimeda*.

Of the 13 species of *Cylindrobulla* described (Table 1), 3 have been transferred to *Ascobulla* (Marcus, 1972). The radular teeth of *C. fischeri* Adams & Angas, 1864 are distinctly ascoglossan (Gascoigne & Sartory, 1974), and this species should also be transferred to the genus *Ascobulla*. On the other hand the teeth of *C. xishaensis* are rather similar to those of *C. beauii* (Lin, 1978). Recently *C. pusilla* Nevill & Nevill, 1869 and *C. sculpta* Nevill & Nevill, 1869 were synonymized with *Ascobulla fischeri* and *C. turtoni* Bartsch, 1915 was synonymized with *Volvatella laguncula* Sowerby, 1894 (Gosliner, 1987). After examining the original descriptions of *C. pusilla* and *C. sculpta*, I consider it likely that the latter species belongs to the genus *Cylindrobulla*, because the drawing clearly shows a curved outer shell-lip (Nevill & Nevill, 1869). The original figure of *C. souverbiei* (Montrouzier, 1874) shows a straight outer shell-lip (Souverbie & Montrouzier, 1874), as does that of *C. systremma* Melvill, 1918. Also, *C. fragilis* has distinctly ascoglossan teeth and must be transferred to *Ascobulla* (Thompson *et al.*, 1985). Thus *Cylindrobulla* presently consists of only 4 species worldwide, including *C. phuketi*.

Although *Cylindrobulla phuketi* has a uniseriate radula with equally long ascending and descending limbs and a postpharyngeal nerve-ring, it is definitely not an ascoglossan. The shape of the radular teeth are more similar to the median teeth of some Diaphanidae than to the teeth of Ascoglossa. Also, the number of teeth is much larger than in ascoglossans.

Cylindrobulla possesses many primitive opisthobranch traits. It has a short foot, not extending the full length of the shell. It has a long, narrow, folded gill which is attached to the mantle roof over its entire length. It also has a very narrow neck region. It can withdraw completely into the shell. It has a well developed cephalic shield, and it does not have parapodial lobes or extensions of the mantle fold covering any external part of the shell. However, it also has some advanced characters. The nerve-ring is postpharyngeal. There are no jaws and no gizzard. This may be a consequence of suctorial feeding. There is no pallial caecum, and the inhalent and exhalent currents are only functionally separated by the posterior adhesive band.

Pruvot-Fol (1954) assigned the genus *Cylindrobulla* to the family Akeridae on the basis of the cylindrical shell and postpharyngeal nerve-ring, whereas Boettger (1954) incorporated it in the family Diaphanidae because it lacked jaws and gizzard-plates. The present study has shown that it cannot be assigned to the family Akeridae, because it does not have gizzard-plates, the radula is uniseriate, the gill is attached throughout its length, and parapodia are absent. Apparently *Cylindrobulla* can neither be assigned to the family Diaphanidae because the uniseriate radula has a long descending limb, there are no lateral teeth, and the nerve-ring is postpharyngeal. The radular teeth of *Cylindrobulla* have rather strong, thick bases, quite unlike the central teeth of most Diaphanidae, which are thin and flattened. The genus *Newnesia* has been assigned to the family Diaphanidae, but apart from a uniseriate radula its anatomy is very poorly known (Eliot, 1906; Boettger, 1954; Gascoigne & Sartory, 1974).

The genus *Ascobulla* has so many ascoglossan features that it must be assigned to the order Ascoglossa, and probably to the family Volvatellidae. However, The genus *Cylindrobulla* cannot be transferred to the order Ascoglossa, and must remain in its own family Cylindrobullidae, at the primitive base of the Cephalaspidea.

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