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Tunic Cuticular Protrusions in Ascidians (Chordata, Tunicata): A Perspective of Their Character-State Distribution

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ABSTRACT—Fine structure of the ascidian tunic cuticle was surveyed for minute protrusions in 30 species of the suborder Aplousobranchia, 10 species of Phlebobranchia and 23 species of Stolidobranchia (Ascidiacea, Chordata). The present results, combined with previously published data, describe the fine structure of the tunic cuticle in 116 species of ascidians covering all the families and subfamilies of the class Ascidiacea except for the phlebobranch families Octacnemidae and Plurellidae. These data confirm the general stability of the character-state distribution (presence or absence) of the tunic cuticular protrusions within families or subfamilies. Cuticular protrusions occur only in Polyclininae and Clavelininae of the order Enterogona (Aplousobranchia and Phlebobranchia) and in all families of the order Pleurogona (Stolidobranchia and Aspiraculata). A few exceptions were the occurrence of cuticular protrusions in *Polycitor proliferus* (Polycitorinae), *Distaplia dubia* (Holozoinae), *Pterygascidia longa* (Cionidae) and *Adagnesia vesiculiphora* (Agneziidae), and the absence in some styelids and pyurids that have hard tunics. Possible character-state changes in the ascidian phylogenies are discussed briefly.

INTRODUCTION

Urochordates (tunicates) constitute one of the three subphyla in the phylum Chordata, and are characterized by the presence of the tunic, an integumentary tissue wholly covering the epidermis. The tunic contains cellulose fibers linked with proteins (De Leo *et al.*, 1977; Van Daele *et al.*, 1992), which makes it unique among metazoan tissue. All ascidians have this integumentary tissue but it varies greatly in structure between the various groups and thus may be important to study comparatively from the viewpoint of ascidian phylogeny.

The outermost layer of the tunic is called the tunic cuticle, underlain by the fibrous matrix furnished densely with mesenchymal free cells (tunic cells). Under an electron microscope, the tunic cuticle is revealed to be a continuous layer composed of electron dense materials. The cuticle is often furnished with minute protrusions over the surface, and/or with semi-electron dense layers (subcuticle) beneath it. In some species, the cuticular surface has numerous protrusions (Katow and Watanabe, 1978; Milanesi *et al.*, 1978). We have examined these fine structures from the viewpoint of ascidian taxonomy and phylogeny. On the basis of a survey of 51 species covering 13 families out of the 15 recognized ones in all suborders of the class Ascidiacea (i.e., Aplousobranchia, Phlebobranchia, Stolidobranchia and Aspiraculata), Hirose *et al.* (1992) recognized the following patterns in the occurrence of cuticular protrusions: (1) Cuticular protrusions occur in only a few aplousobranch families; (2) In the Phlebobranchia, protrusions occur only in *Pterygascidia longa* and *Adagnesia vesiculiphora* among the 17 species examined; (3) In the Stolidobranchia, most of the examined species have protrusions except a few styelid species. These patterns suggest that the character-state distribution (presence or absence of the protrusions) in the tunic cuticle may be a valid clue to ascidian phylogeny. To verify this, however, further extensive surveys of additional species were necessary.

In the present study, we examined 30 additional aplousobranch species, including those belonging to two subfamilies not previously studied, and two subfamilies so far represented only by a single species. Similarly, we also investi-

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gated the cuticular fine structures in 10 additional phlebobranchs and 23 stolidobranchs. As a result we now have a perspective of the character-state distribution of tunic cuticular protrusions in 116 species covering all the families and subfamilies included in a traditional classification (see Table 1), except for the phlebobranch families Octacnemidae and Plurellidae.

MATERIALS AND METHODS

Animals

Thirty species of aplousobranchs, 10 phlebobranchs and 23 stolidobranchs were examined. Collection sites are listed in Table 1. The specimens collected in Australia and Hawaii were generous gifts from Dr. Hiromichi Koyama (Yokohama City University).

Electron microscopy

Tunic pieces were prefixed and stored in (A) 2.5% glutaraldehyde-0.1 M cacodylate-0.45 M sucrose (pH 7.4), (B) 2.5% glutaraldehyde-0.14 M NaCl-0.2 M Millonig's phosphate buffer (pH 7.4), (C) 2% paraformaldehyde plus fix B, or (D) 10% formalin-seawater. In some cases, the specimens were anesthetized with menthol-seawater or 6% $MgCl_2$-menthol for several hours at 12-14°C, before fixation. After brief rinsing through 0.1 M cacodylate-0.45 M sucrose (pH 7.4), the specimens were postfixed in 1% osmium tetroxide-0.1 M cacodylate (pH 7.4) for 1.5 hr, and dehydrated through an ethanol series. The specimens were cleared with *n*-butyl glycidyl ether and embedded in low viscosity epoxy resins. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-9 transmission electron microscope.

In some didemnid and polycitorid specimens, the tunic was impregnated densely with spicules of calcium carbonate. Such specimens were decalcified before postfixation by incubating in 5% EDTA-2Na (ethylenediamine tetra acetic acid, disodium salt) for 2-3 days. The EDTA treatment had no detectable effects on the fine structure of the tunic in control experiments applied to the spicule-free *Botryllus schlosseri* (Fig. 1) and *Botrylloides fuscus* with cuticular protrusions of 100 nm height (Hirose *et al.*, 1990), and to the spicule-bearing *Didemnum moseleyi* and *Leptoclinides echinatus* which had been shown to lack any tunic protrusions using SEM without the EDTA treatment (Hirose *et al.*, 1990, 1992). Approximate height of cuticular protrusions was directly measured from the negatives of electron micrographs.

Phylogenetic considerations

These data were tabulated on a traditional ascidian classification based on the systems given by Berrill (1950), Monniot and Monniot (1972), Kott (1985) and Nishikawa (1986). Due attention was paid to the newly proposed system of Kott (1990, 1992).

RESULTS

Our present results are shown in Table 1 in combination with the previously published data (Hirose *et al.*, 1990, 1992; Cloney, 1994; Turon and Vázquez, 1996).

Aplousobranchia

In the family Polyclinidae, all the examined *Aplidium* species (subfamily Polyclininae) have cuticular protrusions of 30-60 nm (Fig. 2, Table 1). On the other hand, 3 species from 3 different genera in the subfamily Euherdmaniinae have only a very thin cuticular layer lacking the protrusions completely (Fig. 3).

In the Didemnidae, the currently examined 7 species and 3 previously observed species from 5 genera invariably lacked protrusions, whether they were spicule-bearing or not. These 10 species were quite similar to one another in their tunic ultrastructure.

The family Polycitoridae includes three subfamilies: Polycitorinae, Clavelininae and Holozoinae. In the Polycitorinae, only *Polycitor proliferus* had been previously examined and was found to have distinct protrusions of 40 nm. The 7 additional species used here were revealed to lack them. It follows that the presence of cuticular protrusions in *P. proliferus* may be unusual in the Polycitorinae. *Eudistoma gilboviride* has a thicker cuticle than the other 2 congeners (Figs. 4 and 5), though all lack protrusions. In the Clavelininae, the 3 *Clavelina* spp. examined here were very similar in the size of protrusions (30-40 nm) to the previously observed species, but their embossed cuticle was corrugated densely at an amplitude of 100-200 nm (Fig. 6). In the Holozoinae, *Distaplia dubia* has 20-30 nm high protrusions (Fig. 7), while the other 5 examined species including its two congeners lack them (Fig. 8).

Phlebobranchia

All 10 newly examined species have a flat cuticle without any protrusions, consistent with most of the previous results from phlebobranchs (Table 1). As the information about cuticle structure in the family Agneziidae had been limited only to the single species *Adagnesia vesiculiphora*, we examined another species, *Agnesia himeboja*, but unsuccessfully; the tunic was not well-preserved. Further, we failed to examine well-preserved material from a sand impregnated species of Plurellidae and a deep water species of Octacnemidae. In the suborder Phlebobranchia, the tunic of many species is gelatinous, and its cuticle is thinner with less electron density (Fig. 9) than ascidians of the other suborders. In *Chelyosoma*, however, the tunic is rather thick and hard, and its cuticle is thicker than usual for phlebobranchs (Fig. 10).

Stolidobranchia

Character-state distributions of the cuticular protrusions in the newly examined 23 stolidobranchs were consistent with those in the previous results (Table 1) for Botryllidae, Polyzoinae (Styelidae) and Molgulidae (Figs. 11, 15, 16). Further, the previously detected occurrence of both character states in the Styelinae (i.e., present in some species, absent in others) was also true of the newly examined 6 species (Fig. 12). The only exception concerned the Pyuridae, in which *Microcosmus curvus* lacked protrusions while all the other previously or presently analyzed species had them.

Leathery or cartilaginous tunic in some species tended to have a thicker and more electron-dense cuticular layer than the gelatinous tunic of other species. Even in the same individual, the cuticular layer was often thicker where the tunic was harder, and the mode of occurrence of the protrusions varied in some cases. In *Halocynthia igaboja*, the cuticular protrusions of the tunic proper were larger than those of the

Table 1. Occurrence and height of tunic cuticular protrusions in ascidians

Species	Height of protrusions	Fixation[†]	Collection[§] site	References
Order Enterogona				
Suborder Aplousobranchia				
Family Polyclinidae				
Subfamily Polyclininae				
Aplidium californicum	40	A	1	present study
Aplidium benhami	30-40	A	2	present study
Aplidium phortax	30-40	A	2	present study
Aplidium pliciferum	30			Hirose *et al.*, 1990
Aplidium solidum	30	A	1	present study
Aplidium yamazii	60			Hirose *et al.*, 1990
Subfamily Euherdmaniinae				
Placentela crystallina	absent	D	3	present study
Pseudodistoma kanoko	absent	D	4	present study
Ritterella pulchra	absent	A	1	present study
Family Didemnidae				
Diplosoma listerianum	absent	A	1	present study
Diplosoma midori	absent	A	5	present study
Diplosoma mitsukurii	absent			Hirose *et al.*, 1990
Didemnum molle	absent	A[‡]	5	present study
Didemnum moseleyi	absent			Hirose *et al.*, 1990
Didemnum sp.	absent	A[‡]	5	present study
Leptoclinides echinatus	absent			Hirose *et al.*, 1992
Lissoclinum sp.	absent	A[‡]	5	present study
Polysyncraton thallimorpha	absent	A[‡]	5	present study
Tridemnum strangulatum	absent	A[‡]	1	present study
Family Polycitoridae				
Subfamily Polycitorinae				
Cystodytes lobatus	absent	A[‡]	1	present study
Cystodytes sp.	absent	D[‡]	6	present study
Eudistoma gilboviride	absent	A	5	present study
Eudistoma glaucus	absent	A	5	present study
Eudistoma purpuropunctatum	absent	A	1	present study
Eudistoma sp.	absent	A	5	present study
Polycitor proliferus	40			Hirose *et al.*, 1990
Sigillina signifera	absent	D	7	present study
Subfamily Clavelininae				
Clavelina cyclus	30-40	A	5	present study
Clavelina cylindrica	30-40	B	8	present study
Clavelina huntsmani	40-50			Cloney, 1994 (Fig. 7c)
Clavelina miniata	30			Hirose *et al.*, 1990
Clavelina viola	30-40			Hirose *et al.*, 1992
Clavelina sp.	30-40	A	5	present study
Subfamily Holozoinae				
Distaplia dubia	20-30	D	9	present study
Distaplia occidentalis	absent	A	1	present study
Distaplia systematica	absent	D	10	present study
Hypsistozoa fasmeriana	absent	A	2	present study
Sycozoa kanzasi	absent	D	11	present study
Sycozoa sp.	absent	C	8	present study
Suborder Phlebobranchia				
Family Cionidae				
Syndiazona grandis	absent			Hirose *et al.*, 1992
Ciona edwardsi	absent			Hirose *et al.*, 1992
Ciona intestinalis	absent			Hirose *et al.*, 1992
Ciona savignyi	absent			Hirose *et al.*, 1990
Ciona roulei	absent			Hirose *et al.*, 1992
Pterygascidia longa	60			Hirose *et al.*, 1992
Family Perophoridae				
Perophora annectens	absent	A	1	present study
Perophora japonica	absent			Hirose *et al.*, 1990
Perophora multiclathrata	absent			Hirose *et al.*, 1990
Family Ascidiidae				
Ascidia ahodori	absent			Hirose *et al.*, 1992
Ascidia archaia	absent	A	12	present study
Ascidia gemmata	absent			Hirose *et al.*, 1992
Ascidia mentula	absent	A	13	present study
Ascidia paratropa	absent	A	1	present study
Ascidia sydneiensis	absent			Hirose *et al.*, 1990
Ascidia zara	absent			Hirose *et al.*, 1992
Ascidia sp. (cf. *tapni*)	absent			Hirose *et al.*, 1992
Ascidiella aspersa	absent	A	2	present study
Phallusia mammillata	absent	A	13	present study
Family Agneziidae				
Adagnesia vesiculiphora	20			Hirose *et al.*, 1992
Family Corellidae				
Chelyosoma productum	absent	A	1	present study
Chelyosoma siboja	absent			Hirose *et al.*, 1992
Chelyosoma yezoense	absent			Hirose *et al.*, 1992
Corella eumyota	absent	A	2	present study
Corella inflata	absent	A	1	present study
Corella willmeriana	absent	A	1	present study
Corella sp. (cf. *japonica*)	absent			Hirose *et al.*, 1992
Order Pleurogona				
Suborder Stolidobranchia				
Family Botryllidae				
Botryllus primigenus	100			Hirose *et al.*, 1990
Botryllus scalaris	100			Hirose *et al.*, 1990
Botryllus schlosseri	100			Hirose *et al.*, 1990
Botryllus sexiens	100			Hirose *et al.*, 1990
Botrylloides fuscus	100			Hirose *et al.*, 1990
Botrylloides lentus	100			Hirose *et al.*, 1990
Botrylloides simodensis	100			Hirose *et al.*, 1990
Botrylloides violaceus	100			Hirose *et al.*, 1990
Botrylloides sp. (cf. *magnicoecum*)	100	C	8	present study
Family Styelidae				
Subfamily Polyzoinae				
Amphicarpa meridiana	70	B&C	8	present study
Metandrocarpa taylori	100-130	A	1	present study
Metandrocarpa uedai	100			Hirose *et al.*, 1990
Polyandrocarpa misakiensis	100			Hirose *et al.*, 1990
Polyandrocarpa stolonifera	100			Hirose *et al.*, 1992
Polyandrocarpa zorritensis	30	B	14	present study
Polyzoa vesiculiphora	100			Hirose *et al.*, 1990
Protostyela longicauda	100			Turon & Vázquez, 1996
Stolonica socialis	150	A	13	present study
Symplegma reptans	100			Hirose *et al.*, 1990
Subfamily Styelinae				
Asterocarpa humilis	100	A	2	present study
Cnemidocarpa bicornuta	30>?	A	2	present study
Cnemidocarpa clara	100			Hirose *et al.*, 1992
Cnemidocarpa finmarkiensis	absent	A	1	present study
Cnemidocarpa irene	absent			Hirose *et al.*, 1992
Cnemidocarpa nisiotis	absent	A	2	present study
Polycarpa cryptocarpa kroboja	absent			Hirose *et al.*, 1992
Polycarpa maculata	absent			Hirose *et al.*, 1992
Styela clava	30			Hirose *et al.*, 1992
Styela coriacea	absent	A	1	present study
Styela gibbsii	absent	A	1	present study
Styela plicata	absent			Hirose *et al.*, 1990
Family Pyuridae				
Boltenia villosa	80	A	1	present study
Halocynthia igaboja [spine/ body]	40/70	A	1	present study
Halocynthia roretzi (Type A)	50[#]			Hirose *et al.*, 1990
Herdmania momus	100			Hirose *et al.*, 1990
Microcosmus curvus	absent	A	12	present study
Pyura haustor	70	A	1	present study
Pyura mirabilis	100			Hirose *et al.*, 1992
Pyura spinifera [body/stalk]	>30/absent	B&C	14	present study
Pyura sp. (aff. *vittata*)	50>	A	12	present study
Family Molgulidae				
Eugyra arenosa	40	A	13	present study
Eugyrioides glutinans	30			Hirose *et al.*, 1992
Molgula bleizi	50	A	13	present study
Molgula complanata	50	A	13	present study
Molgula echinosiphonica	70	A	13	present study
Molgula occulta (1 day juvenile)	30	A	13	present study
Molgula oculata (4 day juvenile)	30-40	A	13	present study
Molgula manhattensis	40-50			Hirose *et al.*, 1992
Molgula tectiformis	40-50			Hirose *et al.*, 1992
Suborder Aspiraculata				
Family Hexacrobylidae				
Sorbera unigonas	80			Hirose *et al.*, 1992

[#] Protrusions are not papillate.
[†] A-D indicate the pre-fixation media described in "materials and methods".
[‡] Spicules were demineralized with EDTA-2Na after prefixation.
[§] 1, Friday Harbor, Washington, USA; 2, Otago Peninsula, New Zealand; 3, Kochi, Japan; 4, Kerama, Okinawa, Japan; 5, Sakimotobu, Okinawa, Japan; 6, Zamami, Okinawa, Japan; 7, Kuroshima. Okinawa, Japan; 8, Port Phillip, Melbourne, Australia; 9, Otsuchi, Iwate, Japan; 10, Tanabe, Wakayama, Japan; 11, Oki, Shimane, Japan; 12, Chatan, Okinawa, Japan; 13, Roscoff, France; 14, Pearl Harbor, Hawaii, USA.

somewhat hardened tunic spines (Fig. 13). In *Pyura spinifera*, the protrusions were detectable on the tunic of the body proper, while completely absent on that of the much hardened stalk (Fig. 14).

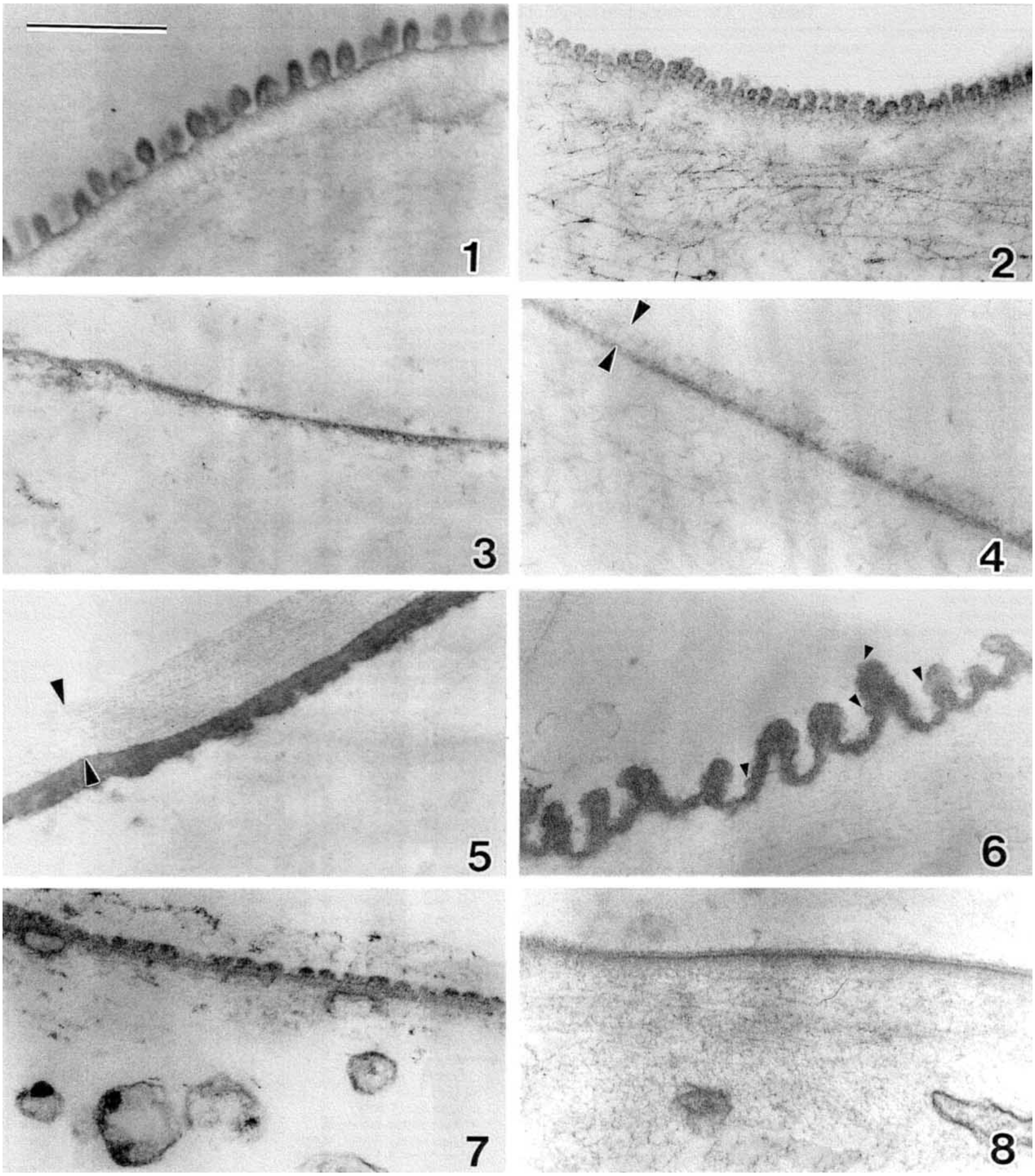

Plate I. Transmission electron micrographs of the tunic cuticle in some aplousobranchs. Fig. 1, *Botryllus schlosseri* (treated with EDTA); Fig. 2, *Aplidium solidum*; Fig. 3, *Placentela crystallina*; Fig. 4, *Eudistoma glaucus*; Fig. 5, *Eudistoma gilboviride*; Fig. 6, *Clavelina cyclus*; Fig. 7, *Distaplia dubia*; Fig. 8, *Distaplia occidentalis.* Arrowheads in Fig. 4 and 5 indicate the epibiont layer covering the outside of cuticle. Small arrowheads in Fig. 6 show the protrusions over the corrugated cuticle (for details see the text). Magnifications are the same in all figures. Scale bar (Fig. 1)=0.5 µm.

DISCUSSION

Stability of the character-state

Table 1 supports our previous conclusion that the presence of cuticular protrusions appears to have a phylogenetic significance in many cases (Hirose *et al.*, 1992), because of the general stability of the character-state (presence or absence) within the families or subfamilies in the traditional classification adopted here. A few exceptions are *Distaplia dubia* (Holozoinae), *Polycitor proliferus* (Polycitorinae), *Pterygascidia longa* (Cionidae), *Microcosmus curvus* (Pyuridae), and the subfamily Styelinae. The cases of *D. dubia* and *M. curvus* are

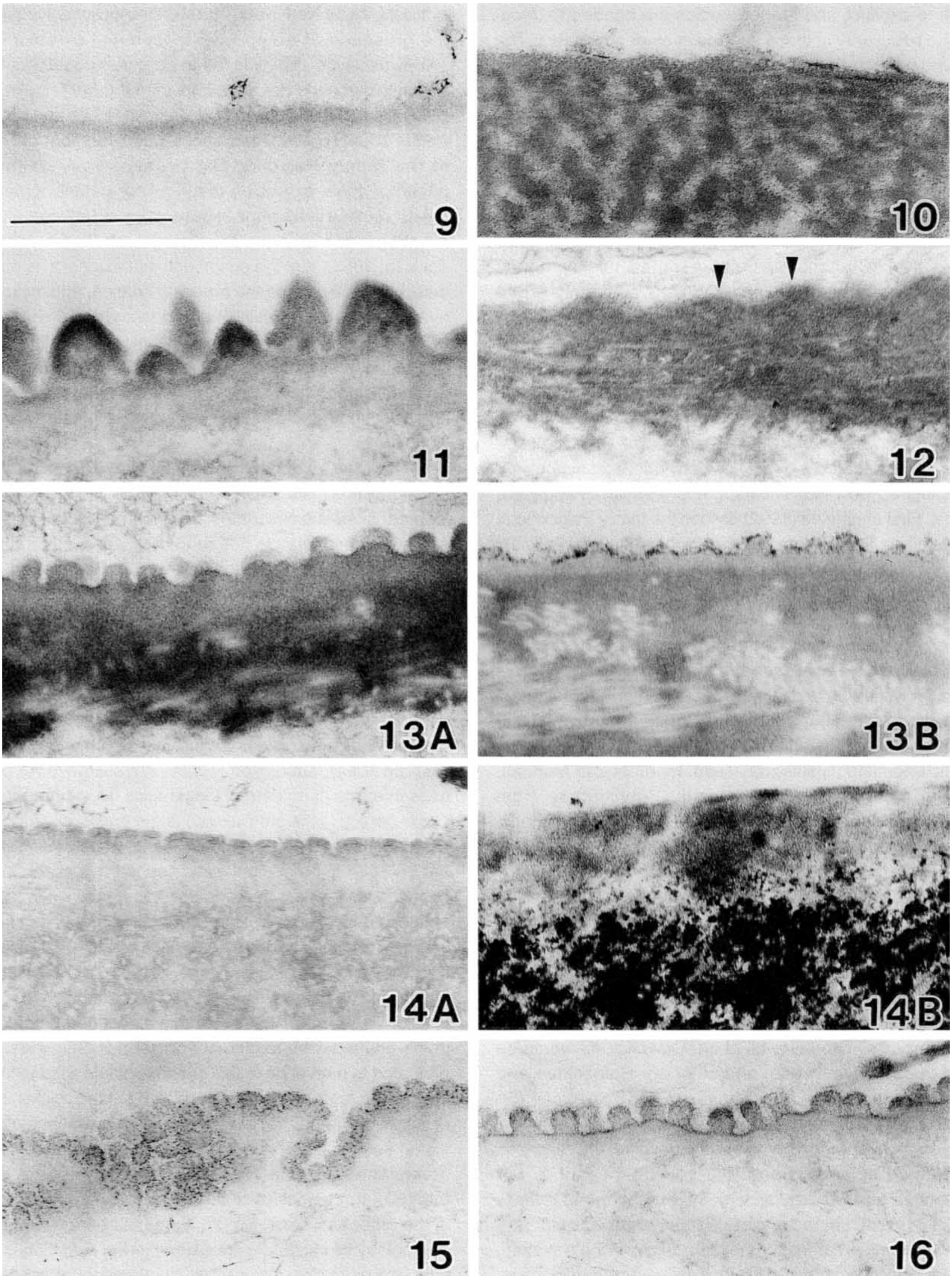

Plate II. Transmission electron micrographs of the tunic cuticle in some phlebobranchs and stolidobranchs. Fig. 9, *Ascidia paratropa*; Fig. 10, *Chelyosoma productum*; Fig. 11, *Stolonica socialis*; Fig. 12, *Cnemidocarpa bicornuta*; Fig. 13, *Halocynthia igaboja* (A, body tunic; B, tunic spine); Fig. 14, *Pyura spinifera* (A, body tunic; B, stalk tunic); Fig. 15, *Molgula bleizi*; Fig. 16, *Eugyra arenosa*. Arrowheads in Fig. 12 indicate projections of uneven surface of the tunic cuticle. Magnifications are the same in all figures. Scale bar (Fig. 1)=0.5 μm.

difficult to explain, and further studies are needed to document the peculiarity of these species in other characters. The observed heterogeneity in character-state distribution in the Styelinae and even within its genera *Cnemidocarpa* and *Styela* might be due to a certain variability in this feature in the concerned clades, as discussed below.

In the Phlebobranchia, cuticular protrusions are absent in most species, but there are two major exceptions: *Pterygascidia longa* in the Cionidae and *Adagnesia vesiculiphora* in the Agneziidae. This fact may support Kott's opinion that "Ciallusiinae", represented by the genus *Pterygascidia*, should be included as a subfamily of the Agneziidae (Kott, 1969, 1985).

The case of *Polycitor proliferus* is worthy of consideration in relation to its somewhat controversial taxonomic position. Kott (1990, 1992) made a major modification in the traditional aplousobranch classification by erecting several new families and raising several subfamilies to family rank. Although she has not yet presented a conclusive phylogenetic hypothesis supporting her new classification, it is relevant to apply our data to it. First of all, Kott (1992) divided the family Polyclinidae into 6 families: Polyclinidae sensu strict (containing *Aplidium* spp. in the present study), Placentelidae (containing *Placentela crystallina* examined here), Protopolyclinidae (no examined species here), Ritterellidae (*Ritterella pulchra* and *Polycitor proliferus*), Euherdmaniidae (no species here) and Pseudodistomidae (*Pseudodistoma kanoko*). According to this system, Polyclinidae is distinguishable from the remaining 5 families by the atrial aperture not 6-lobed and having well-developed cloacal systems. These differences were used by other taxonomists (Nishikawa, 1986; Monniot and Monniot, 1972) to distinguish the subfamily Polyclininae from Euherdmaniinae in the traditional Polyclinidae. Significantly, these character-states parallel the presence or absence of cuticular protrusions, with the single exception of *P. proliferus* which is discussed below. We agree with Kott's delimitation of the family Didemnidae which treats it in the traditional manner.

If we were to follow Kott's (1990) scheme, all of the polycitorid species we examined (Table 1), except *P. proliferus* (see below), of the subfamily Polycitorinae would be included in her family Polycitoridae, all of our Clavelininae would be moved to her Clavelinidae, and all of our Holozoinae and *Sigillina signifera* of our Polycitorinae to her Holozoidae. So far as *S. signifera* (without cuticular protrusions) is concerned, her new system is consistent with our present results, because the absence of protrusions is a consistent trait in our Polycitorinae and Holozoinae with the exception of *Distaplia dubia*. At present, character-state distribution of cuticular protrusions, however, can not completely support Kott's system, because it does not cover all of her aplousobranch families. Therefore, we still prefer to use the traditional phylogenetic system until a comprehensive study is developed in this group.

Polycitor proliferus (Oka, 1933), thus far recorded from Japanese warm waters on the Pacific coasts (Nishikawa, 1995), was transferred to the genus *Ritterella* of the family Ritterellidae by Kott (1969, 1992). This judgment is based on the presence of a short postabdominal extension of *P. proliferus* which she believed to be a true postabdomen because the gonads are situated within it (see Tokioka, 1953, pl. 25, fig. 6). On the other hand, Tokioka (1953) and Nishikawa (1986, 1995) regarded this species as a member of *Polycitor* of the family Polycitoridae because they believe the postabdominal extension is not a true postabdomen. Oka (1942) clarified in his original description of *Polycitor mutabilis*, now regarded as a junior synonym of *P. proliferus* (see Tokioka, 1953), that the postabdominal extension is markedly detectable only during the breeding season, and includes the ovary and testicular follicles but "does not contain the heart" (p. 159). The heart remains in its usual position along the rear end of the intestinal loop (Ebara, 1951). *P. proliferus* seems unusual among its congeners, in which the gonads are located within or along the posterior part of intestinal loop (see Kott, 1990)

Thus, the taxonomic position of *P. proliferus* remains an open question in terms of morphology. In so far as the cuticular protrusions are concerned, as shown in Table 1, their presence in *P. proliferus* is not shared by Kott's Ritterellidae or Placentelidae or our Polycitorinae, but is shared by our Polyclininae, Clavelininae and *Distaplia dubia* in the Holozoinae. We hope that the taxonomic significance of this similarity will be verified by molecular phylogenetics.

Brief phylogenetic considerations

Only a few attempts have been made to present phylogenetic trees of ascidians exclusively on a morphological basis (see Millar, 1966; Kott, 1969). We still have no detailed trees in terms of modern phylogenetics. However, the genus *Ciona* (which lacks protrusions) is generally regarded as the most primitive among extant ascidians, while Stolidobranchia are believed to be monophyletic and to have arisen more recently. If these assumptions are accepted, stolidobranchs obtained cuticular protrusions during their derivation from the common ancestor of ascidians, which would have lacked protrusions as in the modern *Ciona*. In the stolidobranch clade, all families have species with cuticular protrusions.

It follows, then, that the above mentioned heterogeneity in the character-state distribution seen in Styelinae can be attributed to a reversal of this character-state within the clade. Loss of the protrusions may be related to tunic thickening and/or hardening. And the apomorphic state (presence of protrusions) seen in many non-stolidobranchs may have occurred several times in different lineages. Some may well ask whether the tunic protrusions of stolidobranchs are identical to those in the other ascidians. So far, we have no ideas against the homoplasy of cuticular protrusions in the Ascidiacea. However, we must take this possibility into account especially in determining their functional aspects, which are as yet unknown.

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