# A Detailed Description of the Long-Overlooked Tunicate Ascidia protecta (Ascidiacea), Based on the Type and Non-Type Specimens from the Gulf of California

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Overlooked since its establishment, *Ascidia sydneiensis protecta* Van Name, 1945, originally described as having mantle musculature comprising short parallel fibers restricted to the dorsal margin, compared with musculature along the entire margin (except for a central muscle-free area) found in the so-called "*A. sydneiensis* group", is treated as a full species, *Ascidia protecta*. A detailed examination of the type and non-type specimens of the latter, all collected from the Gulf of California, confirmed the mantle musculature arrangement in the species, as well as revealing several new features (particularly in the alimentary tract), although not supporting a protective function of the anterior tunic to retracted siphons, as suggested in the original description. A comparison of *A. protecta* was made with congeneric species with similar musculature.

### Introduction

Ascidia protecta Van Name, 1945 was established by Van Name (1945) for ascidians collected from the Gulf of California, under the trinomen Ascidia sydneiensis protecta, based on the similarity of "a heavy [mantle] musculature of short parallel fibers" characteristic of A. sydneiensis Stimpson, 1855. However, the musculature in Ascidia sydneiensis protecta was described as occupying only the dorsal margin, with "little musculature" in the remaining part of the mantle (excepting siphons), compared with "border[ing] the right side of the body in A. sydneiensis" (Van Name 1945: 190). Furthermore, the possibility was suggested that "during life they [=a pair of long elevated ridges bordering siphonal area of tunic] could close together and serve to protect entirely the retracted tubes, like the two shells of a bivalve mollusk" (Van Name 1945: 190); the dorsal heavy mantle musculature can be supposed helpful for such a retraction. Such a "protective" tunic structure seems to be unique in the family Ascidiidae, but is highly reminiscent of the genus Rhodosoma Ehrenberg, 1828 (family Corellidae), which has an openable lid completely covering the lateral tunic siphonal area. Such structures and functional similarity, if truly comparable, may have resulted from parallel evolution.

Ascidia sydneiensis protecta has remained overlooked during the 75 years since its original description, even being omitted from the Ascidiacea World Database and World Register of Marine Species (WoRMS) (Shenkar et al. 2020). However, we recently located two specimens similar to the original description of *A. s. protecta*, collected from northern-most Gulf of California in 1991 by the late Professor Shigeko Ooishi, a taxonomist of copepods parasitic to ascidians (Damkaer 2015). The specimens were offered to TN and subsequently registered in the Department of Zoology, National Museum of Nature and Science, Tsukuba (NSMT-Pc). Examination of the types of *A. s. protecta*, deposited in the American Museum of Natural History (AMNH), revealed their conspecificity with the non-type material in the AMNH and with the two specimens in 1991. The specimens are described in full herein and their taxonomic status reconsidered. Reference is also made to the "protective" nature of the siphonal area, for comparison with the "lid" structure found in Japanese specimens of *R. turcicum* (Savigny, 1816).

#### Materials and Notes on Type Status

Type-series of *Ascidia sydneiensis protecta*: AMNH Chordata 1711, labelled "<u>Ascidia sydneiensis protecta</u> Van Name, Conception Bay, Gulf of California, [Mexico], 29-III-1940-1711, Holotype & Syntype", 2 specimens, 49.0 and 61.7 mm; the shorter specimen thread-labelled "HOLOTYPE" (typewritten) and "Type" (handwritten) was designated here as the lectotype (see below), the longer specimen, thread-labelled "SYNTYPE" (typewritten) and "Cotype" (handwritten) becoming the paralectotype. Collected by E. F. Ricketts, according to Van Name (1945). The original description noted "Type locality, Conception Bay (two specimens) ... just below low-water level (type, A. M. N. H. No. 1711). A further reference to "Other specimens" (Van Name 1945: 191) indicated that the two AMNH 1711 specimens may have been considered as syntypes, though labelled as above (see International Commission on Zoological Nomenclature 1999: Art. 72.4.7); thus the lectotype and the paralectotype have been designated as above. The AMNH catalogue lists the two specimens incorrectly as "holotype".

Non-type specimens of A. sydneiensis protecta, indicated as such in the original description or collected subsequently: AMNH Chordata 1739, 58.0 mm, labelled "Ascidiacea, Ascidia sydneiensis protecta new subspecies, Puerto Escondido, Gulf of California, Coll. E. F. Ricketts, Leg. March 25-26, 1940, Det. Dr. W. G. Van Name" (typewritten), and "Ascidia sydneiensis protecta new subspecies, Puerto Escondido, Gulf of California, Mar. 25-26, 1940" (handwritten), and "PARETYPE" [sic] (handwritten) (specimen with similar tunic and mantle musculature morphology to type specimens, branchial sac badly damaged) (locality in original description given in error as "Puerto Refugio on Angel de la Guardia Island in the northern part of the Gulf" (Van Name 1945: 191); NSMT-Pc 4481, 26.0 mm and NSMT-Pc 4482, 34.0 mm, both collected from Puerto Penasco, Sonora, Mexico, 0 m depth, 18 December 1991, S. Ooishi coll.

Note on AMNH Chordata 1740: specimen catalogued as "paratype" of *A. sydneiensis protecta*. This specimen differs from the above material, being highly reminiscent of *Acidia interrupta* Heller, 1878, characterized by a dense meshwork of mantle musculature on the right side, and is accompanied by conflicting labels—"<u>Ascidia interrupta</u> det. D. P. Abbott, 1965" (handwritten), "<u>Ascidia sydneiensis protecta</u> Van Name, Gulf of California, April 2, 1940–1740" (typed with the subspecific name handwritten), "PARATYPE", "Puerto Refugio Angel de la Guardia, Gulf of Calif. April 2, 1940" (handwritten), and "Ascidia sydneiensis protecta new subspecies" (handwritten). Included in the original description



Fig. 1. Map showing localities of *Ascidia protecta* Van Name, 1945, including unconfirmed "Angel de la Guardia Island".

under "other specimens", AMNH Chordata 1740 has no type status and was not considered further.

All specimen localities given in Figure 1. All measurements throughout text refer to body length, unless otherwise stated.

### **Taxonomic Accounts**

Family **Ascidiidae** Herdman, 1880 **Ascidia protecta** Van Name, 1945 (Figs. 1–2; Table 1)

Ascidia sydneiensis protecta Van Name, 1945: 190–191.

**Description.** Body 26.0–61.7 mm long, with oval outline, flattened laterally, and attached to substratum usually by entire right (usually more or less depressed) side (entire left side in paralectotype). Both siphons conspicuous, 6-lobed, completely surrounded by a longitudinally elongate, conspicuously ridged oval ring (Fig. 2A, F) (a low ridge only on right side in AMNH Ch. 1739), with minute conical or round papillae, up to 0.5 mm tall (Fig. 2A) (type specimens), or elongate conical projections extending onto ridge and siphon vicinity, up to 3 mm long (NSMT-Pc 4481) (Fig. 2F). Tunic cartilaginous; thick (up to 7 mm), rather tough, translucent pale yellowish (49.0–61.7 mm specimens), thin but tough, translucent white (26.0–34.0 mm specimens). Surface sometimes covered with hydrozoan colonies.

Mantle body often damaged on right side at time of collection (Fig. 2C, H). Branchial siphon terminal, base of more or less elongated atrial siphon at anterior two-fifths of mantle proper (3 larger specimens), near midpoint of mantle (2 smaller specimens); both siphons 6-lobed. Siphonal musculature very well developed; mantle musculature developed only along entire dorsal edge, comprising short, thick, dense, yellowish or fleshy pink transverse fibers (Fig. 2B, C, G, H); muscles absent around neural complex. Epidermis and connective tissue of mantle wall narrowly thickened around siphonal area (Fig. 2B), probably underlying oval tunic ridge. Tentacles simple, mostly elongated of variable size, distribution crowded, numbering ca. 100 (smaller specimens) to ca. 180 (largest specimen); prebranchial zone narrow, smoothsurfaced. Ciliated groove basically C-shaped with both horns rolled inwards (Fig. 2D), but often somewhat complicated (Fig. 2E); a narrow longitudinal groove behind dorsal tubercle 1.5 to 3.5 times as long as tubercle, a neural complex along groove. Edge of dorsal lamina markedly serrated by tips of ribs projecting beyond laminal edge, each rib derived from adjoining transverse vessel. Number of inner longitudinal vessels 36 (L) and >40 (R, uncertain due to damage) in lectotype, 44 (L) and 44 (R) in paralectotype, 24 (L) and 35 (R) in NSMT-Pc 4481, 45 (L) and 50 (R) in NSMT-Pc 4482; up to 140 transverse vessels; secondary papillae distinct, but lacking intermediate papillae. Up to 12 stigmata per mesh in larger specimens, 3-4 stigmata in smaller specimens. Stomach usually oval (Fig. 2B), rarely cylindrical (Fig. 2G), located at postero-dorsal corner of mantle body; internal



Fig. 2. *Ascidia protecta* Van Name, 1945 from the Gulf of California. A–D, lectotype (AMNH No. 1711, 49.0 mm); E, paralectotype (AMNH No. 1711, 61.7 mm); F, NSMT-Pc 4481; G–H, NSMT-Pc 4482. A, tunic, right side, arrow indicates cut made during original examination; B, G, left side of mantle body, LMT and RMT indicate left and right mantle thickening, respectively (probably underlying siphonal area tunic ridge) (both omitted for G); C, H, right side of mantle body (RMT of H omitted); D, E, ciliated groove; F, tunic, left side, hydrozoan colonies over surface omitted. Scales for A–C indicate 10 mm; for D–E, 1 mm; F–H, 5 mm.

G

plication obscure. Intestine almost isodiametric (except in lectotype—bottom part of second loop weakly swollen; Fig. 2B). Visceral mass occupying posterior 75% (lectotype) to 90% of mantle body proper, usually anteriorly well beyond anterior base of atrial siphon (Fig. 2G) (except lectotype—visceral mass extending to midlevel of atrial siphon; Fig. 2B). A tunic vessel issuing from mantle near stomach in lectotype (Fig. 2B) and paralectotype. Bottom (=distal) half of first intestinal loop bent dorsad at 90 degrees or deeper, its axis almost parallel to that of second loop (long axis) of stomach (Fig. 2B, G). Second loop very deep, narrow and closed, its axis passing through middle to end of proximal (pre-bent) half of ascending branch of first loop. Rectum short, with bi-lobed anus. Gonad more or less mature in bottom half of first loop, even in smallest specimen (NSMT-Pc 4482,

34.0 mm long).

**Remarks.** The examined specimens from the Gulf of California can all be regarded as conspecific on the basis of similarities in the tunic depression around the siphonal area with distinct siphons, the mantle musculature (excepting siphonal musculature) situated only along the entire dorsal edge and comprising dense short thick transverse fibers, and a massive visceral mass with a markedly curved first intestinal loop and deep narrow second loop (see also Table 1).

#### Discussion

Distinctness of *A. protecta* from "*A. sydneiensis* group". The mantle musculature of *A. protecta* is markedly differ-

Characters	A. protecta	A. dorsalis	A. papillata
Body length (mm)	26.0-61.7	Up to 20	41
Tunic siphons	Remarkable	Remarkable	?
Surface depression around siphons	Distinct	Distinct only on right	Absent
Tunic surface of siphons	Papillated	Papillated	?
Tunic	Thickened	Firm but thin	Thickened
Location of atrial siphon base on mantle body	Anterior two-fifths or middle	Anterior one-third	Anterior two-fifths*
Muscle-free around dorsal complex	Yes	Yes?*	No*
Approximate number of tentacles	100-180	<50	260
Prebranchial zone surface	Smooth	Papillate	Papillate
Ciliated groove	C-shaped, sometimes complicated	C-shaped	U-shaped
Length of narrow groove behind dorsal tubercle	1.5-3.5 times tubercle length	ca. 10 times* tubercle length	6 times* tubercle length
Free margin of dorsal lamina	Serrated	Serrated	Serrated
Intermediate papillae	Absent	Absent	Absent
Number of longitudinal vessels	24-45 (L), and 35-50 (R)	35 on each side	36 (L) and 41 (R)
Number of stigmata per mesh	Up to 12	4-8	3–5
Visceral mass occupancy in mantle body proper	Posterior 75 to 90%	Posterior half	Posterior two-thirds*
Anterior margin of visceral mass	Anterior to level of atrial siphon	At level of atrial siphon or lower*	At level of atrial siphon*
Tunic vessel issue location	Near stomach	Near middle of ascending branch of intestine	?
Stomach shape	Essentially oval	Oval or cylindrical*	Round
Thickness of intestine	Almost isodiametric	Isodiametric?*	Isodiametric
Curvature in bottom half of first intestinal loop	90 degrees or deeper	Absent	70 degrees*
Orientation of bottom part of first intestinal loop	Parallel to axis of second loop	Antero-posteriorly	Parallel to axis of second loop
Second intestinal loop	Deep and narrow	Deep and open*	Deep and wide*
Axis of second loop (passes through)	Middle to end of proximal half of first loop ascending branch	Pyloric end of stomach*	Middle of proximal half of first loop ascending branch*
Anus	Bi-lobed	Bi-lobed or smooth	?
Locality and depth	Gulf of California, 0 m	New Caledonia, Fiji, and East Australia, to 5 m	Brazil, 3–4 m
References	Van Name (1945), present study	Kott (1985), Monniot (1987)	Bonnet and Rocha (2011)

Table 1. Comparison of morphological features among *Ascidia* species with the mantle (except siphonal) musculature comprising only short dense transverse fibers along the dorsal edge.

\* Determined from illustrations.

ent from that of the so-called "Ascidia sydneiensis group", the latter having "a band of short parallel muscles encircling the right side of the body" (Bonnet and Lotufo 2015), with an apparently muscle-free central region. In fact, the latter region is often provided with thin, almost transparent colorless fibers connecting visible thick marginal muscle bundles (Tokioka 1967: text-fig. 50b; Nishikawa, unpublished observation). Therefore, the Gulf of California specimens described herein should be removed from the "A. sydneiensis group" sensu Kneer et al. (2013) and Bonnet and Lotufo (2015), and given full specific rank as A. protecta. Although its musculature is reminiscent of that of Phallusia recifensis (Millar, 1977) from the Brazilian Shelf (see Millar 1977: 206-208), the morphological distinction is clear between the genera Ascidia Linnaeus, 1767 and Phallusia Savigny, 1816 (both family Ascidiidae), as shown by the lack (in the former) or presence (in the latter) of secondary ciliated grooves opening into the peribranchial cavity.

**Comparison of** *A. protecta* with other *Ascidia* species with similar mantle musculature. Table 1 shows a comparison of *Ascidia* species with the mantle musculature (excepting siphonal musculature) comprising only short dense

transverse fibers along the entire dorsal edge, i.e., A. protecta described herein, A. dorsalis Monniot, 1987 described by Kott (1985: 43-44, fig. 14, pl. Ic) and Monniot (1987: 9-11, fig. 4), and A. papillata Bonnet and Rocha, 2011 by Bonnet and Rocha (2011: 814-816, fig. 2). Ascidia prona Monniot and Monniot, 1994 from Senegal differs in having the mantle musculature with an antero-ventral series of short transverse fibers as well as dorsal musculature (see Monniot and Monniot 1994: 80-81, fig. 4). Table 1 clearly shows that the former three species are distinguishable from one another mainly by differences in the course of the intestine, the curvature of the bottom half of the first loop being very deep (90 degrees or more) and the second intestinal loop deep and narrow in A. protecta, compared with curvature absent and the loop deep but open in A. dorsalis, and curvature of ca. 70 degrees and the loop deep but wide in A. papillata. The species also differ in the number of tentacles (100-180, less than 50, and 260, respectively), the overall morphological differences apparently being sufficient for differentiation of the three species, although their validity for species delimitation should be further examined morphologically (using more material) and by molecular analysis.

Unlikely protective function of siphonal area in *A. protecta.* The initial suggestion of a protective function of an elevated ridge around the siphonal area closing entirely to protect the retracted tubes in *A. protecta* seemed highly unlikely, so far as the preserved specimens were concerned, since the ridge around the siphonal area was not elevated sufficiently for complete coverage of the siphonal area. Such a function, if possible, should necessitate contraction of the underlying transverse muscle fibers, but in fact, their contribution appeared to be insufficient.

The mantle musculature of A. protecta is markedly different from that of the genus Rhodosoma, the only ascidian genus with the ability to cover the tunic siphonal area completely by using openable tunic lips; the left (=lower) lip is fixed, while the right (=upper) lip is movable as a lid margin. A detailed examination of some well-preserved Japanese specimens of R. turcicum disclosed that the musculature consisted of two groups of 5-7 very thick longitudinal bands (number depending on individual), running approximately along the dorsal and ventral aspects, respectively, of the anterior half of the mantle body; many of the bands terminated at the right lip to form sucker-like expansions, the remainder branching and anastomosing anteriorly to widely cover both siphons and the immediate vicinity on the left side. On the right side, the siphonal musculature included a single thick and many thin bundles of short fibers originating from the right lip and ramifying to the siphonal apertures (Kott 1985: 85-86; Nishikawa, unpublished observation). It is suggested here that the left lip is devoid of muscles, the mantle (and siphonal) musculature associated with the right lip being responsible for the opening (due to muscle contraction) and closing (muscle relaxation) movements of the lid. Further functional morphological analyses of the mantle musculature should provide more light on the mechanisms of lid movement.

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