

Three sibling species of didemnid ascidians from northern Norway: *Didemnum albidum* (Verrill, 1871), *Didemnum polare* (Hartmeyer, 1903), and *Didemnum romssae* sp.nov.

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Abstract: Recent advances in taxonomy have disclosed the prevalence of cryptic sibling species in marine systems. In didemnid ascidians, morphological variation between species is often slight, and many species may have been overlooked. Species boundaries within this group are often equivocal, and patterns among taxa are reminiscent of those formed by reticulate evolution in plants. Specimens of the sessile colonial ascidian *Didemnum albidum* (Verrill, 1871) were examined for life-history and morphological characters and found to constitute at least three sympatric sibling species. One of these, *Didemnum romssae*, is described here as a new species. Colonies were collected from hard-bottom assemblages in Troms and Finnmark counties in northern Norway. Zooid morphology in *D. romssae* is similar to that of *D. albidum*, but varies within each species, even for traits usually considered specific. *Didemnum romssae* is proposed as a distinct species on the basis of consistent differences in (i) the shape and size of calcium carbonate spicules within the common test; (ii) larval size and the number of lateral ampullae; (iii) timing of reproduction; and (iv) the absence of a seasonal nonfeeding, overwintering stage, which occurs in *D. albidum*. Examination of specimens from museum collections considered synonymous with *D. albidum* confirmed the presence of a third species, *Didemnum polare* (Hartmeyer, 1903), which differed from the other two species in zooid and larval morphology as well as spicule shape. Complexes of sibling species such as these provide a tractable system for studying the consequences of life-history variation among closely related taxa.

Résumé : Des travaux taxonomiques récents ont révélé l'existence d'espèces soeurs difficiles à distinguer dans les systèmes marins. Chez les ascidies didemnides, la variation morphologique d'une espèce à l'autre est parfois ténue et l'existence de plusieurs espèces a pu passer inaperçue. Les lignes de démarcation entre les espèces sont équivoques chez ce groupe et les relations entre les taxons rappellent celles qui prévalent chez les plantes apparues par évolution en réseau. L'étude de la biologie et des caractères morphologiques chez des spécimens de l'ascidie coloniale sessile *Didemnum albidum* (Verrill, 1871) a révélé l'existence d'au moins trois espèces soeurs sympatriques. L'une d'elles, *Didemnum romssae*, dont on trouvera ici la description, est nouvelle. Des colonies ont été récoltées à même les communautés des fonds durs dans les comtés de Troms et de Finnmark dans le nord de la Norvège. Chez *D. romssae*, les caractéristiques morphologiques des zoïdes sont semblables à celles que l'on observe chez *D. albidum*, mais varient au sein de l'espèce, même celles que l'on considère spécifiques. *Didemnum romssae* est considérée comme une espèce distincte à cause de différences constantes (i) dans la forme et la taille des spicules de carbonate de calcium dans la partie commune du test; (ii) dans la taille des larves et le nombre d'ampoules latérales; (iii) dans le moment de la reproduction; et (iv) de plus, il n'y a pas, comme chez *D. albidum*, de stade saisonnier hivernal qui ne s'alimente pas. L'examen de spécimens de musée considérés comme synonymes de *D. albidum* a confirmé l'existence d'une troisième espèce, *Didemnum polare* (Hartmeyer, 1903) qui diffère des deux autres par la morphologie des zoïdes et des larves et par la forme des spicules. De tels complexes d'espèces soeurs donnent lieu à un système facilement accessible pour étudier les conséquences de différences démographiques entre des taxons très apparentés.

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Introduction

Understanding variability in nature hinges on the identification of groups of organisms that act as independent evolutionary or ecological units. In addition, recognizing cryptic

sibling species in the sea is a prerequisite for understanding the processes underlying speciation and for the assessment of marine biological diversity (Butman and Carlton 1995). Recent detailed studies utilizing morphological features, additional characters, and character states (e.g., life history, reproductive biology, mate-recognition system, allelochemical compatibility, molecular evidence) have revealed the widespread presence of sibling species in marine environments (Knowlton 1993; Knowlton and Jackson 1994a; Jackson and Cheetham 1990). The ubiquity of sibling species should come as no surprise. First, if the gradual accumulation of small changes is one route to the formation of new

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Table 1. Species in the family Didemnidae previously reported from Norwegian waters.

Sources	Distribution in Norway
<i>Didemnum albidum</i> (Verrill, 1871)*	
Hartmeyer 1903 (<i>Leptoclinum albidum</i>)	Spitzbergen, North and West Norway, south to Bergen
Van Name 1945	
Millar 1966	
Lützen 1967	
<i>Didemnum</i> sp.	
(? <i>coriaceum</i> (Van Drasche, 1883))	
Millar 1966 (<i>D. helgolandicum</i>)	South and West Norway, north to Bergen
Lützen 1967 (<i>D. helgolandicum</i>)	
<i>Leptoclinides faeroensis</i> Bjerkan, 1905	
Bjerkan 1905	Spitzbergen, Bear Island, West Norway, south to Bergen;
Van Name 1945	mostly >200 m depth
Millar 1966	
Lützen 1967	
<i>Lissoclinum aureum</i> Verrill, 1871	
Hartmeyer 1903, 1923	Spitzbergen, North Norway†
(<i>Diplosomiodes dubium</i> , <i>D. bathyphylum</i>)	
Van Name 1945	
Millar 1966	
<i>Trididemnum tenerum</i> (Verrill, 1871)	
Hartmeyer 1923	Spitzbergen, Bear Island, North, West, and South Norway
Van Name 1945	
Millar 1966	
Lützen 1967	
<i>Diplosoma listerianum</i> (Milne Edwards, 1841)	
Millar 1966	South and West Norway, north to Lofoten
Lützen 1967	

*Includes *D. polare* and *D. romssae*.

†Reported only from Spitzbergen but common in North Norway (personal observation).

species (Templeton 1989; Gosling 1994), one would expect to find some closely related fossil and extant species that are either incompletely or only recently separated from one another. Rather than a neat world of discretely packaged species, evolutionary and ecological processes yield a continuum of taxa that reflects the dynamic nature of speciation. Second, there is no a priori reason to assume that morphological traits evolve more rapidly than life-history, behavioral, or biochemical characters. Indeed, morphologies may be conserved by selection or developmental constraints. In addition, divergence in, for example, life-history traits without concurrent morphological change can result in the evolution of one or more sibling species. Using life-history traits and morphology, I provide evidence that the didemnid ascidian fauna of northern Norway consists of several unrecognized sibling species.

Ascidians are dominant members of many subtidal, rocky-bottom assemblages. In arctic and boreo-arctic Norwegian waters, six species of colonial ascidians of the family Didemnidae are reported to occur commonly (Table 1). Despite their abundance, the taxonomy of these species is poorly defined.

Within the family Didemnidae, morphological differences among species are often minor. Classification is complicated by the high degree of phenotypic plasticity, which confounds the distinction between species and ecotypes. Species are separated solely on the basis of slight structural differences

in the shape of the gonads, atrial languet, or spicules (see Fig. 1), characters that may vary even within a single colony; thus, assignment to taxon or rank sometimes seems almost arbitrary. This combination of highly conserved morphology across species (and even genera), coupled with extreme phenotypic variability within each species, suggests that traditional methods of classifying this family are inadequate. It is well known that a single morphological "species" may actually comprise a complex assemblage of biological entities (Abbott et al. 1985; White 1978; Templeton 1989) and taxonomists have often failed to recognize many of these cryptic species. The patterns among taxa in this family resemble the product of high levels of hybridization and reticulate evolution seen in many plants (Templeton 1989). Indistinct species boundaries render this group particularly suitable for studies of speciation and of the evolution of phenotypic plasticity and breeding systems (e.g., mictic versus amictic life cycles in clonal organisms) (Carvalho 1994; Knowlton and Jackson 1994b). As many genera of didemnid ascidians have a cosmopolitan distribution, mapping species boundaries within this group may also provide a tractable system for examining global rates of evolution (Rohde 1992).

Didemnum albidum is ubiquitous on subtidal vertical rock walls along the northern Norwegian coast, where it may dominate hard-bottom assemblages at depths below 15 m. *Didemnum albidum* is considered by most taxonomists to be a circumpolar species that exhibits extreme variation in colony

Fig. 1. General representation of the main morphological features of a mature zooid (A) and a didemnid larva (B) (drawn from *Didemnum romssae*).

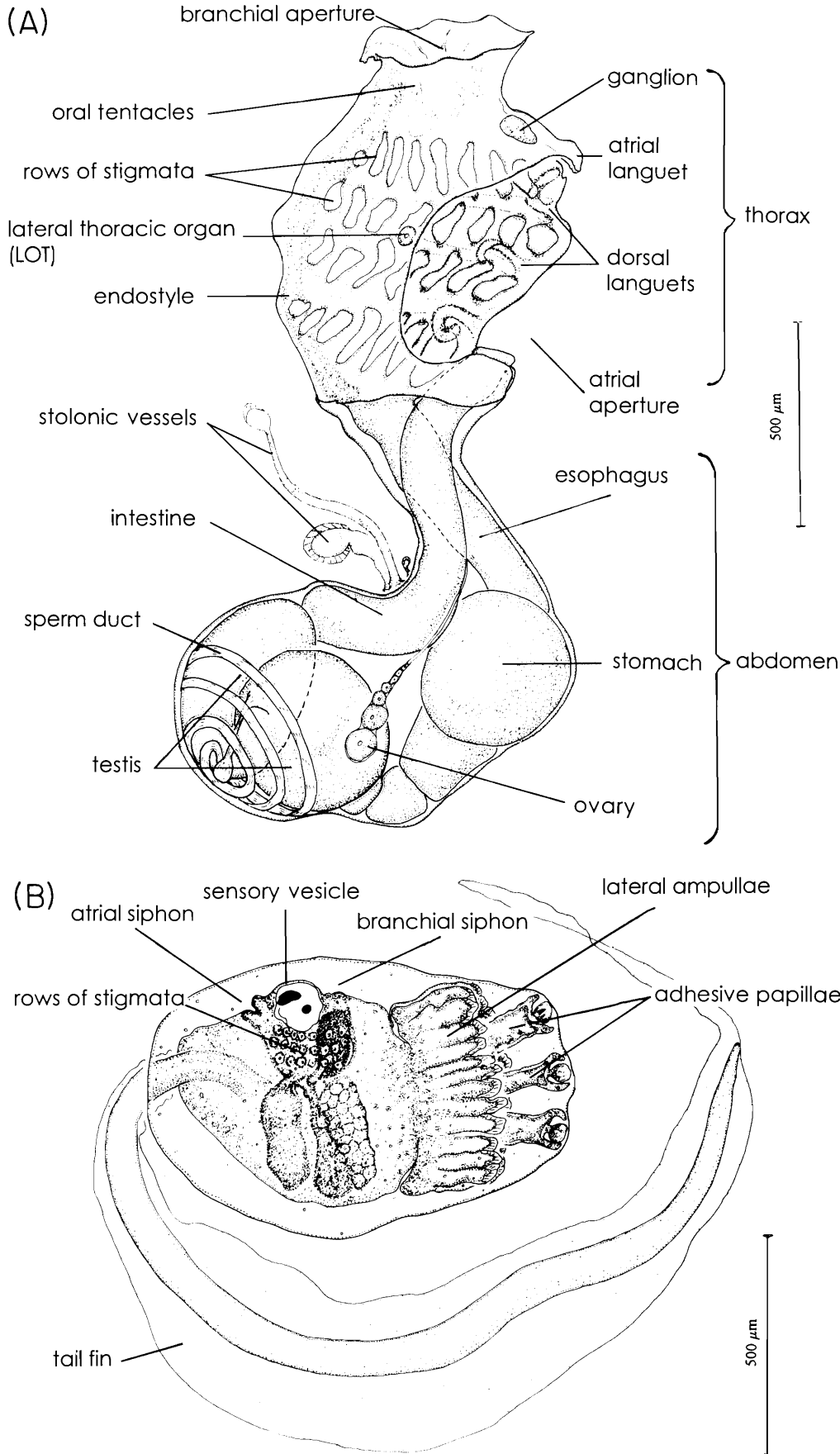
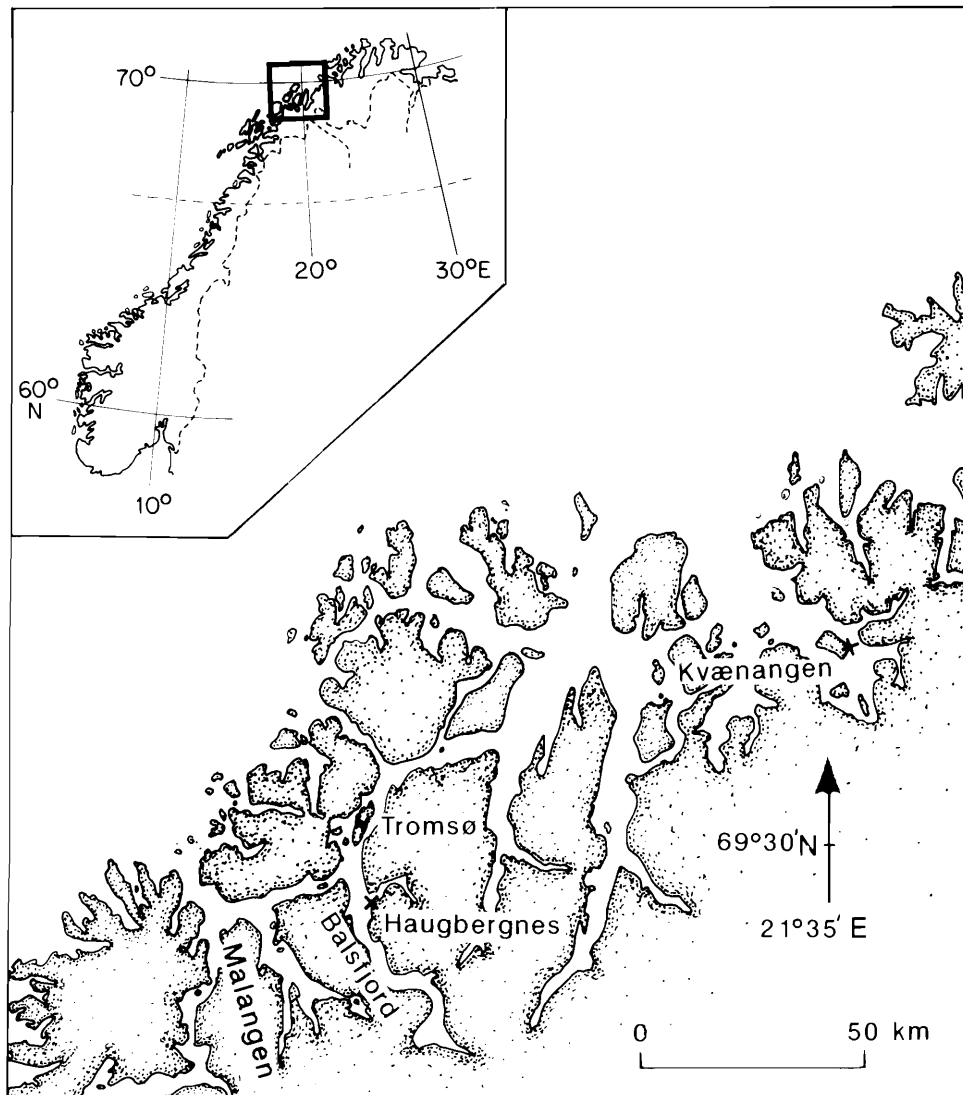


Fig. 2. Map showing collecting sites and the main study site at Haugbergnes in Ramfjorden. Inset: Norway.



color, size, and shape as well as variable spicule morphology. The oldest description of *D. albidum* (Verrill, 1871) is that of M. Sars (1851), who collected it from West Finnmark, Norway, under the name of *Didemnum roseum*. In 1871, Verrill described the same species as *Leptoclinum albidum* (*D. albidum*) from the New England coast of North America. As the name *D. roseum* was already used for another, unrelated species (*D. roseum*, Delle Chiaje, 1828), Verrill's name, "type species," and "type locality" were adopted.

Hartmeyer (1903) described a second species from Spitzbergen (*Leptoclinum polare* (*Didemnum polare*)), based partly on differences from *D. albidum* in spicule shape. Later, he concluded that differences in spicule shape were due to environmental variability, (e.g., depth) and synonymized *D. polare* with *D. albidum* (Hartmeyer 1923). However, the observations which I report here indicate such great variability between colonies that a revision of *D. albidum* is warranted: (i) co-occurring colonies differed in spicule shape and size at several localities in northern Norway, and thus this variation could not be accounted for by depth;

(ii) a degenerated, nonfeeding stage was observed occasionally in colonies at several study sites. Such a resting stage occurred only in some of the colonies and had not previously been described for the Didemnidae.

The aim of this work is to describe the morphological and life-history variation that occurs within *D. albidum*, justifying its division into three species: *D. albidum*, *Didemnum romssae* n.sp., and *D. polare*. The study is based on morphological examination of over 400 colonies from several sites in Norway, the U.S.A., and the Faeroe Islands, as well as specimens from museum collections in Tromsø, Bergen, and Oslo, Norway, and the U.S.A. Additionally, I assessed the seasonal patterns of reproduction and the presence or absence of an annual nonfeeding stage by sampling a putative population of *D. albidum* monthly for 1 year. This paper is part of a larger study on the ecology and systematics of *D. albidum*. A detailed description of the periodic thoracic regeneration that characterizes the seasonal resting stage in this species, along with a discussion of the significance of resting stages in the Didemnidae, will be presented elsewhere.

Materials and methods

Colonies were collected from various sites in Balsfjorden, Malangen, and Kvænangen in northern Norway. The main study site was a subtidal vertical rock wall at Haugbergnes in Ramfjorden (69°31'N, 19°1'E), 15 km south of Tromsø (Fig. 2).

Species belonging to the genus *Didemnum* are polymorphic for many traits. Over 400 colonies from several depths and seasons were sampled to determine which traits represented morphological variation within and between colonies and which traits were species-specific. In addition to samples from northern Norway, four samples identified as *D. albidum* from the coast of Maine, U.S.A., were examined, as well as specimens from the Faeroe Islands. Ascidian colonies and their substrates were collected using SCUBA (0–40 m, Balsfjorden and Kvænangen) or with a dredge (20–400 m, Balsfjorden and Malangen).

Animals were anaesthetized and fixed following the method described by Lafargue and Wahl (1987). Colonies and their substrates were placed in individual containers in aquaria with running seawater until zooids were fully extended. The water current was gradually reduced and a thin layer of menthol crystals was added without disturbing the colonies. When colonies failed to respond to slight mechanical disturbance (ca. 6–8 h), containers were transferred to a freezer and cooled until ice began to form on the water surface. Formaldehyde was then added, to a final concentration of approximately 10%, and colonies were left in this solution for several weeks. Colonies were stored in 4% buffered formaldehyde (v/w in seawater). Anaesthetized colonies to be used for electron microscopy or serial sectioning were transferred directly into a solution of 2.0% glutaraldehyde / 2.5% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and refrigerated.

Isolated zooids were stained using Masson's acid haemalum (Lafargue and Wahl 1987). Formalin-fixed material was rinsed 3 times in distilled water before staining and again after the desired degree of staining was achieved (about 1 min). Samples conserved in glutaraldehyde fixative were rinsed in (0.1 M) sodium cacodylate buffer prior to staining. All samples were then dehydrated (3 × 70% ethanol; 3 × 100% *n*-butanol), mounted on slides with Canada balsam, and dried for 24 h at 60°C.

Isolated larvae were fixed in either the glutaraldehyde–paraformaldehyde solution or a 1:1 solution of 4% formalin and 4% propylene glycol in seawater. Larvae were stained and mounted as described above for zooids.

Specimens identified as *D. albidum* and all probable synonyms were examined from the collections of the Tromsø Museum, the University of Bergen Museum of Zoology, and the University of Oslo Zoological Museum. Verrill's syntypes and hypotypes of *D. albidum* were examined from the collection of the Peabody Museum of Natural History, New Haven, Conn., U.S.A. For all museum specimens, zooid and larval morphology and spicule form were noted. Whenever possible, tissue samples were taken in order to verify spicule shape.

To determine spicule shape, scanning electron micrographs were taken of the spicules of all didemnid species examined. Spicules were isolated by burning samples of colony tissue in a muffle oven at 450°C for 5 h or until only ash remained. This temperature incinerates all organic tissue but leaves the calcium carbonate spicules intact (Harvell and Suchanek 1987). Spicules were rinsed in 70% ethanol, allowed to dry, and mounted on stubs with silver glue. They were then sputter-coated (C/Au) and examined using a JEOL JSM-840 scanning electron microscope.

Spicules from all forms of *Didemnum* spp. were measured to the nearest micrometre to see if spicule sizes differed among colonies of different species and among conspecific colonies. Spicules were obtained by burning tissue samples as described above and then mounted on slides using double-sided sticky tape. Slides were prepared from five colonies of each Norwegian form (*D. romssae* and all forms and color morphs of *D. albidum*) as well as from the four

colonies from Maine, U.S.A. (*D. albidum*). Measurements were made using a microscope at 400×; 100 spicules were counted on each slide.

There is strong evidence that spicule production in the Didemniidae occurs solely in the lateral organs of the thorax (LOTs) present on either side of the thorax, although spicule growth may continue in the common tunic (Lafargue and Kniprath 1978; Kniprath and Lafargue 1980). Seasonal spicule production was assessed by recording the presence or absence of spicules in the LOTs of *Didemnum* colonies collected at Haugbergnes from December 1989 to August 1990.

Seasonal patterns of reproduction were assessed by collecting samples of *Didemnum* spp. from Haugbergnes every 4–8 weeks from August 1989 to August 1990. For each sample, spicule shape was evaluated and colonies were scored for the presence of ovaries, testis, and fertilized eggs as well as brooded immature larvae and fully developed larvae just prior to release. Characterization of the reproductive state of a colony was based on examination of not less than 50 zooids (unless limited by colony size).

Samples for seasonal studies were taken at depths between 20 and 35 m, along 75-m horizontal transects within the main study area. *Didemnum* colonies grow as uniformly flat sheets over the substrate and most, at this depth, grow directly attached to the rock wall. Thus, the majority of samples collected were either whole colonies or portions of colonies (10 × 10 cm, sampled midway between the center and edge of a colony) that were collected by scraping the colonies off the rock wall with a knife. Colonies removed from their substrate in this manner were contracted, could not be relaxed with menthol, and died if kept in laboratory aquaria for more than a few days. Most material for this portion of the study was therefore examined fresh rather than fixed and stained.

Many of the colonies at the study site periodically underwent a resting stage, signaled by degeneration of the thoraces and a glossy surface pellicle, which sealed all the in- and out-current openings. To assess the occurrence of this state of nonfeeding, samples from Haugbergnes were designated "active form" or "resting form."

Results

Species descriptions

Family Didemniidae

Didemnum albidum (Verrill, 1871)
Figs. 3A–3J, 4A, 5A, 5B, 6, 7, 8

Synonymy:

Didemnum albidum Hartmeyer, 1909 (in part), p. 1449; 1921 (in part), p. 81; 1923 (in part), p. 144, Table 1, Fig. 21; Huus 1933 (in part), p. 66 (Hoel's Greenland Expedition); Van Name 1945, p. 80, Figs. 33, 34, Plate 1, Fig. 5; ?Tokioka 1951, p. 2; Millar 1966 (in part), p. 32, Fig. 19; 1988; Lützen 1967 (in part), p. 64; ?Kozloff 1987, p. 473, Fig. 23.13; Romanov 1989, p. 173, Figs. 347, 348.

Didemnum roseum M. Sars, 1851 (in part), p. 153; 1858 (in part), p. 66; Huitfeldt-Kaas 1896 (in part), p. 6; not *D. roseum* Delle Chiaje 1828.

Leptoclinium albidum Verrill, 1871, p. 446; 1872, p. 212; 1874a; 1874b; Hartmeyer 1912 (in part), p. 279.

Leptoclinium luteolum Verrill, 1871 (in part), p. 446; 1872, p. 212; 1874a; 1874b.

Leptoclinium roseum Hartmeyer, 1903 (in part), p. 361, Table 6, Figs. 17, 18; Bjerkan 1908 (in part), p. 98.

Not *Leptoclinium structum* Gottschaldt, 1894.

Tetradidemnum albidum Van Name, 1910, p. 380, Figs. 13, 14, 15, Plate 35, Fig. 2, Plate 39, Fig. 13.

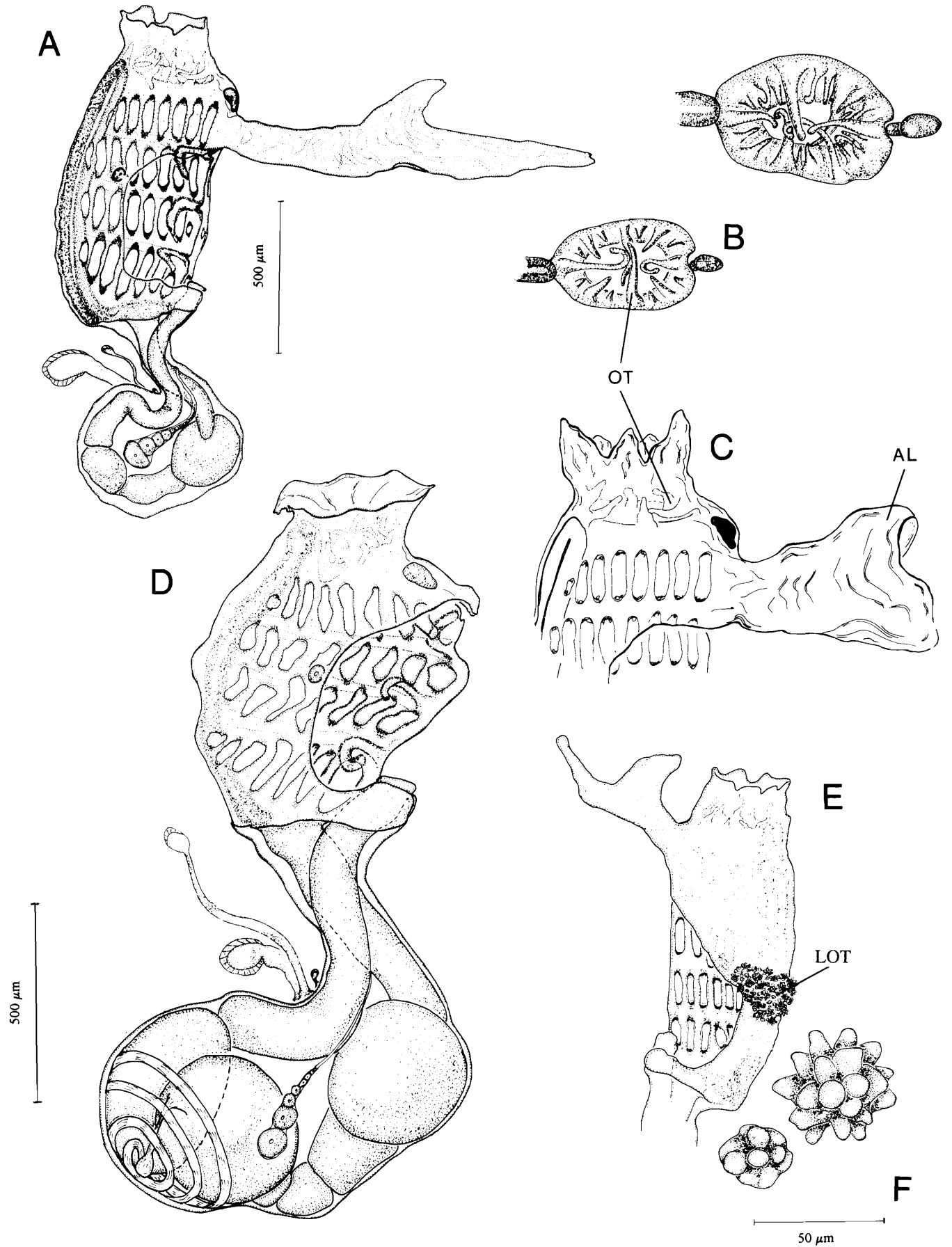
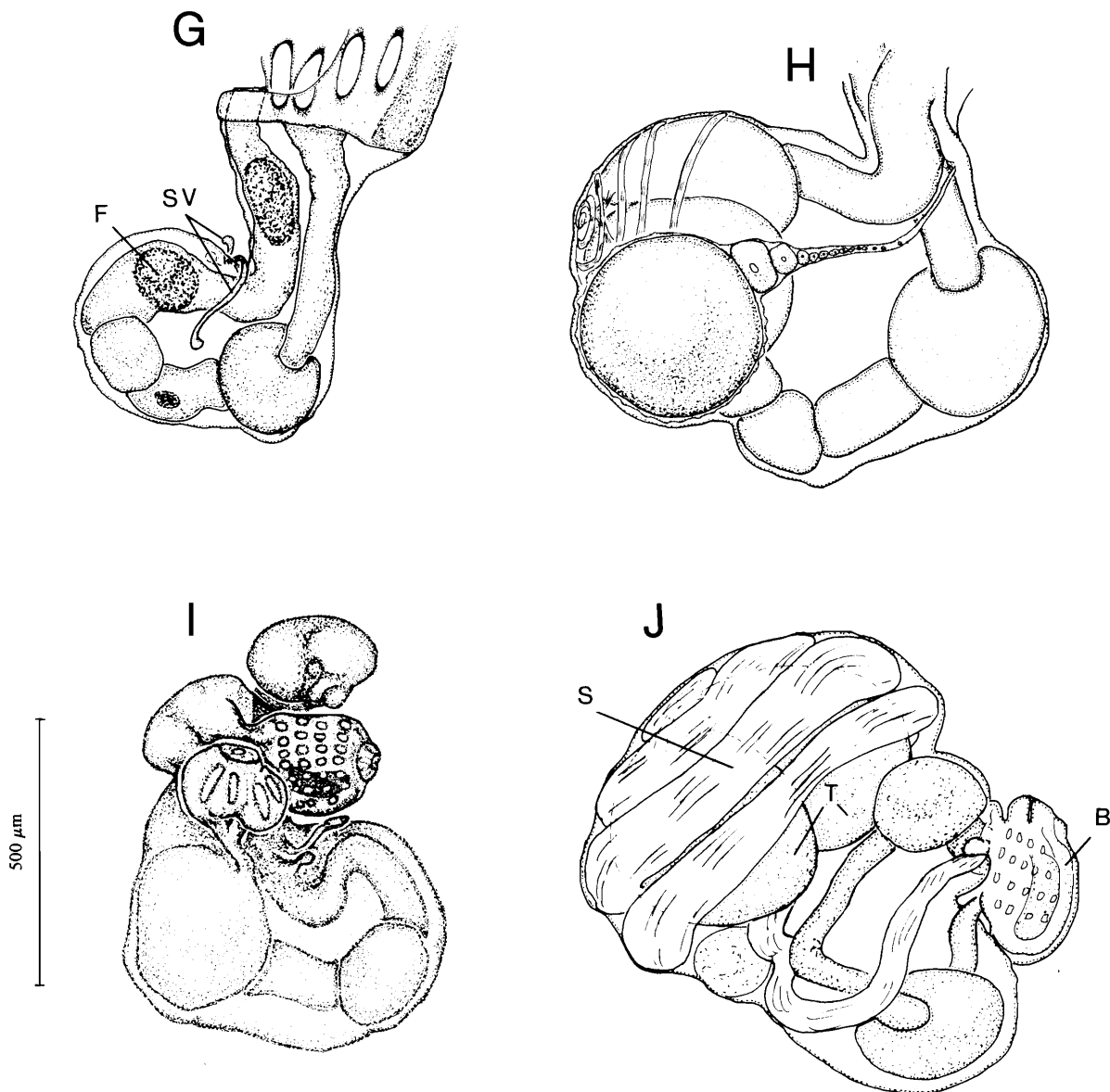


Fig. 3. *Didemnum albidum* and *D. romssae*. (The morphology of mature, adult zooids of these two species is similar.) (A and D) Zooids, orientation "to the left." (B) Tentacular crown; two typical arrangements of oral tentacles (OT). (C) Placement in thorax. (E) Thorax, showing lateral organs of the thorax (LOT) packed with spicules. (F) Partially and fully formed spicules dissected from the spiculagenic organ. Note the variation in the form of the atrial languet (AL) found in both species.

Fig. 3 (concluded). (G) Abdomen from a nonreproductive colony, showing the digestive system and zooid orientation "to the right." SV, stolonial vessels; F, food particle in the gut. (H) Abdomen with mature testis and ovary, zooid orientation "to the left." Drawings A–C, G, and H are from specimens of *D. romssae*; D–F are from *D. albidum*. (I) Resting state of *D. albidum* with no gonads and several budding thoraxes, and (J) with mature testis (T), sperm duct (S), and a single regenerating thorax bud (B).



See Van Name (1945) for a synopsis of North American synonyms.

TYPE MATERIAL: Syntypes: Colonies in Peabody Museum of Natural History, New Haven, Conn., U.S.A. Cat. Nos. YMP 1356 and YMP 1358.

TYPE LOCALITY: Bay of Fundy, Eastport, New Brunswick, Canada.

MATERIAL EXAMINED: One hundred and fifty colonies col-

lected from Haugbergnes in Ramfjorden, North Norway, and surrounding area (12–45 m), 1986–1990; 15 colonies from Kvænangen, North Norway (15–40 m), 1987, 1989; 10 colonies from Malangen and Balsfjorden (20–400 m), 1987–1991; 4 colonies from New England, U.S.A. (collected by K. Sebens): Nahant, Mass., 12 April 1980; Nahant, (40 m), 1 Aug. 1980; Nahant, 12 Aug. 1980; Ram Island, Maine, 14 May 1981. In collection of Tromsø

Museum, University of Tromsø: No. 837, Helgø (90–110 m), 5 July 1925; No. 596, Porsanger (Repvåsneset, 45–60 m), 24 Aug. 1956; No. 122, Bear Island (56–60 m), 30 Aug. 1957; No. 279, Spitzbergen (Dickson Fjord, 12–44 m), 7 July 1958. In collection of Zoological Museum, University of Oslo: No. H1297, labeled “Tromsø & Hammerfest,” 1849 (1 specimen of 7 “syntypes”² of *Leptoclinium roseum* M. Sars, 1851); No. H393, Vegasund (Nordland, 250 m), 15 Aug. 1930; No. H390, H395, Vegasund (Nordland, 190–250 m, 250 m, 2 specimens), 17 Aug. 1930. In collection of Peabody Museum of Natural History, Yale University, New Haven, Conn., U.S.A.: No. 1356, Bay of Fundy, Eastport, New Brunswick, Canada, 1870 (10 specimens, syntypes of *Leptoclinium albidum* Verrill, 1871); No. 1357, Bay of Fundy, Eastport, New Brunswick, Canada, 1868 (20 specimens, hypotype of *Leptoclinium albidum* Verrill, 1871); No. 1358, Bay of Fundy, Eastport, New Brunswick, Canada, 1868 (20 specimens, syntypes of *Leptoclinium luteolum* Verrill, 1871); No. 1360, Bay of Fundy, Eastport, New Brunswick, Canada, 1870 (10 specimens, hypotype of *Leptoclinium luteolum* Verrill, 1871); No. 1361, Grove Point, Lubec, Maine, U.S.A., 1968 (2 specimens, hypotype of *Leptoclinium luteolum* Verrill, 1871); No. 1382, Newfoundland Banks (8 specimens, hypotype of *Leptoclinium albidum* Verrill, 1871).

Simplified diagnosis of the species

Colonies orange to salmon, or white. From late August to May, most colonies are in a nonfeeding stage, recognizable by the thick, glossy surface pellicle that covers all in- and out-current openings.

Zooids have a simple atrial opening with an atrial languet (may be short) and four rows of stigmata in the branchial basket.

The tentacular crown usually has 16 branchial tentacles, in three complete orders.

A round, flattened or spherical LOT is present at a level between the third and fourth rows of stigmata.

The sperm duct is coiled 4–8 times around the testis, which is single or divided in two.

There is no thoracic appendix.

Three stolon vessels are present.

Larvae have three adhesive papillae and 10–17 lateral ampullae per side. They are non-gemmparous and have three rows of stigmata in the branchial basket, with up to seven stigmata per row.

Spicules are stellate, with blunt rays, and range from 20 to 135 μm in diameter (sizes vary somewhat with color morph; see below).

Colony morphology

Colonies collected during this study occurred in two color morphs: the “orange morph” in various shades of either orange or salmon pink and the less common “white morph,” which sometimes had a pinkish hue. Colonies from Maine, U.S.A., were only white or pink (K. Sebens, personal communication). Color morphs are hereafter designated according to both color and location where they were collected: orange Norway (ON), white Norway (WN), or white U.S.A.

(WU). Unless otherwise specified, observations refer to the orange morph from Norway. Common cloacal openings are easily visible in the colony surface, reaching a diameter of 7 mm in colonies with actively pumping zooids.

Zooid morphology

Zooids (Figs. 3A, 3D) range from 1.5 to 2.1 mm in length (thorax 0.6–1.5 mm; abdomen 0.4–0.7 mm; abdomen with well-developed testis and ripe eggs, isolated from “resting stage” colonies, ca. 1.3 mm (Fig. 3J)).

The branchial basket has four rows of stigmata and three curved, posteriorly directed dorsal languets situated to the left of the median dorsal vessel.

The branchial aperture is 6-lobed and the branchial (oral) tentacles are simple. The number of branchial tentacles (Fig. 3B) varies with zooid size; 16 is most common but smaller zooids often have 8–16 tentacles in three orders of size, and very large zooids have up to 26.

The atrial aperture is simple (no siphon), with an atrial languet arising from its anterior margin. The form of the atrial languet varies. Many zooids have a short, narrow atrial languet (Fig. 3D), while in others the languet is drawn out into a long, blunt or forked tongue (Figs. 3A, 3C, 3E). The shape of the atrial languet is sometimes similar in all zooids within an individual colony and sometimes varies among zooids.

A spherical spiculogenic organ (the LOT) is present on each side of the thorax, either as a simple, flattened protrusion (Figs. 3A, 3D) or as a dense mass of spicules (Fig. 3E), at a level between the second and third rows of stigmata. The LOT is an epidermal evagination of the body wall with no connection to the branchial basket, so its relative position along the rows of stigmata varies slightly with the contraction of the thorax.

There is no thoracic appendix (muscular retractor process).

Three stolon vessels of varied length protrude from the center of the abdomen (Figs. 3A, 3D, 3G) below the zooid’s “waist.”

The digestive loop is divided into five parts (oesophagus, stomach, post-stomach, midgut, and intestine) as in other didemnids.

The sperm duct is coiled 4–8 times around the testis (Figs. 3D, 3H, 3J), which is either a single rounded gland or divided in two. As the number of testicular glands is often used as a taxonomic character, it is noteworthy that zooids with a single-lobed testis and zooids with a bilobed testis may occur within a single colony.

The ovary (Figs. 3A, 3D, 3H) is elongated and, when still small, situated beside the ascending portion of the sperm duct. The most posterior eggs in the ovary are most developed. Small, immature eggs are nearly transparent, while larger eggs (>250 μm) contain yolk and are bright orange (white in the white morph). No more than two eggs with yolk are present in the ovary at any time, and only one of these is fully mature (Fig. 3H). Mature eggs reach a diameter of 450 μm .

The arrangement of the digestive loop and gonads is usually of the *Didemnum* type (i.e., oriented “to the left,” sensu Lafargue and Laubier 1980). The intestine crosses over the oesophagus, with the stomach on the right side when the gonads are on the side nearest the observer. The ovary lies near the stomach and the testis is terminal, with the

² Sars incorrectly designated these 7 samples, from two localities, as “syntypes” and preserved them in a single container.

Table 2. Diameters of spicules from all forms of *Didemnum albidum* and *D. romssae* examined in this study.

Source of spicules	Diameter (μm)			<i>n</i>
	Minimum	Maximum	Mean \pm SD	
<i>D. albidum</i>				
Orange morph, "active form," Norway	23.65	133.30	61.8 \pm 17.9	500
Orange morph, "resting form," Norway	24.38	126.14	63.4 \pm 14.8	500
White morph, "active form," Norway	20.14	63.60	41.5 \pm 6.5	500
White morph, "active form," Maine, U.S.A.	26.50	94.46	52.1 \pm 10.9	400
<i>D. romssae</i>	15.05	88.00	34.2 \pm 7.6	500

sperm duct rolled around it counterclockwise from the testis apex (Figs. 3A, 3D, 3H). However, colonies can be found in which some zooids are oriented with the intestine *parallel* to the oesophagus (i.e., "to the right"; Fig. 3G), while neighboring zooids are oriented as described above.

Larval morphology

Larvae (Figs. 4A, 8) are large (trunk length 1.0–1.4 mm, length with tail extended 2.70–2.75 mm) and are non-gemmiparous, with no sign of blastozoid development before metamorphosis. They possess a rudimentary adult pharynx, siphons, and gut, as well as a sensory vesicle with a well-developed otolith and ocellus. Larvae close to hatching have three rows of stigmata in the pharynx, with up to seven stigmata per row. Three adhesive papillae are present, surrounded by 10–17 lateral ampullae per side. Bladder cells (ca. 100 μm) are conspicuous under the cuticle.

Larvae from the white morph differ from those of the orange morph only in size (trunk length 0.7–0.95 mm for specimens from Norway, 0.75–0.95 mm for samples from the coast of Maine, U.S.A.).

Spicule shape

Spicules are stellate, with smooth, blunt projections radiating from a central point (Fig. 5A). Although variation in spicule shape occurs within each colony (Fig. 6), all spicules follow the same basic design. Spicules from "active" colonies are identical in shape with those from "resting-stage" colonies.

The white morph (from Norway and Maine, U.S.A.) has stellate spicules resembling those of the orange morph, but with slightly shorter and blunter or bifurcated projections (Figs. 5B, 7).

Spicule size

Spicules are 20–135 μm in diameter (mean 61.7 \pm 17.9 μm) (Table 2). Spicules from the white morph of *D. albidum* (from Norway and Maine, U.S.A.) are slightly smaller than those of the orange morph from Norway (Table 2).

Reproduction

It is noteworthy that there was always at least a small proportion of colonies (ON) with male and female gonads at each sampling date, and fertilized eggs were present throughout most of the year (Fig. 9A). The period of active sperm pro-

duction may be overestimated, as no distinction was made between different stages of spermatogenesis. Colonies brooded larvae from December until May, and actively released larvae over the same period but in slightly lower percentages (Fig. 9B). The highest proportions of colonies both brooding and releasing larvae occurred in December.

Norwegian colonies of the white morph (WN) with brooded larvae were observed in March, August, and October. Two white colonies from Maine, U.S.A. (WU), collected in April and August, were brooding larvae.

Resting stage

Didemnum albidum exhibits a periodic, colony-wide renewal of zooids. Colonies undergo an annual period of nonfeeding, during which the thoraces completely degenerate and subsequently regenerate from buds on the persisting abdomen (Figs. 3I, 3J). This process occurs primarily during the fall and winter (ca. 5 months' duration) and appears to be an overwintering adaptation. It differs from the cyclical regeneration of parts in many other colonial animals (e.g., polypoid regression in bryozoans) in which the colony itself remains actively feeding. Not a form of true dormancy (developing larvae are often brooded in the common test of nonfeeding colonies), this process is termed a resting stage. A detailed description of the timing and morphology of the resting stage, and its significance, is provided elsewhere (Marks 1993, 1996).

The white morph of *D. albidum* (WN) has a resting stage similar in appearance (except color) to that of the orange morph. Resting-stage colonies have been collected in August and October. One colony from Maine, U.S.A. (WU), collected in August, also appeared to be in a resting stage.

Distribution

In this study, I have not attempted a detailed investigation of the distribution of *D. albidum* in northern Norway. Samples obtained by diving during the study indicate that this species occurs in Ramfjorden, along with other sites in Balsfjorden and in Kvænangen (Fig. 2), in hard-bottom assemblages. *Didemnum albidum* (ON) was commonest below 15 m at these locations, growing on primary substrate (bare rock wall) or secondary substrate (other organisms, mussels). Colonies were also collected by trawling at sites in Malangen

and Balsfjorden (Fig. 2), on boulders and secondary substrate, between 20 and 400 m.

The white morph (WN) was found at nearly all of the same locations in Norway as the orange morph. Additionally, it occurred as epibiotic growth on scallops (*Chlamys islandica*) at several locations where the orange morph was absent.

Museum specimens of *D. albidum* confirm reports of this species from Spitzbergen and Bear Island. Other reports in the literature, lacking voucher specimens, are difficult to interpret, owing to the confusion of the three species described herein. Accounts that include a description or illustration of spicule shape suggest that *D. albidum* is fairly common along the Norwegian coast as far south as Bergen. It has been found from Gullmarsfjorden, Sweden (Ärnback Christie-Linde 1934), Scotland (Millar 1988), and Japan (Tokioka 1951), though these are isolated reports. It has also been described from the coasts of Greenland, Iceland, and the Faeroe Islands, but these reports now need to be corroborated to determine the species.

Didemnum albidum (WU) occurs along the eastern North American coast from Canada south to Cape Cod (Massachusetts, U.S.A.), though it is common only as far south as Boston. With few exceptions, specimens reported south of Cape Cod probably represent another species (*Didemnum candidum lutarium* (Van Name, 1945)).

Kozloff (1987) provides the only published report of *D. albidum* from the west coast of North America, and assigns to this species the commonest didemnid of the San Juan Islands and the Puget Sound region, but further investigation is needed in order to verify the identify of this species there.

Ecology

Actively feeding colonies of the orange morph from Norway (ON) occasionally reach a diameter of 40 cm, and a diameter of 20 cm is common. Colonies grow as flat, encrusting sheets on most available types of hard substrate. Colony thickness varies between 3 and 15 mm in "active colonies," while "resting-stage" colonies can be as thin as 2 mm. The resting stage is recognizable by its smooth, glossy surface and the absence of both in- and out-current openings. While actively feeding colonies are usually epibiont-free, resting-stage colonies often have heavily fouled free surfaces.

Colonies of the white morph (WN) are generally small, rarely reaching a diameter greater than 10 cm.

Didemnum romssae new species

Figs. 3A–3H, 4B, 4C, 5C

Synonymy:

Didemnum albidum Hartmeyer, 1921 (in part); 1923 (in part), p. 144; Huus 1933 (in part), p. 66 (Hoel's Greenland Expedition); Millar 1966 (in part), p. 32; Lützen 1967 (in part), p. 64.

Didemnum roseum M. Sars, 1851 (in part), p. 153; 1858 (in part), p. 66; Huitfeldt-Kaas 1896 (in part), p. 6; not *D. roseum* Delle Chiaje 1828; Romanov 1989, p. 169, Figs. 336–342.

Leptoclinium roseum Hartmeyer 1903 (in part), p. 361, Table 14, Fig. 17; Bjerkan 1908 (in part), p. 98.

TYPE MATERIAL: Holotype and six paratypes consisting of

fragments of several large colonies representing various stages of reproductive maturity. Deposited in the Tromsø Museum collection (University of Tromsø, Norway) Nos. ASC 971–977.

TYPE LOCALITY: Vertical rock wall at Haugbergnes in Ramfjorden, North Norway (69°31'N, 19°1'E), 20–30 m deep.

ETYMOLOGY: The specific name refers to *Romssa*, the Samisk language name for Tromsø, Norway, where this species was first collected.

Material examined

One hundred and eighty colonies collected from Haugbergnes in Ramfjorden, northern Norway (12–40 m), and surrounding area (1986–1990); 15 colonies from Kvænangen, northern Norway; 55 colonies from Malangen and Balsfjorden at 20–400 m (35 from scallop bed at Berg in Balsfjorden at 20–60 m). In collection of Tromsø Museum: No. 311, "Tromsø II" (35–45 m), 1 Sept. 1917; No. 246, Vargsund (Alta, 40–50 m), 25 July 1931; No. 247, Porsangerfjord (260 m), 28 July 1931; No. 248, Varangerfjord (25 m), 17 July 1937; No. 836, Balsnes (Troms, 28 m), 1 Oct. 1954; No. 840, Tisnes (Troms, 18–35 m), 5 Oct. 1954; No. 589, Tisnes (Troms, 30–37 m), 8 July 1955; No. 591, Kvalsund (20–30 m), 14 July 1955; No. 515, Spitzbergen (50–65 m), 18 Aug. 1958; No. 280, Lyngholmen (26–44 m), 26 Aug. 1959; No. 593, Breisund (100 m), 14 Sept. 1959. In collection of Zoological Museum, Oslo: No. H1297, "Tromsø & Hammerfest," 1849 (6 specimens of 7 "syntypes" of *Leptoclinium roseum* M. Sars, 1851); Saltstraumen, 23 July 1894; Kaasvik, 1894; Skrova, 1897; Ramfjorden (Troms, 18–30 m), 16 Aug. 1903; Kongsfjord (Spitzbergen, 25–30 m), 28 Aug. 1905; H391, Franz Josefsfjord (170 m), 12 Aug. 1932.

Simplified diagnosis of the species

Colonies are orange to salmon in color and may reach up to 40 cm in diameter. No degenerated, resting stage occurs in this species and colonies remain actively feeding year-round.

Mature zooids are morphologically similar to those of *D. albidum*.

Larvae are smaller than those of *D. albidum*, with three adhesive papillae, and 9–12 lateral ampullae per side (arranged in a crown).

Spicules are spherical, with numerous pointed rays, and are 15–90 µm in diameter.

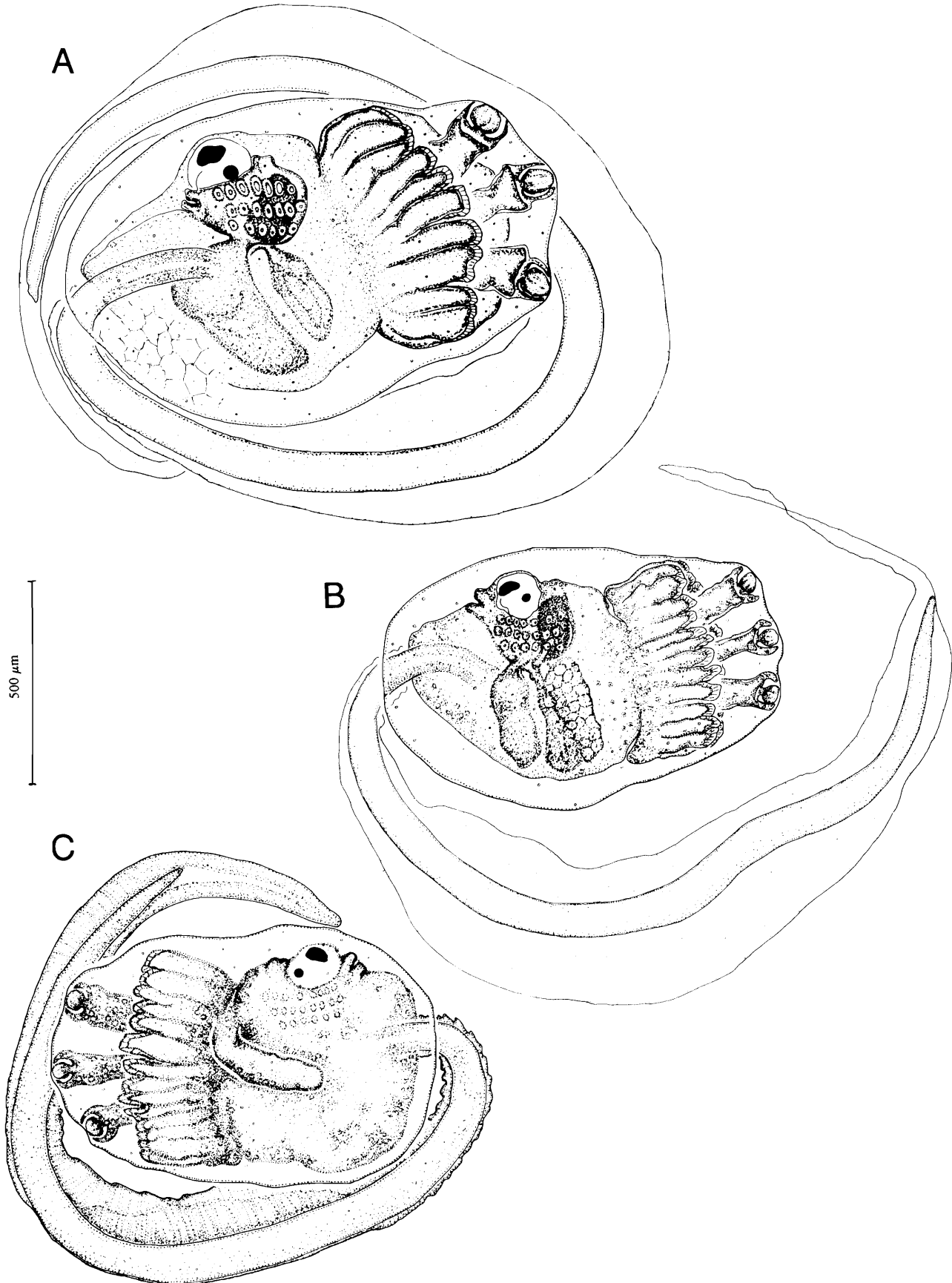
Colony morphology

All samples of *D. romssae* were bright orange or salmon color in life. Colonies are similar in morphology and size to "active" colonies of the orange morph of *D. albidum*, and the two are usually indistinguishable in the field.

Zooid morphology

Mature, fully developed zooids of *D. romssae* are identical with those of *D. albidum* (above), and the morphology of individual zooids in this species also varies greatly within and between colonies. Morphological variations illustrated in Figs. 3A–3H are thus also representative of *D. romssae*.

Fig. 4. (A) *Didemnum albidum* larva. (B and C) *Didemnum romssae* larva, showing internal (B) and surface (C) morphology. For an explanation of larval structures see Fig. 1B.



Larval morphology

Larvae measure 0.8–1.15 μm (trunk length; mature eggs to 350 μm) (Figs. 4B, 4C) and have three adhesive papillae surrounded by 9–12 lateral ampullae per side.

Spicule shape

Spicules are round, with many small pointed spines projecting from a spherical mass (Fig. 5C), differing dramatically from those of *D. albidum*. All spicules within a colony had this characteristic shape.

Spicule size

Spicules are 15–90 μm in diameter (mean $34.20 \pm 7.56 \mu\text{m}$ (Table 2)).

Reproduction

Colonies with male and female gonads first appeared in December (Fig. 10A) and their numbers peaked in March. Colonies brooding larvae and those releasing larvae were present in small numbers in December, increased until March (90% of colonies were releasing larvae in March 1990), and then gradually decreased; no colonies releasing larvae were observed from May to December (Fig. 10B).

Resting stage

Synchronous colony-wide degeneration and regeneration of zooids do not occur in this species; all colonies remain actively feeding throughout the year.

Distribution

The local distribution of *D. romssae* is similar to that of *D. albidum*. *Didemnum romssae* occurs in all the habitats and depths described above, as well as in several hard-bottom sites (scallop beds) where *D. albidum* is not found.

This species also occurs along the coasts of Spitzbergen and Bear Island and along the Norwegian coast as far south as Saltstraumen, Nordland County. Additionally, some descriptions of *D. albidum* from other localities may, in part, refer to *D. romssae*.

Ecology

Colonies grow as encrusting sheets on most hard-bottom substrates. They may reach a diameter of 40 cm and thickness of ca. 10–15 mm (when actively pumping water). During the winter months, *D. romssae* is more easily distinguishable from *D. albidum* in the field, as it has no degenerated, resting stage.

Didemnum polare (Hartmeyer, 1903)

Fig. 5D

Synonymy:

Didemnum albidum Hartmeyer, 1921 (in part); 1923 (in part), p. 144, Table 1, Figs. 22, 23; Huus 1933 (in part), p. 66. (Hoel's Greenland Expedition); Millar 1966 (in part), p. 32; Lützen 1967 (in part), p. 64.

Didemnum albidum polare Van Name, 1945, p. 82.

?*Didemnum caudliculatum* Romanov, 1989, p. 167, Figs. 329–335.

Didemnum polare Hartmeyer, 1909 (in part), p. 1451.

Didemnum tenue Hartmeyer, 1909 (in part), p. 1451; 1912, p. 279.

Leptoclinium gelatinosum M. Sars, 1851, p. 154; ?1858, p. 66; not *L. gelatinosum* Milne Edwards 1841.

Leptoclinium polare Hartmeyer, 1903, p. 363, Plate 14, Figs. 18–21.

Leptoclinium roseum Bjerkan, 1908 (in part), p. 98.

?*Leptoclinium structum* Gottschaldt, 1894, p. 357, Table 24, Figs. 4a, 4b; Table 25, Fig. 9.

Leptoclinium tenue Herdman, 1886 (in part, specimens from the North Atlantic only), p. 281, Plate 40, Figs. 3–5; Hartmeyer 1912, p. 279; not *L. tenue* Sluiter, 1898.

Not *Leptoclinium albidum* nor *L. a.* var. *luteolum* Herdman, 1886, pp. 287, 290, Table 40, Figs. 10–14.

Material examined

Three colonies from Haugbergnes in Ramfjorden, North Norway (ca. 25 m). Four colonies from the Faeroe Islands, epibiotic on *Stryphnus ponderosus* (collected by A. Klitgaard): Biological Investigations on the Faeroe Islands (BIOFAR) Stn. 453, 62°45'N, 07°10'W (400 m, 2 colonies), 3 June 1989; Stn. 540, 61°33'N, 08°16'W (480 m, 2 colonies), 31 July 1989. In collection of Tromsø Museum: No. 837 (b, in part), Helgø (90–110 m), 5 July 1925; No. 590, Glomfjord (100–200 m), 8 Aug. 1955; No. 281, Bear Island (Straumrevet, 24 m), 29 Aug. 1957; No. 558, Storlisnaget (180–190 m), 1 Aug. 1960; No. 831, east of Storlisnaget (200 m), 1 Aug. 1960. In collection of University of Bergen Museum of Zoology: No. 31811 (in part), Florvaagskjær (150 m), 1921; No. 31812 (in part), Herløfjord, Bergen (130–190 m), 1921; No. 31813, Osterfjord, Bergen (550 m), 1921; No. 45586, Kvinnheradsfjord (390–670 m), 1956; No. 50207, Ålfjorden (Vikebygd, 180 m), 1957. In collection of Zoological Museum, University of Oslo: No. H1298, Hammerfest, 1849 (holotype of *Leptoclinium gelatinosum* M. Sars, 1951); (2 specimens; 20–100 fathoms (1 fathom = 1.828 m)), 1907; No. H379, Nathorstfjord (137 m), 4 Aug. 1932.

Simplified diagnosis of the species

Colony morphology is similar to that of *D. albidum*, described above, except that the colonies are less compact. Thin strands of tunic substance connect the zooids to a lower, denser layer of tunic, rather than completely surrounding the zooids. In some colonies, the oral openings are star-shaped and ringed with spicules. Colonies from Norway were white or orange, while those from the Faeroe Islands were orange.

Zooids have a simple atrial opening, with no atrial languet. Four rows of stigmata are present in the branchial basket.

An elongated, wing-shaped LOT is present at the level of the fourth row of stigmata.

The sperm duct is coiled 7–10 times around the testis, which may be single or bilobed.

A thoracic appendix (muscular retractor process) is present at the peduncular insertion.

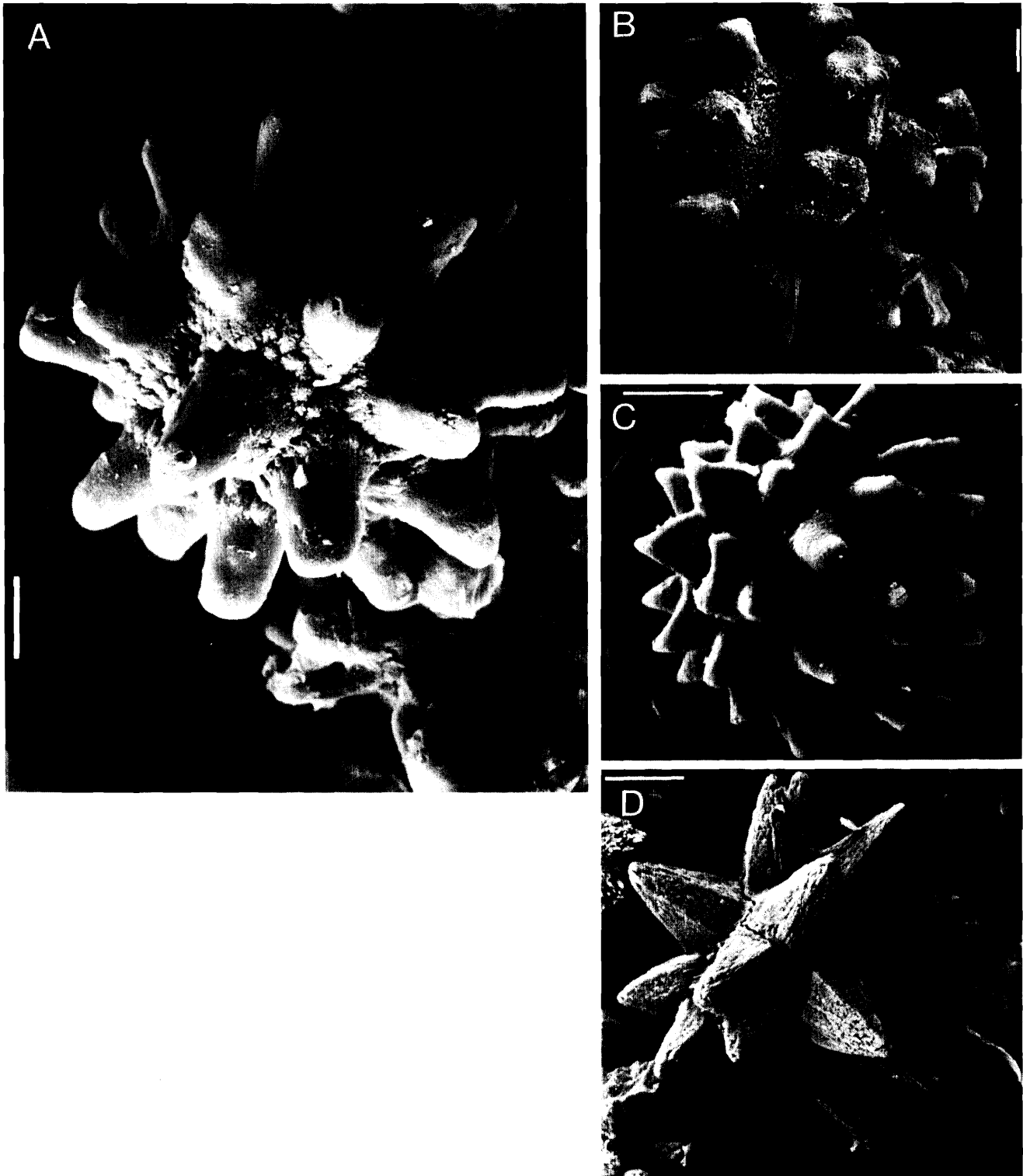
Larvae have three adhesive papillae and seven or eight lateral ampullae per side.

Spicules are stellate, with a few long, pointed and crested rays (Fig. 5D) and are 20–55 μm in diameter.

Distribution

Based on data from museum collections and reports by Hartmeyer (1903, 1923), *D. polare* occurs around the Faeroe Islands, Spitzbergen, and Bear Island as well as along the

Fig. 5. Scanning electron micrographs of spicules. (A) *Didemnum albidum*, orange morph. (B) *Didemnum albidum*, white morph. (C) *Didemnum romssae*. (D) *Didemnum polare* (specimen from the Zoological Museum, Oslo). Spicule rays are etched from preservation in formalin. Scale bar = 10 μm .



coast of the Norwegian mainland as far south as Hardangerfjord. As colonies are superficially indistinguishable from *D. albidum*, it is probably also included in descriptions of this species from other localities.

Hartmeyer describes *D. polare* as an exclusively arctic,

deep-water species, occurring only at depths greater than 100 m. Material presented here shows that this species also occurs in shallow water. However, *D. polare* is reported from greater depths (up to 1040 m) than either *D. albidum* or *D. romssae*.

Fig. 6. Scanning electron micrograph of *Didemnum albidum* spicules, showing intracolony variation in spicule size and shape. Scale bar = 10 μm .

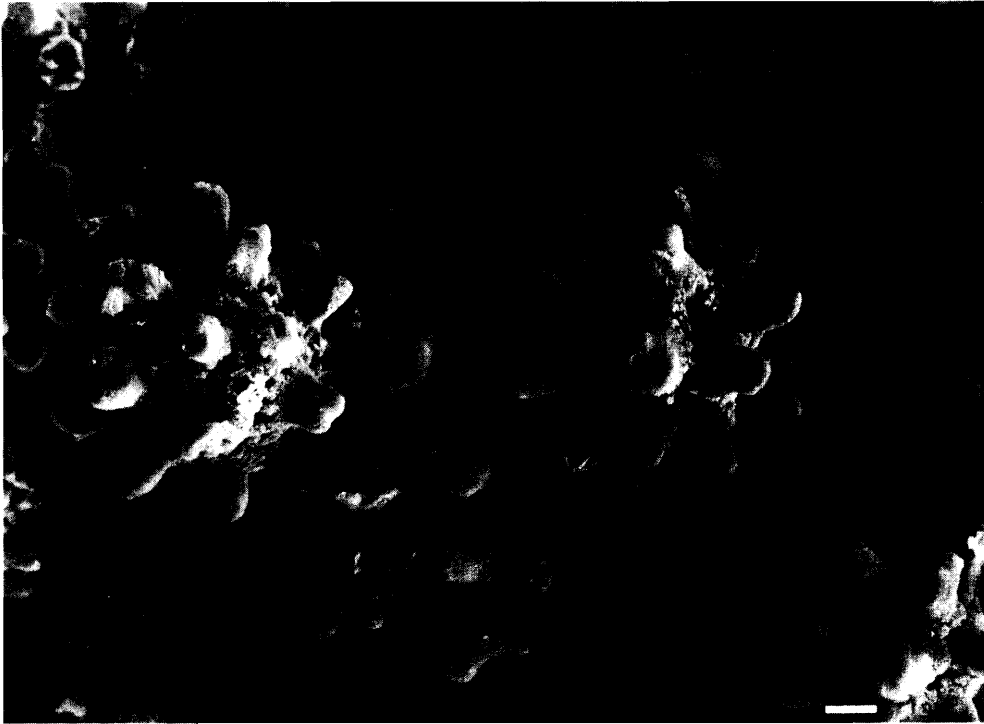
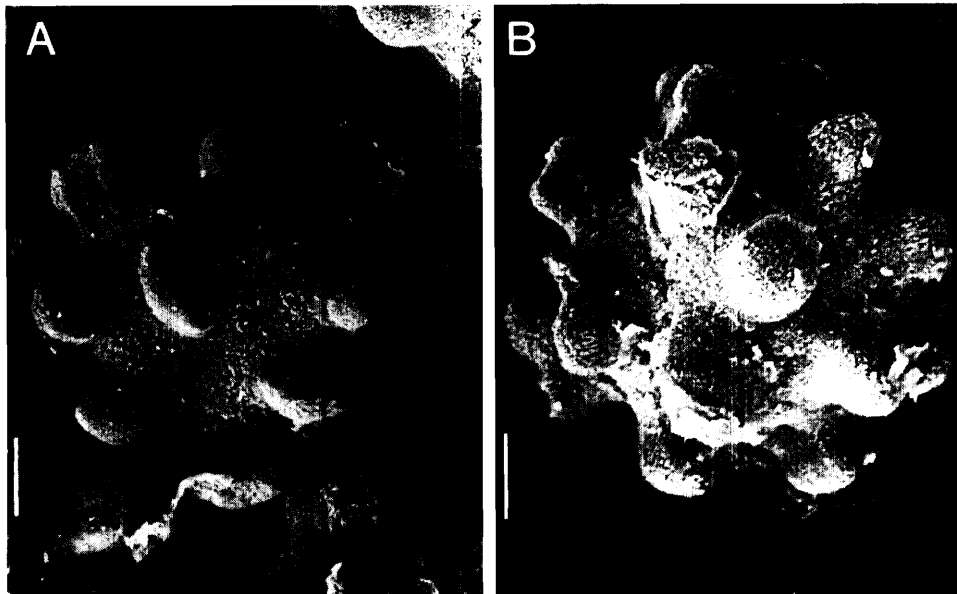


Fig. 7. Scanning electron micrographs of spicules from white morphs of *Didemnum albidum* from Norway (A) and Maine, U.S.A. (B). Scale bar = 10 μm .



Comparison between *Didemnum albidum* and *D. romssae*

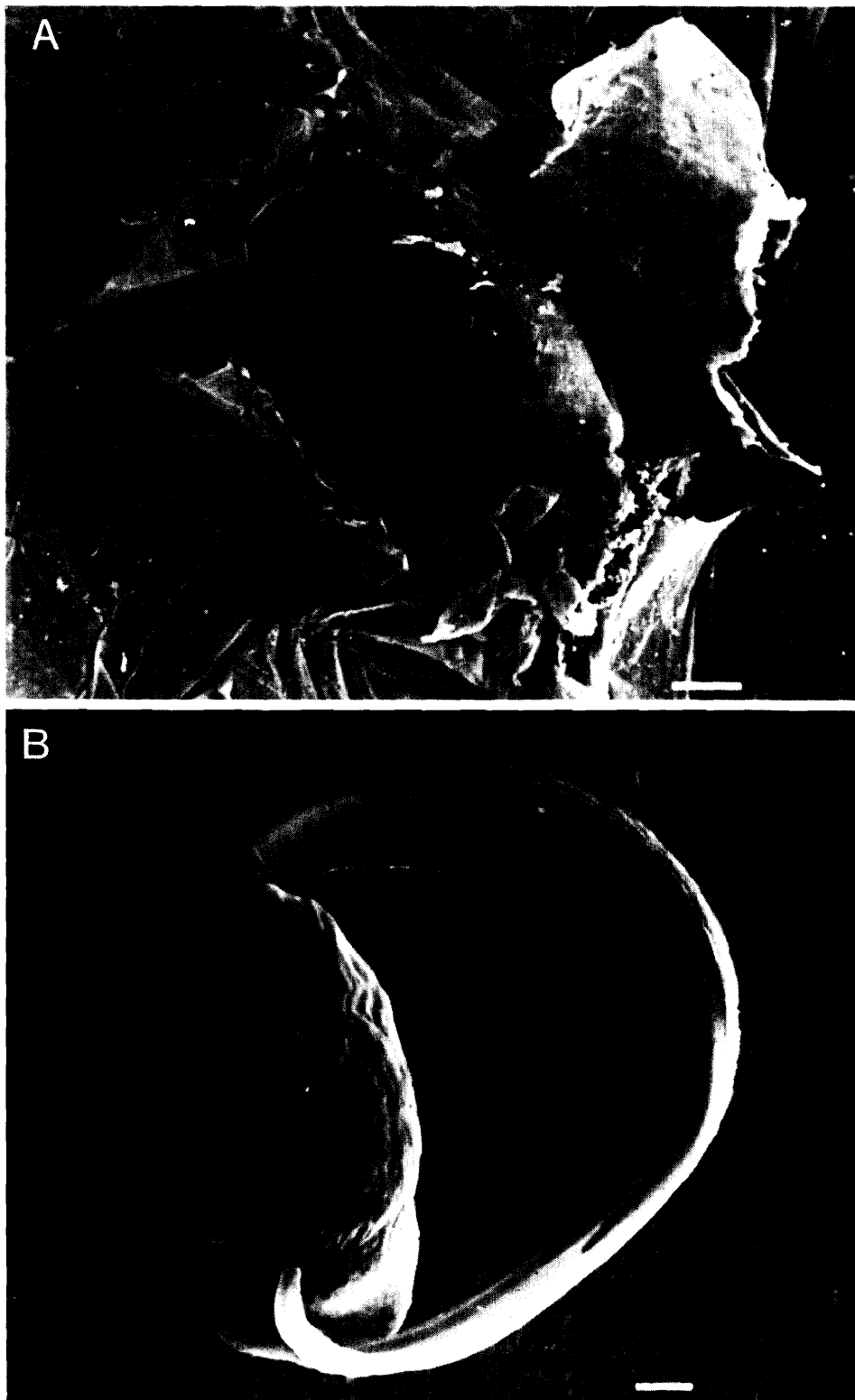
Table 3 summarizes morphological and life-history traits of all forms of *Didemnum* examined in this study. No consistent differences were observed between the mature, fully developed zooids of *D. albidum* and *D. romssae*, although zooid morphology was highly variable both within and between colonies. These two species differed, however, in (i) larval size and morphology, (ii) spicule shape, (iii) maximum spic-

ule size, (iv) reproductive timing, and (v) the presence of a degenerated "resting stage" in only one species.

Larval morphology

Larvae produced by these two species can be distinguished by visual inspection (Fig. 4). The eggs and larvae of the orange morph of *D. albidum* are noticeably larger than those of *D. romssae*, although size ranges do overlap. Larvae of

Fig. 8. Scanning electron micrographs of a resting-stage colony of *Didemnum albidum*. (A) Brooded larva rupturing the colony surface. (B) Larva. Scale bar = 100 μ m.

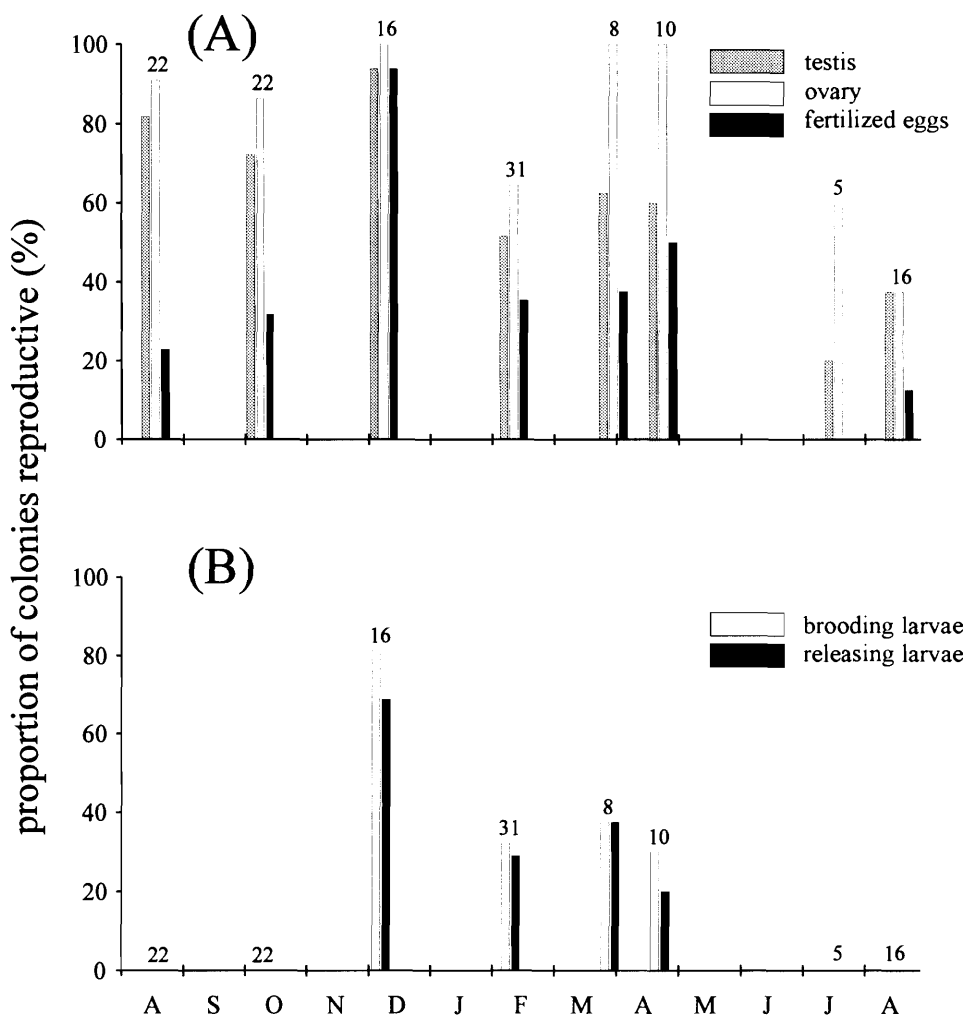


D. albidum can often be distinguished by the number and arrangement of lateral ampullae (10–17 per side, compared with 9–12 per side, arranged in a crown, in *D. romssae*). In addition, *D. albidum* larvae have conspicuous test cells, not clearly visible in larvae of *D. romssae*.

Spicule shape and size

As with most species in the family Didemnidae, calcium carbonate (aragonite) spicules were abundant in the common test of all specimens examined. Size distributions for spicules from all forms are summarized in Table 2. Spicule shape was

Fig. 9. Proportion of *Didemnum albidum* colonies with testis, ovaries, or fertilized eggs (A) and colonies brooding and releasing larvae (B) from August 1989 to August 1990. The sample size is given above each column. Sampling dates less than 2 weeks apart with small sample sizes were pooled (August, October, December, February).



characteristic for each species (Fig. 5, Table 3). No colony was observed with spicules intermediate in shape between those typical of *D. albidum* and *D. romssae*, although the exact shape of spicules varied slightly between color morphs and with geographic area.

Both mean and maximum spicule size were also characteristic for each species, although size ranges overlapped (Fig. 11, Table 2). The white morphs of *D. albidum* from both Norway (WN) and the U.S.A. (WU) had mean spicule diameters intermediate between those of the orange morph (ON) and *D. romssae*. There was no significant difference between the size-frequency distributions of *D. romssae* and either white morph (WN, WU) of *D. albidum*, but all three differed significantly from the orange morph of *D. albidum* (Kolmogorov–Smirnov two-sample test, $p = 0.05$). Frequency distributions are shown in Fig. 11.

There was no significant difference between the mean diameters of spicules from ‘active’ and ‘resting’ colonies of *D. albidum* (orange morph, ON) (Kolmogorov–Smirnov two-sample test, $p = 0.05$). However, spicules from these

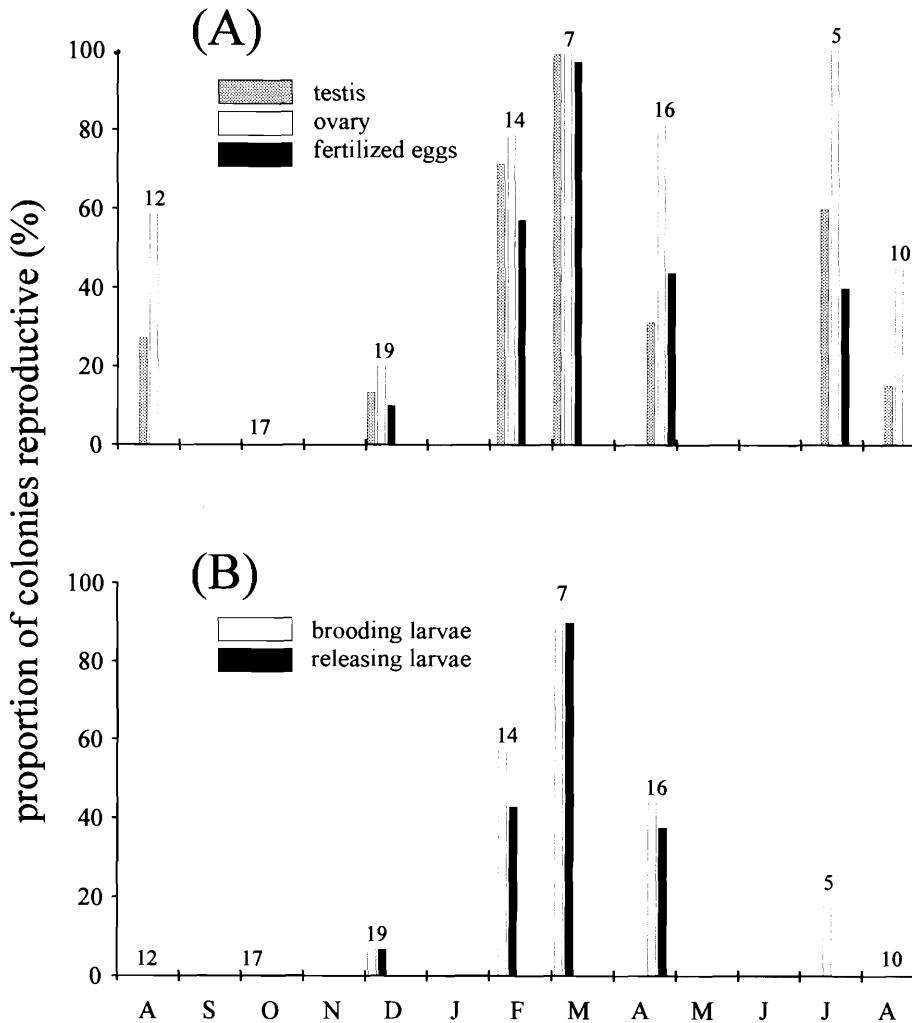
two forms differed in the shape of their size-frequency distributions; the ‘active’ form had a greater number of spicules in the smaller size classes. This could be a consequence of the fact that resting-stage colonies were not actively growing and thus not actively producing spicules.

Spicule production

Seasonality of spicule production, as assessed by the presence of spicules in the LOTs, was similar in these two species. *Didemnum albidum* did not produce spicules during the winter months (Fig. 12A). Spicule production was first observed in April, increased throughout the summer, and began to decrease in late August (when the sampling period ended). Because resting-stage colonies of *D. albidum* lack a thorax (and thus a LOT), spicule production is reported as the proportion of colonies with thoraces (active form) that had spicules in the LOT. Not surprisingly, spicule production coincided with periods of active colony growth (based on unpublished growth rates).

Spicule production in *D. romssae* was low during the winter

Fig. 10. Proportion of *Didemnum romssae* colonies with testis, ovaries, or fertilized eggs (A) and colonies brooding and releasing larvae (B) from August 1989 to August 1990. The sample size is given above each column. Sampling dates with small sample sizes that were less than 2 weeks apart were pooled (August, October, December, March).



months, increased throughout the spring to 100% in June and July, and then decreased again in the fall (Fig. 12B). No connection was seen between spicule production and any trait characterizing the feeding activity of a colony.

Reproduction

As is typical of colonial asidians, *D. albidum* and *D. romssae* are hermaphroditic and produce short-lived nonfeeding larvae. Fertilization is internal and single larvae are brooded in lacunae in the common tunic and are released by rupturing the tunic wall (Fig. 8). Development of the ovary slightly preceded that of the testis in both species (unusual in that most didemnids are slightly protandric hermaphrodites (Lafargue and Wahl 1987)). The timing of gonad production and larval brooding and release differed between *D. albidum* and *D. romssae* during the study period. In *D. albidum*, the greatest proportion of colonies releasing larvae was observed during the winter, while most *D. romssae* colonies released larvae in the spring. Reproductive seasons did, however, overlap (Figs. 9, 10).

Resting stage

Didemnum albidum underwent a seasonal colony-wide degeneration and regeneration of zooids ("resting form"), while no synchronous zooid degeneration was observed for *D. romssae*. A detailed description of the morphology and timing of the resting form is provided elsewhere (Marks 1993, 1996).

Museum collections

To verify my identifications of the didemnids described above, as well as to gain information on the taxonomy and distribution of these species, the specimens on which M. Sars based his original descriptions of *D. albidum* (in the collection of the Zoological Museum at the University of Oslo) were examined. All specimens under the name *D. albidum* and all probable synonyms in the collections of the Zoological Museum at the University of Oslo, the Museum of Zoology, Bergen, and the Tromsø Museum, Tromsø, Norway, were also examined (Table 4). Although samples from the museum collections were often in poor condition,

Table 3. Comparison of selected characteristics of *Didemnum albidum*, *D. romssae*, and *D. polare*.

	<i>D. albidum</i>			<i>D. romssae</i>	<i>D. polare</i> †
	Orange morph	White morph	U.S.A. samples*		
Zooids					
Zooid size (mm)	1.5–2.1	1.5–2.1	1.5–2.1	1.5–2.1	1.2–1.8
Thoracic appendix?	No	No	No	No	Yes
No. of oral tentacles	16	16	16	16; 27	?
Larvae					
Larval size (mm)‡	1.0–1.4	0.7–0.95	0.7–0.95	0.8–1.15	0.6–0.9
No. of lateral ampullae (per side)	10–17	(10–17?)	(10–17?)	9–12	7–8
Spicules					
Shape	Stellate; blunt rays	Stellate; short blunt rays	Stellate; short blunt rays	Spherical; pointed rays	Stellate; long pointed rays
Size (µm)§	40–85	35–50	40–65	25–45	20–55
Life history					
Period spent incubating larvae	Winter–spring	(March, October, August)	(April, August)	Spring–summer	?
Resting stage?	Yes	Yes	Yes	No	Yes?
Time of resting stage	Peak in fall–winter	(August, October)	(August?)	—	?

Note: Data in parentheses represent limited observations.

*Four samples only.

†Museum samples only.

‡Trunk length.

§Of spicules measured, 90% fall in this range.

Table 4. Summary of species identifications, depths, and distribution of museum specimens under the name *Didemnum albidum* examined from the collections of the zoological museums of Bergen, Oslo, and Tromsø.

Museum	<i>Didemnum albidum</i>	<i>Didemnum romssae</i>	<i>Didemnum polare</i>
Tromsø	4	12	5
Oslo	4	12	3
Bergen	0	0	5
Total	8	24	13
Depth (m)	12–250	18–260	24–670
Distribution	Nordland coast, Troms, Finnmark, Bear Island, Spitzbergen	Nordland coast, Troms, Finnmark, Bear Island, Spitzbergen, Franz Joseph's Fjord	Hardangerfjord, Bergen, Troms, Finnmark, Bear Island

and thus identification based on zooid morphology was not always possible, the following distinctions were made.

Colonies contained three spicule types, which corresponded to *D. albidum*, *D. romssae*, and *D. polare* and matched the descriptions of these species given above. Sars' (1851) original specimens of *D. albidum* (under the name *D. roseum*) consisted of several colonies representing both *D. albidum* and *D. romssae*.

In the absence of type material for *D. polare*, specimens described by Hartmeyer (1923) as exemplifying the "typical" *D. polare* spicule form were examined. These specimens were consistent with *D. polare* with regard to both spicule shape (stellate with a few long, pointed spines) (Fig. 5D) and zooid and larval morphology (the presence of a thoracic appendix, a simple atrial opening, an elongated wing-shaped LOT, and larvae with six or six to eight lateral

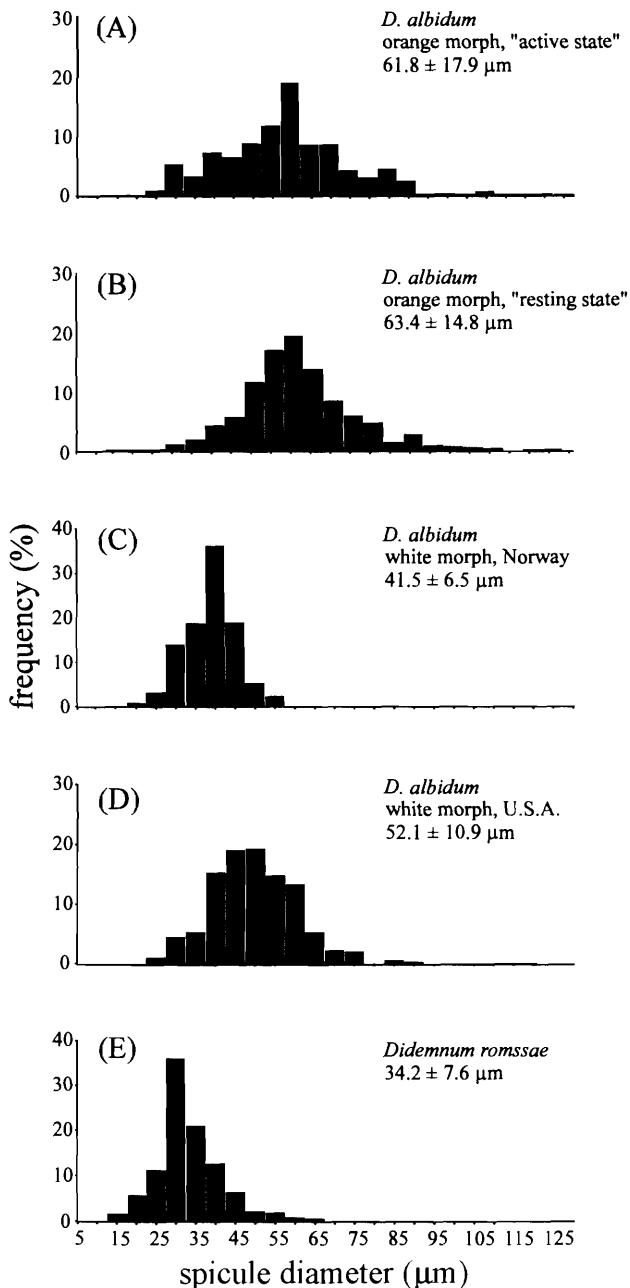
ampullae per side). *Didemnum polare* accounted for 29% of museum samples under the name *D. albidum*.

Verrill's syntypes and hypotypes of *L. albidum* and *L. luteolum* (= *D. albidum*; collected 1868–1870) in the collection of the Peabody Museum of Natural History, Yale University, were also examined. Colony, zooid, and spicule morphology was consistent with that of *D. albidum* as described in this paper. Spicule shape and size most closely matched those of the white morph of *D. albidum* from Norway.

Discussion

The results show that *D. albidum* in Norway consists of at least three species. *Didemnum romssae*, n.sp. is superficially similar to *D. albidum* but actually possesses a set of traits that

Fig. 11. Spicule sizes (mean \pm SD) and frequency distributions of spicule diameters for all *Didemnum albidum* forms (A–D) and for *D. romssae* (E). Values on the x axis are midpoints of the classification intervals ($n = 500$ for A–C and E; $n = 400$ for D).



consistently differ from those of *D. albidum*, which justifies their separation into two species. These traits include (i) spicule morphology and maximum spicule size, (ii) larval size and morphology, (iii) timing of reproduction, and (iv) the presence or absence of an annual nonfeeding stage. The white morph differed from the orange morph of *D. albidum* only slightly in spicule form and color, and, in the absence of life-history data, was classified as a color morph only. Museum specimens of *D. albidum* revealed a third species, found only rarely in my samples, that meets the criteria of *D. polare* (Hartmeyer, 1903), previously considered a synonym or subspecies of *D. albidum*.

Spicules

It is well established that the calcareous aragonite spicules of the Didemnidae are taxonomically diagnostic (Monniot 1970; Lafargue and Kniprath 1978; Kniprath and Lafargue 1980; Lafargue and Laubier 1980; Turon 1986; Brookfield 1988). Less certain is the taxonomic specificity of spicule shape, size, and mineral composition. Lafargue and Laubier (1980) and Turon (1986) studied the spicules of the Didemnidae from the coasts of France and northeastern Spain. From both studies it was concluded that spicule morphology (shape and number of rays) remains fairly constant within each species throughout these geographic areas. Species with unlike spicules are generally grouped as one if this is the only distinguishing characteristic. More work, however, is needed to elucidate the amount of intra- and inter-specific variation in spicule form that is due to environmental versus genetic factors.³ This would strengthen the position of spicule form as a useful diagnostic tool for classifying both living and fossil didemnids. Differences in spicule morphology may parallel divergence in sibling species or morphotypes. The patterns of spicule size and shape reported here were found to be consistent in co-occurring colonies over a range of geographically and environmentally varied habitats.

The genus *Didemnum* is notable for the similarity in spicule morphology between different species (Turon 1986). Species in this genus characteristically have round or stellate spicules with pointed rays. *Didemnum albidum* is thus easily recognizable by the smooth, blunt rays of its spicules, and (given the difficulties that burden didemnid identification) has often been identified on this basis alone. Spicule form varies slightly between the white and orange morphs of *D. albidum*. However, the variation in spicule shape within the orange morph (ON) encompasses the shapes observed in the white morphs from both Norway (WN) and the U.S.A. (WU). In the absence of any other morphological distinctions, the diversity of spicule shape and size observed in all morphs of *D. albidum* examined in this study is considered to represent intraspecific variation.

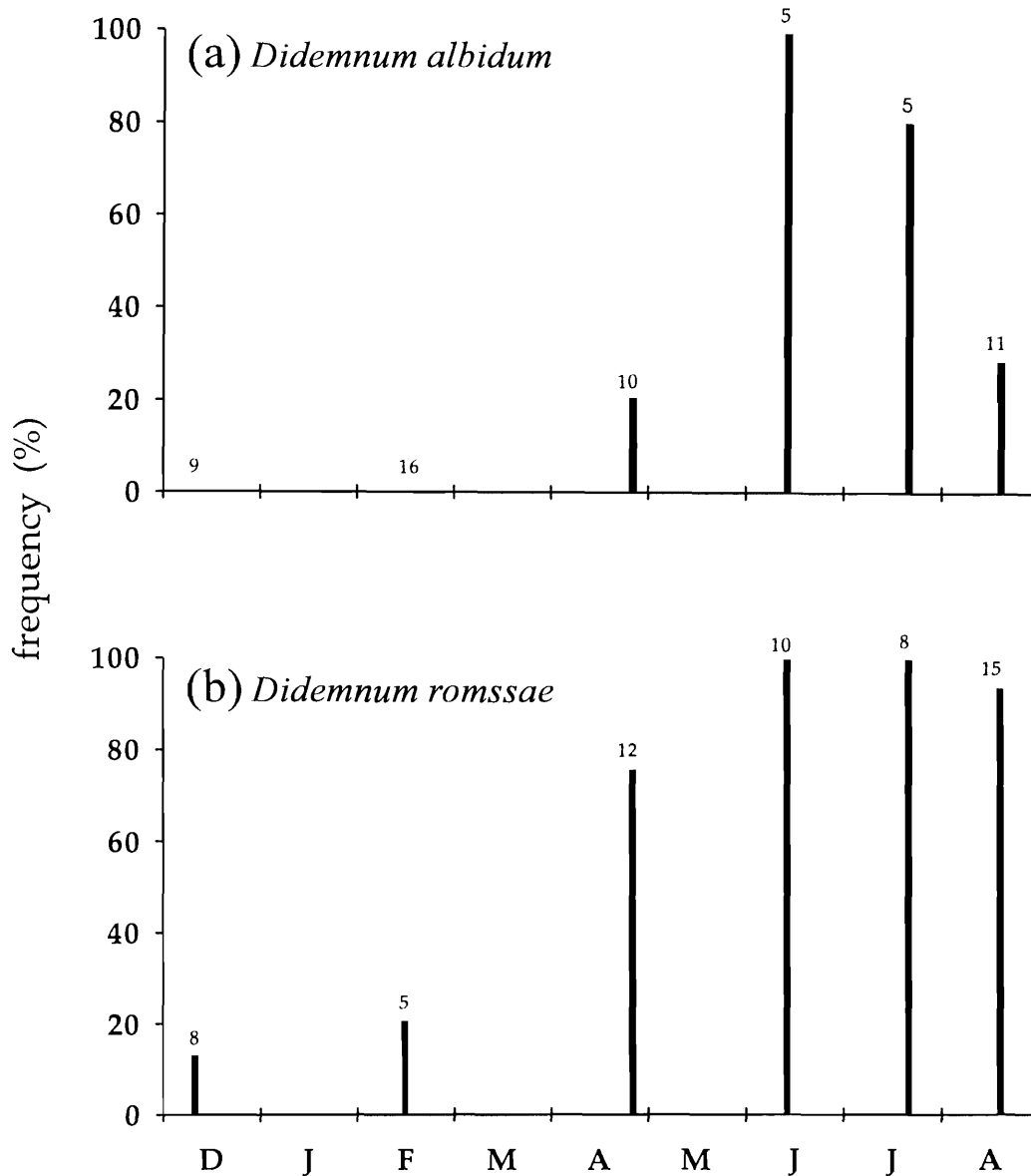
Didemnum romssae and *D. polare* have spicule shapes that differ dramatically from each other and from those of *D. albidum*, which supports the conclusion that these represent distinct species.

Spicule production

The LOT is sometimes only a tiny flattened protrusion on the body wall. At other times, it is a compact oval clump (450 μm) of tiny spicules, which is very conspicuous on either side of the thorax. Lafargue and Kniprath (1978) report that the spiculogenic organs (LOTs, Kniprath and Lafargue 1980; spiculogenic organ, Lafargue and Wahl 1987; thoracic lateral organs, lateral organs, Van Name 1945) are the only source of initial spicule production in didemnids. In 1980, Kniprath and Lafargue showed that spicules in *Trididemnum cereum* (Giard, 1872) are produced intracellularly in the LOTs, and covered with a double cell membrane. Spicules and "tunic substance" are produced by scleroblasts in the LOTs, and spicules then migrate to

³ Van Name (1945) illustrates nine very different spicule shapes belonging to different colonies of *Didemnum candidum*, but suggests that these may actually comprise a complex of sibling species.

Fig. 12. Seasonal variation in the proportion of colonies producing spicules from December 1989 to August 1990. The sample size is given above each column. For *Didemnum albidum*, only "active" colonies (with functional thoraces) were used for the analysis.



their permanent positions in the common tunic, where they may continue to grow. The actual organ is an invagination of the exterior ectoderm into the body wall (Kniprath and Lafargue 1980).

Monniot et al. (1991) propose that spicules in didemnids are of extracellular origin, forming as calcium carbonate precipitates of a reaction between seawater and ascidian blood in the common tunic. They maintain that the LOTs cannot be sites of intracellular spicule production because they are not differentiated glands, and because the tiny spicules often associated with these organs are also found in contact with other parts of the zooid (near the oral siphon and the abdomen).

Regardless of its function, the location of the LOT is characteristic for any species (Monniot et al. 1991; Lafargue and Kniprath 1978). In both *D. albidum* and *D. romssae*, the LOT lies between the second and third rows of stigmata.

Despite some slight variation in their exact position along the branchial basket, the location of the LOTs is a reliable taxonomic trait, but not one that distinguishes between these two species.

In *D. albidum*, the presence or absence of spicules in the LOTs is correlated with the seasonal degeneration and regeneration of the thorax, while in *D. romssae* the same cycle appears to occur, despite the lack of a nonfeeding stage (Fig. 12). This may be an indication that there is some sort of seasonal regeneration of zooids, without a complete resting stage. Alternatively, it could simply indicate that the colony stops growing during the winter months, or at least produces no new spicules then. The absence of newly formed spicules in both nonfeeding-stage colonies of *D. albidum* (in which the in- and out-current openings are completely sealed) and in actively feeding colonies of *D. romssae* during the winter suggests that spicules are not formed as extracellu-

Table 5. Comparison of major morphological traits of *Didemnum* and *Polysyncraton*; *D. albidum* and *D. romssae* lie midway between these genera (shared traits are shown in boldface type).

	<i>Polysyncraton</i>	<i>D. albidum</i> and <i>D. romssae</i>	<i>Didemnum</i>
Zooids			
Tentacle crown	20–26	16–26	16 (3 complete orders)
Coiled sperm duct	4–5 loose coils	4–7 loose coils	8–9 tight coils
Testicle	Divided; usually >2	Divided (2) and single	Single
LOTs*	Spherical, above third row of stigmata	Spherical, between second and third rows	Wing-shaped, below third row of stigmata
Thoracic appendix	Present or absent	Absent	Present
Cloacal languet	Present	Present	Absent
Larvae			
Budding	Gemmiparous	Non-gemmiparous	Non-gemmiparous
Branchial basket	Four rows of stigmata	Three rows of stigmata	Three rows of stigmata
No. of adhesive papillae	3	3	2 or 3
No. of lateral ampullae (per side)	Numerous; usually >9, arranged in a crown	11–19, arranged in a crown	Few; usually <9, arranged in rows

*Lateral organs of the thorax.

lar precipitates. In the species examined here, the LOTs appear to be the sole site of spicule production. No tiny spicules were observed at any other point in contact with the zooid. All zooids within a colony generally have LOTs either with or without spicules, with the exception of resting-form colonies, which may have “old thoraces” with spicules as well as newly budded thoraces under the surface that have no spicules.

Nomenclature

Didemnum albidum (Verrill, 1871)

Didemnum albidum from the coasts of Norway and Spitzbergen and those from the northeast coast of North America (Verrill 1871) are presumed to be synonymous. Variation in color and slight variation in spicule form exist between colonies from different geographic locations, and these populations potentially represent distinct species or subspecies. These differences alone, however, do not justify such a division at present. A comparative analysis of populations from different geographic locations is needed, as little is known about genetic or life-history variation (reproduction, resting stages) within this species.

Didemnum romssae new species

Although this species has often been confused with both *D. albidum* and *D. polare*, it is clearly a previously undescribed species.

Didemnum polare (Hartmeyer, 1903)

Hartmeyer originally described *D. polare* as distinct from *D. albidum* on the basis of spicule shape, zooid size, and the lack of an atrial languet. In the absence of type material, specimens described by Hartmeyer (1923) as having typical *D. polare*-type spicules, were examined. Spicule shape and zooid morphology of these specimens were consistent with the species description provided here for *D. polare* (above). Thus, this name is given priority, despite Hartmeyer's (1903) inaccurate description of the morphology. In addition

to many samples from Norway, samples from the Faeroe Islands also matched this description. Many records of *D. albidum* in these waters may actually refer to *D. polare*.

Systematic position

Didemnum albidum and *D. romssae* are intermediate between the closely related genera *Didemnum* and *Polysyncraton*, sharing traits of both. Table 5 lists the major morphological traits that characterize these genera from examination of the French species (Lafargue and Wahl 1987). Monniot et al. (1991) provide numerical keys for the Didemnidae in which the only distinction between these two genera is that *Didemnum* does not combine the presence of an atrial languet and a divided testis in any one species. Both *D. albidum* and *D. romssae* combine these two features (having two testicular glands when mature). The basis on which these two genera are divided may eventually prove too tenuous to justify their separation (see Monniot et al. 1991; Monniot 1993).

Speciation

The results of this study raise questions about the extent to which genetic differences are reflected by phenotype in the genus *Didemnum*. Is the pattern of speciation within this genus one of reproductive isolation with negligible morphological differentiation, or of widely distributed species with a high degree of environmental variability? While the answer to this question is not clear, indistinct species boundaries among taxa in the Didemnidae suggest that the evolutionary processes involved in speciation in these organisms may resemble the reticulate evolution common in plants (Harper 1977; Templeton 1989) and found in some corals (Willis et al. 1992; Knowlton and Jackson 1994a). Gross morphological characters may be highly conserved, with divergence occurring instead in life history, chemical-recognition systems (allelochemical compatibility), mate recognition, reproductive timing, or ultrastructure. Variability in character states could reflect differences in function; the presence or absence of an atrial languet may be determined by water

currents through a colony. Even suites of morphological traits that co-occur in different genera may result from functional constraints such as those imposed by zooid size (e.g., the degree of sperm duct coiling increases and the number of rows of stigmata decreases with decreasing zooid size (Lafargue and Wahl 1987)). Consequently, the standard, solely morphological, phylogenetic approach has failed to accurately describe many species. Additionally, the traditional focus on morphological species descriptions within the Didemnidae obscures the need for studies of speciation and of the evolution of morphological plasticity invoked by the presence of fuzzy species boundaries in this group.

What maintains the genetic boundaries between sympatric sibling species such as those described here? The evolutionary processes involved in speciation in clonal marine organisms with limited dispersal, such as didemnids, are in some ways reminiscent of speciation in plants. For example, barriers to hybridization in the form of gametic incompatibility in marine organisms show similarities to the genetic isolating mechanisms of terrestrial plants (Palumbi 1992). These barriers may have arisen as pleiotropic effects of genetic systems whose primary function is to prevent self-fertilization (Palumbi 1992). Didemnid ascidians are simultaneous hermaphrodites with internal fertilization, whose individual zooids simultaneously produce sperm and eggs. They are potentially capable of intrazooid selfing, interzooid selfing, and out-crossing between separate colonies. While the atrial opening of each zooid is tiny, usually less than 1 mm, the entire colony surface may span several centimetres to nearly a metre in diameter. Sperm released by a zooid in the center of such a colony must be transported over the incurrent siphons of many other zooids before reaching another colony. Additionally, many didemnids undergo fragmentation, and thus, groups of neighboring colonies may actually comprise a single genetic individual. Despite these life-history variables that suggest high levels of inbreeding in larviporous ascidians (Knowlton and Jackson 1994b), cross-fertilization is prevalent in several species (Cohen 1990; Grosberg 1991; Bishop and Ryland 1993; Knowlton and Jackson 1994b), including one didemnid (Ryland and Bishop 1990). If out-crossing is the rule, barriers to selfing may be particularly important in these organisms. Such barriers could provide the raw material for the rapid development of reproductive isolation. Knowledge of breeding systems, and evidence for divergence of reproductive recognition systems at the gametic level, should be areas of priority in future evolutionary studies involving sibling species complexes.

In this study, I demonstrate the presence of three sympatric cryptic species of *Didemnum* from northern Norway. While morphological differences between these species are slight, their life histories differ. Without supportive molecular or biochemical genetic evidence, interbreeding experiments, or knowledge of life histories, questions of relatedness among other species in this group cannot be answered unequivocally. I predict that further study will reveal similar patterns in other widely distributed morphological "species" within the Didemnidae. Caution should be used in making phylogenetic conclusions based merely on morphological characters. The widespread presence of sibling species in marine systems (Knowlton and Jackson 1994a) underscores the need for taxonomists to examine a combination of characters when

attempting to identify species, and illuminates the complexity of biodiversity in the sea. While proliferation of cryptic species descriptions would confound the taxonomic literature, it would provide a new, tractable system for studies of life-history evolution and speciation.

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