Phylogeny of the Aplousobranchia (Tunicata: Ascidiacea) [1]

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ABSTRACT. The phylogenetic relationships of genera and families of Aplousobranchia Lahille (Tunicata, Ascidiacea) is reconstructed based on morphological characters – the first comprehensive morphology-based phylogenetic analysis for the Aplousobranchia. Monophyly of Aplousobranchia and its families were tested with samples of 14 families. The final character matrix comprised 47 characters and 41 genera as terminal taxa. Nine equally most parsimonious trees (length 161, CI = 0.5031, RI = 0.7922) were found. Characters describing replication, colony system formation, and branchial walls were the more important in phylogenetic reconstruction. These characters were more useful than others more traditionally used in ascidian taxonomy, such as: body division, position of the heart, gonads and epicardium. Characters not frequently used in phylogenetic analysis, such as body wall muscles, muscles associated with transversal blood vessels and arrangement of the larval papillae, also have phylogenetic information. Results supported monophyly of the Aplousobranchia *sensu* Lahille, 1887 including only Polycitoridae, Polyclinidae, and Didemnidae. On the other hand, Aplousobranchia including also Cionidae and Diazonidae is not monophyletic since *Perophora* and *Ecteinascidia* were included as ingroups in the cladogram, *Ciona* (now closer to *Ascidia*) was no longer included in Aplousobranchia and the position of *Rhopalaea* and *Diazona* is not resolved. We propose a revised classification based on this phylogenetic analysis, in which Aplousobranchia, with three new families and an indeterminate taxon, now has 15 families.

KEY WORDS. Chordata; classification; morphological characters; systematic; Urochordata.

RESUMO. **Filogenia de Aplousobranchia (Tunicata: Ascidiacea).** O relacionamento filogenéticos de gêneros e famílias de de Aplousobranchia Lahille (Tunicata, Ascidiacea) foi reconstruída com base em caracteres morfológicos – esta constitui a primeira análise filogenética morfológica abrangente para Aplousobranchia. A monofilia de Aplousobranchia e suas famílias foi testada com espécies de 14 famílias. A matriz final compreendeu 47 caracteres e 41 gêneros como taxons terminais. Nove árvores igualmente parcimoniosas (comprimento 161 passos, CI = 0.5031, RI = 0.7922) foram encontradas. Caracteres descrevendo reprodução, formação de sistemas de zoóides nas colônias e relacionados com a parede da faringe foram os mais importantes na reconstrução filogenética. Estes caracteres apresentaram maior sinal filogenético do que outros mais tradicionalmente utilizados na taxonomia de Ascidiacea como divisão do corpo, posição do coração, gônadas e epicárdio. Caracteres pouco utilizados como musculatura da parede do corpo, musculatura associada aos vasos sanguíneos transversais da faringe e arranjo das papilas adesivas da larva também apresentaram informação filogenética. Os resultados suportam a monofilia de Aplousobranchia *sensu* Lahille, 1887 incluindo apenas Polycitoridae, Polyclinidae e Didemnidae. Por outro lado, Aplousobranchia incluindo também Cionidae e Diazonidae não é taxon monofilético já que *Perophora* e *Ecteinascidia* foram incluídos como grupos internos no cladograma; *Ciona* (próximo a *Ascidia*) não pôde ser incluído em Aplousobranchia e não foi possível definir a posição de *Rhopalaea* e *Diazona*. Uma classificação revisada baseada nesta análise filogenética é proposta, na qual Aplousobranchia possui 15 famílias, sendo três novas famílias e um táxon indeterminado.

PALAVRAS-CHAVE. Caracteres morfológicos; Chordata; classificação; sistemática; Urochordata.

Traditionally, pharynx structure provided the basis for ascidian classification (LAHILLE 1887, 1890 *in* KOTT 1969) while later studies included the position of the gonads (PERRIER 1898 *in* KOTT 1969). Based on the structure and development of the pharynx, the class is divided into three Orders: Aplousobranchia (flat pharynx without internal longitudinal vessels); Phlebobranchia (flat pharynx, with numerous internal longitudinal vessels); and Stolidobranchia (folded pharynx with internal longitudinal vessels). Using gonad position, the class is divided into two Orders: Enterogona, with the gonads within or below the intestinal loop (includes Aplousobranchia and Phlebobranchia); and Pleurogona, with gonads attached to the body wall (includes Stolidobranchia).

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Berrill (1936) traced a probable ascidian evolutionary pathway based on the development of the heart, pericardium and epicardium. He suggested that classification based on the pharynx was weak since pharyngeal structure is probably largely due to the size of the organism, and consequently divided the Ascidiacea into Pleurogona and Enterogona. More recently, Hirose (2001), in a study of tunic cell types in 65 ascidians species (including 11 of the 15 traditional ascidian families), showed that two tunic cell types ("tunic bladder cells" and "tunic net cells") supported the Pleurogona and Enterogona division, because they were only found in the Enterogona. Additional analysis, based on 18S rDNA sequences (Wada *et al.* 1992, Wada 1998), also supported this view. In summary, two systems of classification were prominent in the twentieth century: 1) the orders Pleurogona and Enterogona and sub-orders Aplousobranchia, Phlebobranchia and Stolidobranchia (Berrill 1936, 1950, Kott 1985); 2) the Aplousobranchia, Phlebobranchia and Stolidobranchia as orders (Van Name 1945, Monniot & Monniot 1972, Monniot *et al.* 1991). Monophyly of these groups must be determined to better choose between the two classifications. Here, we test the monophyly of the Aplousobranchia.

The Order Aplousobranchia comprises colonial ascidians, with the body divided into two or three parts (thorax, abdomen and, in some cases, posterior abdomen); with simple oral tentacles and the pharynx with neither folds nor internal longitudinal vessels (Van Name 1945). In a comprehensive review of the ascidian genera of the world, Monniot & Monniot (1972) recognized 46 genera of Aplousobranchia in only three families: Polyclinidae, Polycitoridae and Didemnidae. Two additional families, Clavelinidae and Holozoidae, were later accepted (Monniot & Monniot 2001).

Kott (1969, 1985, 1990, 1992, 2001), based on long-term surveys of ascidians from Australia and Antarctica, proposed the most important modifications in the traditional classification of the Aplousobranchia: inclusion of Diazonidae, with the genera *Rhopalaea* Philippi, 1843, *Diazona* Savigny, 1816, *Tylobranchion* Herdman, 1886, *Syndiazona* Oka, 1926 and *Pseudodiazona* Millar, 1963a; and inclusion of Cionidae, with *Ciona* Fleming, 1822. Those families belonged previously within the Phlebobranchia Lahille, 1887. She also proposed new families, without describing the phylogenetic methodology to support her new classification. Following her classification, Aplousobranchia includes 14 families (Tab. I), based on replication processes, aspects of the colony and zooid and larval morphology. Kott (1990) redistributed the genera/species of Polycitoridae in five families, believed to be monophyletic. Kott (1992) also re-defined Polyclinidae restricting it to the genera included in the Polyclininae, and suggested five new families. Didemnidae, however, remained stable in various classifications because of the short size of zooids with four or three rows of stigmata, few testis follicles and large larvae being brooded inside the tunic (Lafargue 1983, Lafargue & Wahl 1987, Kott 1962, 2001), which suggests that this family is indeed a natural group, although its monophyly was never tested.

Very few attempts to reconstruct ascidian phylogeny exist, and the first ones were intuitive and not cladistically based. One of the first comprehensive attempts proposed the origin of a group including *Diazona*, *Rhopalaea*, *Tylobranchion* and the evolution of Aplousobranchia from a *Ciona*-like ancestor (Berrill 1936). Following this hypothesis, Didemnidae would have a common origin with *Distaplia* while Perophoridae is an isolated branch originating directly from *Ciona*. In the tree produced by this hypothesis, Phlebobranchia was clearly polyphyletic. Kott (1962) proposed an alternative that suggested independent origins for Polycitoridae, Polyclinidae and Didemnidae, each with a Diazonidae-like ancestor, and which would make Aplousobranchia polyphyletic. Millar (1966) briefly discussed the main lines of ascidian evolution and proposed a phylogenetic tree based exclusively on morphological characters. He suggested that Clavelinidae might have arisen close to the division between Aplousobranchia and Phlebobranchia.

Apart from these few morphological studies, some phylogenetic hypotheses based on specific characters were proposed in various studies. For example, vanadium as a character was proposed by Hawkins *et al.* (1983). They noted that all the species of Aplousobranchia *sensu* Lahille and of the families Cionidae and Diazonidae contained significant concentrations of vanadium in its oxidized form (oxidation state IV). This character confirms the proximity of those groups and suggests that they share a common ancestor (Kott 1990), but not necessarily that they form a monophyletic group. In all the Phlebobranchia, vanadium appears in oxidation state III while, in Stolidobranchia, significant levels of vanadium were rarely encountered. Trends in the evolution within each of those *taxa* seem to be associated with the loss of the element or changes of the oxidation state of vanadium (IV for III).

Recently, accumulating molecular data have shed new light on the phylogenetic relationships within Deuterostomia (Cameron *et al.* 2000, Turbeville *et al.* 1994, Swalla *et al.* 2000, Swalla 2001) and within Ascidiacea (Wada *et al.* 1992, Wada 1998, Stach & Turbeville 2002, Turon & López-Legentil 2004). Analysis based on 18S rDNA sequences (Wada *et al.* 1992, Wada 1998) inferred that Enterogona (including only Phlebobranchia) is more closely related to Thaliacea than to Stolidobranchia (Wada 1998), which implies that Ascidiacea is itself not monophyletic. The combined analysis with molecular data (18S rDNA) and morphological characters also showed that Phlebobranchia (including *Ascidia*, *Perophora* and *Ciona*) is a sister-group of Thaliacea and that Aplousobranchia is suggested as a sister-group of Appendicularia (Stach & Turbeville 2002). On the other hand, Turon & López-Legentil (2004) focused on the Aplousobranchia, using partial COI sequences of 28 species of ascidians (21 in Aplousobranchia) to reconstruct the phylogeny of the group. Aplousobranchia was shown to be monophyletic with an uncertain relationship with the other two orders.

Here, we test the monophyly of Aplousobranchia and of the families therein. We also propose a phylogenetic recon-

Table I. Traditional classification of families and genera in the Order Aplousobranchia. Numbers in parentheses indicate the approximate number of species in the genus.

Van Name (1945)	Monniot & Monniot (1972); Monniot *et al.* (1991)	Kott (1990, 1992, 2001)
Synoicidae Milne-Edwards, 1841	Polyclinidae Milne-Edwards, 1841	Polyclinidae Milne-Edwards, 1841
Polyclinum Savigny, 1816 (34)	*Polyclinum* Savigny, 1816	*Polyclinum* Savigny, 1816
Synoicum Phipps, 1774 (76)	*Synoicum* Phipps, 1774	*Synoicum* Phipps, 1774
Amaroucium Milne-Edwards, 1841 (3)	*Aplidium* Savigny, 1816 (217)	*Aplidium* Savigny, 1816
Aplidiopsis Savigny, 1816 (14)	*Aplidiopsis* Lahille, 1890	*Aplidiopsis* Lahille, 1890
Pharyngodictyon Herdman, 1886	*Sidneioides* Kesteven, 1909 (4)	*Sidneioides* Kesteven, 1909
Euherdmania Ritter, 1904 (10)	*Sidnyum* Savigny, 1816 (13)	*Morchellium* Giard, 1872 (3)
Sigillinaria Oka, 1933 (5)	*Atopogaster* Herdman, 1886 (1)	Placentelidae Kott, 1992
	Placentela Redikorzev, 1913 (2)	*Placentela* Redikorzev, 1913
	Homoeodistoma Redikorzev, 1927(3)	Ritterellidae Kott, 1992
	Ritterella Harant, 1931 (24)	*Ritterella* Harant, 1931
	Dumus Brewin, 1952 (2)	*Dumus* Brewin, 1952
	Pharyngodictyon Herdman, 1886 (6)	Euherdmaniidae Ritter, 1904
	Euherdmania Ritter, 1904	*Euherdmania* (Ritter, 1903)
	Polyclinella Harant, 1930 (1)	Pseudodistomidae Kott, 1992
	Pseudodistoma Michaelsen, 1924 (29)	*Pseudodistoma* Michaelsen, 1924
	Protopolyclinum Millar, 1960 (1)	*Anadistoma* Kott, 1992 (1)
	Citorclinum Monniot & Millar, 1988 (1)	Protopolyclinidae Kott, 1992
Didemnidae Verrill, 1871	Didemnidae Verrill, 1871	*Monniotus* Millar, 1988 (5)
Echinoclinum Van Name, 1902 (5)	*Echinoclinum* Van Name, 1902	*Condominium* Kott, 1992 (1)
Didemnum Savigny, 1816 (65)	*Askonides* Kott, 1962 (2)	Didemnidae Giard, 1872
Trididemnum Della Valle, 1881 (53)	*Didemnum* Savigny, 1816	*Didemnum* Savigny, 1816
Leptoclinides Bjerkan, 1905 (40)	*Trididemnum* Della Valle, 1881	*Trididemnum* Della Valle, 1881
Lissoclinum Verrill, 1871 (42)	*Polysyncraton* Nott, 1892 (50)	*Polysyncraton* Nott, 1892
Diplosoma Macdonald, 1859 (38)	*Leptoclinides* Bjerkan, 1905	*Leptoclinides* Bjerkan, 1905
Coelocormus Herdman, 1886	*Lissoclinum* Verrill, 1871	*Lissoclinum* Verrill, 1871
	Diplosoma Macdonald, 1859	*Diplosoma* MacDonald, 1859
	Atriolum Kott, 1983 (3)	*Atriolum* Kott, 1983
	Botrydemnum Oka, 1933 (1)	*Clitella* Kott, 2001 (1)
	Coelocormus Herdman, 1886 (1)	
	Sinecloaca (Michaelsen, 1930) (1)	Polycitoridae Michaelsen, 1904
Polycitoridae Michaelsen, 1904	Polycitoridae Michaelsen, 1904	*Exostoma* Kott, 1990 (1)
Polycitor Renier, 1804 (40)	*Polycitor* Renier, 1804	*Polycitor* Renier, 1804
Archidistoma Garstang, 1891 (13)	*Polycitorella* Michaelsen, 1924 (17)	*Polycitorella* Michaelsen, 1924
Eudistoma Caullery, 1909 (119)	*Eudistoma* Caullery, 1909	*Eudistoma* Caullery, 1909
Cystodytes Drasche, 1884 (27)	*Cystodytes* Drasche, 1884	*Cystodytes* Drasche, 1884
Clavelina Savigny, 1816 (47)	*Tetrazona* Michaelsen, 1930 (2)	*Brevicollus* Kott, 1990 (1)
Holozoa Lesson, 1830 (3)	*Protoholozoa* Kott, 1969 (6)	Clavelinidae Forbes & Hanley, 1848
Distaplia Della Valle, 1881 (41)	*Atapozoa* Brewin, 1956 (1)	*Nephtheis* (Drasche, 1882) (1)
Sycozoa Lesson, 1830 (19)	*Clavelina* Savigny, 1816	*Clavelina* Savigny, 1816

Continue

Table I. Continued.

Van Name (1945)	Monniot & Monniot (1972), Monniot *et al.* (1991)	Kott (1990, 1992, 2001)
	Oxycorynia Drasche, 1882	Pycnoclavellidae Kott, 1990
	Podoclavella Herdman, 1890 (9)	*Euclavella* Kott, 1990 (1)
	Pycnoclavella Garstang, 1891	*Pycnoclavella* Garstang, 1891 (10)
	Archidistoma Garstang, 1891	Holozoidae Berrill, 1950
	Archascidia Julin, 1904 (1)	*Polydistoma* Kott, 1990 (3)
	Distaplia Della Valle, 1881	*Distaplia* Della Valle, 1881
	Cyathocormus Oka, 1912 (1)	*Sigillina* Savigny, 1816 (10)
	Hypodistoma Tokioka, 1967 (4)	*Hypodistoma* Tokioka, 1967
	Dimorpha Pérès, 1946 (1)	*Neodistoma* Kott, 1990 (1)
	Hypsistozoa Brewin, 1953 (3)	*Hypsistozoa* Brewin, 1953
	Sycozoa Lesson, 1830	*Sycozoa* Lesson, 1830
	Millarus Monniot & Monniot 1987a (1)	
	Stomozoa Kott, 1957 (3)	Stomozoidae Kott, 1990
		Stomozoa Kott, 1957
Ordem Phlebobranchia	Ordem Phlebobranchia	Cionidae Lahille, 1887
		Ciona Fleming, 1822 (13)
		Rhopalaea Philippi, 1843 (13)
		Pseudodiazona Millar, 1963a (3)

struction and new classification for the group based on morphological characters.

MATERIAL AND METHODS

Character selection and taxonomic sampling

Ascidians usually have few characters which are often difficult to access. As soft-body animals, contraction during fixation often alters the shape and size of some structures. Although size of zooid, ratio between thorax and abdomen, length of siphons are characters usually used in the diagnosis of species, they were not used in this analysis because they were variable and depended on the state of contraction of the specimen. With these tendencies in mind, we included the largest number of possible characters, in addition to those traditionally used for diagnoses of the Ascidiacea (Tab. II). Also, no character was arbitrarily left off the analysis. Situations that made us drop a character were: lack of information for most of the taxa (usually occurred with characters of the larva, presence and shape of the pyloric gland), characters too variable (polymorphic) within many genera (such as presence of a stalk, shape of the atrial languet), autapomorphic characters in the level of the genera, characters altered by the fixation process (presence and number of pigmented spots between the lobes of the siphons), characters invariable and therefore uninformative (the pre-pharyngeal groove is U shape in all *taxa* of the ingroup, except in the Perophoridae).

The characters were collected initially from the literature. However, many descriptions were incomplete or difficult to obtain. Direct observation of biological material was required, both, to confirm and to complement descriptions in the literature. Studied specimens are deposited in collections of the Department of Zoology at the Federal University of Paraná (DZUP), at the National Museum of Natural History – Smithsonian Institution, Washington, DC, USA (NMNH), at the Museum National d'Histoire Naturelle in Paris, France (MNHN), at the Iziko Museums of Cape Town in South Africa, and at the Instituto Oceanográfico and Instituto de Biociências at the University of São Paulo in Brazil. Specimens were also obtained from private collections in Spain (researcher Elsa Vázquez), now deposited in the collection of the Department of Zoology of Universidade Federal do Paraná.

Species were selected from one to three genera of each of the 14 families that comprise the order Aplousobranchia (following Kott 1990, 1992, 2001, Tab. I). A minimum of three species in each genus were studied with the following selection criteria: 1) the type species of the genus, 2) species of different geographical areas 3) species present in the Brazilian coast.

Of the 103 species, 88 are representative of 34 genera of Aplousobranchia, three of *Ascidia* Linnaeus, 1767, one of the *Ecteinascidia* Herdman, 1880, three of the *Perophora* Wiegmann, 1835 (Phlebobranchia), three of the *Pyura* Molina, 1782, three of the *Molgula* Forbes & Hanley, 1848 (Stolidobranchia), and one species of *Branchiostoma* (Cephalochordata). In the list of studied species, the type-species of the respective genus is written in bold and the collection locations are in agreement with the current information in the labels of each sample (Appen-

Table II. The list of morphological characters used to generate the phylogenetic hypothesis in figure 1.

Character *	Character states
1. Life habit	0, Simple: solitary forms that reproduce sexually; 1, Colonial: compound forms comprising individuals (zooids) that originated by budding (asexually), which stay united by a stolon or common tunic.
2. Spicules in the tunic	Calcareous crystalline and small (usually 10-40 µm) structures produced in the tunic. 0, Absent; 1, Present.
3. Position of the intestinal loop and gonads in relation to the pharynx	0, Lateral to the pharynx; 1, Lateral and posterior to the pharynx; 2, Posterior to the pharynx.
4. Body divisions	0, Body undivided, sac like: usually globular; 1, Body divided into thorax (pharynx) and abdomen (digestive tract, gonads and heart); 2, Body divided into thorax (pharynx), abdomen (digestive tract) and posterior abdomen (gonads and heart).
5. Colony form	0, Stalked: colonies comprising a "head" that usually contains the thoracic region of the zooids or the whole zooids and a stalk formed by the common tunic that elevates the animal from the substrate and contains, in some cases, the abdominal and/or the posterior abdominal regions of the zooids (Fig. 2); 1, Globular: colonies taller than wide, attached to the substrate by a smaller surface area (inferior surface; Fig. 3); 2, Cushion: thick colonies (5 mm or more) attached to the substrate by a larger surface are (inferior surface; Fig. 4); 3, Sheet-like: encrusting thin colonies (5 mm or less), attached to the substrate by an extensive area of the tunic (inferior surface; Fig. 5); 4, Expanded encrusting base: colonies with a thick base from which arise digit-like projections (Fig. 6); 5, Stolonic: colony formed by zooids connected at base through stolons. Stolons consist of stolonic vessels covered with tunic (Fig. 7).
6. Openings on the colony surface	0, Both siphons open on colony surface (Figs 8, 9); 1, Only oral siphon opens on colony surface: the atrial siphon opens within the tunic in cavities or channels that communicate with the exterior through a cloaca (Fig. 10).
7. Atrial siphon shape	0, Tubular: the atrial siphon is formed by a cylindrical extension of the body (Figs 11-14); 1, Small aperture: there is no body extension nor tubular siphon, and the atrial opening exposes less than 50% of the pharynx (Fig. 12); 2, Wide aperture: there is no body extension nor tubular siphon, and the atrial opening exposes more than 50% of the pharynx (Fig. 13).
8. Atrial siphon position in colonial forms	0, Apical (Figs 11, 12); 1, Not apical: in the middle or base of the thorax (Figs 15, 16).
9. Oral siphon margin	0, Lobed; 1, Smooth (Fig. 15).
10. Oral siphon lobe shape	0, Rounded (Fig. 16); 1, Sharp (Fig. 17); 2, With projections (Fig. 18).
11. Number of oral tentacles	0, From 11 to 24; 1, 30 or more; 2, Eight or less.
12. Longitudinal muscle - right side of the body	0, From the oral siphon to the base of the thorax; 1, From the oral siphon to the extremity of the abdomen; 2, From the oral siphon to the extremity of the posterior abdomen; 3, From the oral siphon to the middle of the thorax; 4, Absent or inconspicuous.
13. Number of longitudinal thoracic muscle bands or fibers	0, More than 50 bands or fibers in each side; 1, Less than 30 bands or fibers in each side.
14. Orientation of longitudinal thoracic muscle bands	0, Oblique in relation to the endostyle (Figs 19-20); 1, Parallel in relation to the endostyle (Figs 21-23); 2, Oblique, ramified with anastomoses; bands from the area of the siphons and the inter-siphonal space towards the base of the thorax bend toward the dorsal line or mid-ventral line and become slightly transverse as they become separated in several narrow bands (Fig. 24); 3, Irregular (Fig. 25).
15. Thoracic transverse muscles	0, Conspicuous (Fig. 26); 1, Absent or inconspicuous.
16. Dorsal pharyngeal muscle band	Narrow muscle bands, one on each side of the body, concentrated in the dorsal area of the pharynx. In the didemnid these bands join to form the muscular process. 0, Absent; 1, Present (Figs 27, 28).
17. Abdominal prolongation of the dorsal pharyngeal muscle band	0, Absent; 1, Present (Fig. 27).
18. Muscular process	Also denominated retractor muscle. It is the continuation of the dorsal pharyngeal muscle band (23) and projects into the tunic thereby anchoring the zooid within the colony. 0, Absent; 1, Present (Fig. 28).

Continue

Table II. Continued.

Character *	Character states
19. Transverse pharyngeal muscle	Transverse muscle bands on the transverse vessels of the pharynx. 0, Absent; 1, On all transverse vessels (Figs 34, 35).
20. Horizontal rows of pharyngeal stigmata	0, Seventy-five or more; 1, From 5 to 26; 2, Four; 3, Three.
21. Longitudinal pharyngeal vessels	0, Complete (Figs 30, 31); 1, Incomplete (Fig. 32); 2, Absent.
22. Parastigmatic vessels	Fine transverse vessels that cross the stigmata. 0, Absent; 1, Present.
23. Branchial papillae associated with longitudinal vessels	Papillae perpendicular to the plane of the pharynx wall, supporting longitudinal blood vessels in their junctions with the transverse vessels. 0, Present (Fig. 33); 1, Absent.
24. Vestigial branchial papillae	Short and rounded papillae not associated with longitudinal vessels. 0, Absent; 1, Present (Figs 34, 35).
25. Prepharyngeal groove	Ring of one or two membranes that delimit a channel formed by ciliated cells. Located in the anterior limit of the pharynx. 0, Double: two membranes delimit the channel; 1, Simple: one membrane delimits the channel.
26. Neural gland opening	0, C or U shaped, or irregular; 1, Circular or oval.
27. Stomach shape	0, Tubular stomach with poorly defined intestine (Fig. 36); 1, Oval to rectangular (Figs 37-39); 2, Kidney shaped (Fig. 40); 3, Trapezoidal (Fig. 41); 4, Rounded (Fig. 42).
28. Stomach wall	0, Smooth (Figs 36, 39, 41, 42); 1, Smooth with internal folds (Fig. 43); 2, Folded (Figs 37, 38); 3, With longitudinal ornamentations (Fig. 40); 4, With rounded ornamentations.
29. Stomach position in the alimentary tract	0, In the first half of the proximal branch of the gut; 1, In the middle of the proximal branch of the gut; 2, In the posterior half of the proximal branch of the gut.
30. Posterior stomach	The posterior stomach is a swollen portion of the median intestine. 0, Absent; 1, Present.
31. Gastric reservoir	A vesicle located between and connected to the stomach and distal portion of the intestine. 0, Absent; 1, Present.
32. Gonad position relative to the abdomen or posterior abdomen	0, Included in or beside the intestinal loop; 1, In the abdomen, below the intestinal loop; 2, In the posterior abdomen; 3, In a sac that is separate from the abdomen.
33. Ovary and testis position	0, Ovary in the centre of the testis lobes; 1, Ovary anterior to the testis; 2, Ovary posterior to the testis.
34. Testis shape	0, Lobed; 1, Irregular, ramified, elongated; 2, Rounded or pyriform.
35. Sperm duct	0, Straight (Figs 44, 45); 1, Spiral in proximal part; distal part straight (Fig. 46); 2, Convoluted: proximal part is irregularly curved over the testis; distal part is straight (Fig. 47).
36. Number of testis follicles	0, More than 10; 1, From 3-6 follicles; 2, Two follicles; 3, One follicle.
37. Oocyte number	0, More than 30; 1, Fifteen or fewer.
38. Asexual reproduction types	0, Strobilization of the abdomen; 1, Strobilization of the posterior abdomen; 2, Oesophageal budding; 3, Budding of the vascular appendix or vascular stolon.
39. Fertilization	0, External; 1, Internal (viviparous species).
40. Incubation of embryos	0, Absent; 1, Present.
41. Place of incubation	0, In the atrial cavity or oviduct of the zooid; 1, In a special pouch; 2, In the tunic.
42. Length of the larval trunk from the tip of the adhesive papillae to the base of the tail.	0, Up to 0.2 mm; 1, Greater than 0.4 mm.
43. Ectodermal ampullae	Epidermal evaginations in the anterior region of the larvae, which form lobes at the base of the adhesive papillae. 0, Absent; 1, Present (Figs 48-50).
44. Structure of the adhesive papillae	Adhesive papillae are structures that fix the larva to the substrate and are located in the anterior extremity of the larval trunk. 0, Non-everting, conical, adhesive organs (Fig. 50); 1, Everting, adhesive organs (Figs 48, 49).
45. Arrangement of adhesive papillae	0, Triangle (Fig. 50); 1, Linear (Figs 48, 49).
46. Adhesive papillae stalk	0, Absent (sessile papillae) (Fig. 48); 1, Present (Fig. 49); 2, Papillae in a peduncular process: the three papillae have only one peduncle (Fig. 50).
47. Vesicles in larval trunk	Ectodermal projections in the larval trunk, not associated with the adhesive papillae.0, Absent; 1, Present.

* The number of each character is the same number that appears in the data matrix (Tab. III) and in the cladogram (Fig. 1).

dix I). Complementary information for some species was obtained only from literature when it was impossible to locate or to borrow the specimens.

Although the *taxa Tylobranchion nordgaardi* (Hartmeyer, 1922), *Placentela translucida* Kott, 1969, *Polycitor vitreus* (Sars, 1851) and *Tetrazona porrecta* Millar, 1962 were studied, they were not included in the analysis due to lack of information.

The final character matrix comprised 41 genera (including five suitable genera traditionally out of Aplousobranchia and one from Cephalochordata) and 47 characters (Tab. III).

Phylogenetic analysis

Phylogenetic relationships among *taxa* of Aplousobranchia were analyzed following Hennig (1966) and Wiley (1981). All identical states within a character were *a priori* considered homologous in the absence of contrary evidence. The homoplasies (convergences and parallelisms) were determined *a posteriori* (Wiley 1981).

In the data coding, non-applicable characters were coded as "-" and both, absence and polymorphisms were coded by "?" (NDE Software; Page 2000). Characters were treated as non-ordered, equally weighted and optimized by the parsimony method following Fitch (1971). Thus, each state can be derived directly from any other and in any sequence, so that only one step is counted for each transformation along the tree. The non-ordered procedure is suggested for the treatment of multiple-state characters (Amorim 1997).

Trees were constructed under the assumptions of parsimony with PAUP* (Phylogenetic Analysis Using Parsimony), version 4.0b10 (Swofford 2002), using the following options: heuristic search, TBR ("Branch-swapping algorithm: tree-bisection-reconnection"), random addition sequence with 1000 replicates ("stepwise addition = random Nreps = 1000"). A strict consensus tree was generated to show groups that were constant in all the equally parsimonious trees. Zero-length branches were collapsed (MulTrees option in PAUP).

The characters were optimized with ACCTRAN. This option tend to bias the appearance of a character to a very basal position, when the character appear in a *taxa* whose sister-group was not either comparable or had an unknown state. In this case, we preferred to maintain its first appearance in the *taxa* itself.

We first built a cladogram with species as terminal *taxa*, in which most of the genera grouped well. When character states differed between congeneric species they were at first considered absent. Subsequently, analyzing the topography of the retrieved tree it was possible to discover their plesiomorphic states by comparison with the sister *taxon* at the respective node, the functional outgroup (Kornet & Turner 1999). Since we were testing the monophyly of families, we used genera as terminal *taxa*. Only *Polycitor* species (with conflicting character states, suggesting that this genus is not monophyletic) were analyzed separately.

In the absence of phylogenetic hypotheses for the sister-group relationships of Phlebobranchia and Stolidobranchia with Aplousobranchia, we used representatives of both (*Pyura* and *Molgula* in the Stolidobranchia, and *Ascidia*, *Ecteinascidia* and *Perophora* in the Phlebobranchia) as outgroup. We also included

Branchiostoma (representing Cephalochordata) as one outgroup. Appendicularia, considered in some analysis as the sister-group of Aplousobranchia (Stach & Turbeville 2002), was not included because, being planktonic, the body structures were lost or reduced, resulting in insufficient characters for a morphological analysis.

The point of divergence between Cephalochordata and Tunicata (Ascidiacea) was chosen as the root of the cladogram and polarization of the homologous series was determined *a posteriori* by comparison with the outgroup generated by the analysis (Nixon & Carpenter 1993).

The program TreeView (Page 1996) was used for preparation of the cladograms. When discussing more derived *taxa* than those at a particular node we used the following notation: name of the first *taxon* that appeared in the node followed by the symbol +.

RESULTS

The final data matrix included 41 terminal *taxa* and 47 characters (24 binary and 23 multiple state (Tabs II and III). Nine equally parsimonious cladograms were generated by PAUP (161 steps, IC = 0.5031, IR = 0.7922). The strict consensus tree was generated to summarize the information contained in all the trees (Fig. 1).

The consensus tree shows the position of *Diazona* and *Rhopalaea* not resolved (in the trees it appeared either in the base of the Aplousobranchia branch or in the base of the outgroup branch). The taxon *Tylobranchion*+ is monophyletic, but contains *Perophora* and *Ecteinascidia*, traditionally viewed as Phlebobranchia. Both Polycitoridae and Polyclinidae are divided in more taxa and their diagnoses have to be reviewed. Didemnidae is the most derived Aplousobranchia family and it is very stable in all the cladograms retrieved.

DISCUSSION

Distribution of the characters in the cladogram (Fig. 1, Tabs II and III)

The solitary habit (1.0) appeared both in the external group (Cephalochordata and *Ciona*(*Ascidia*(*Pyura*+*Molgula*)) and in the taxa *Rhopalaea*. While there is no information about the regenerative capacity of *Pyura*, *Molgula* and *Ascidia*, the abdomen of the *Ciona intestinalis* (Linnaeus, 1767) has the capacity to regenerate a new thoracic area. *Rhopalaea crassa* (Herdman, 1880) and *R. neapolitana* Philippi, 1843 have a thorax that degenerates from time to time and that is replaced by a new one. This regeneration process may be the intermediate condition between simple ascidians and the formation of colonies (Kott, 1981). Colonialism is present in *Diazona* and in the ingroup and it is one of the most important characters that placed *Perophora*+ *Ecteinascidia* in the internal group.

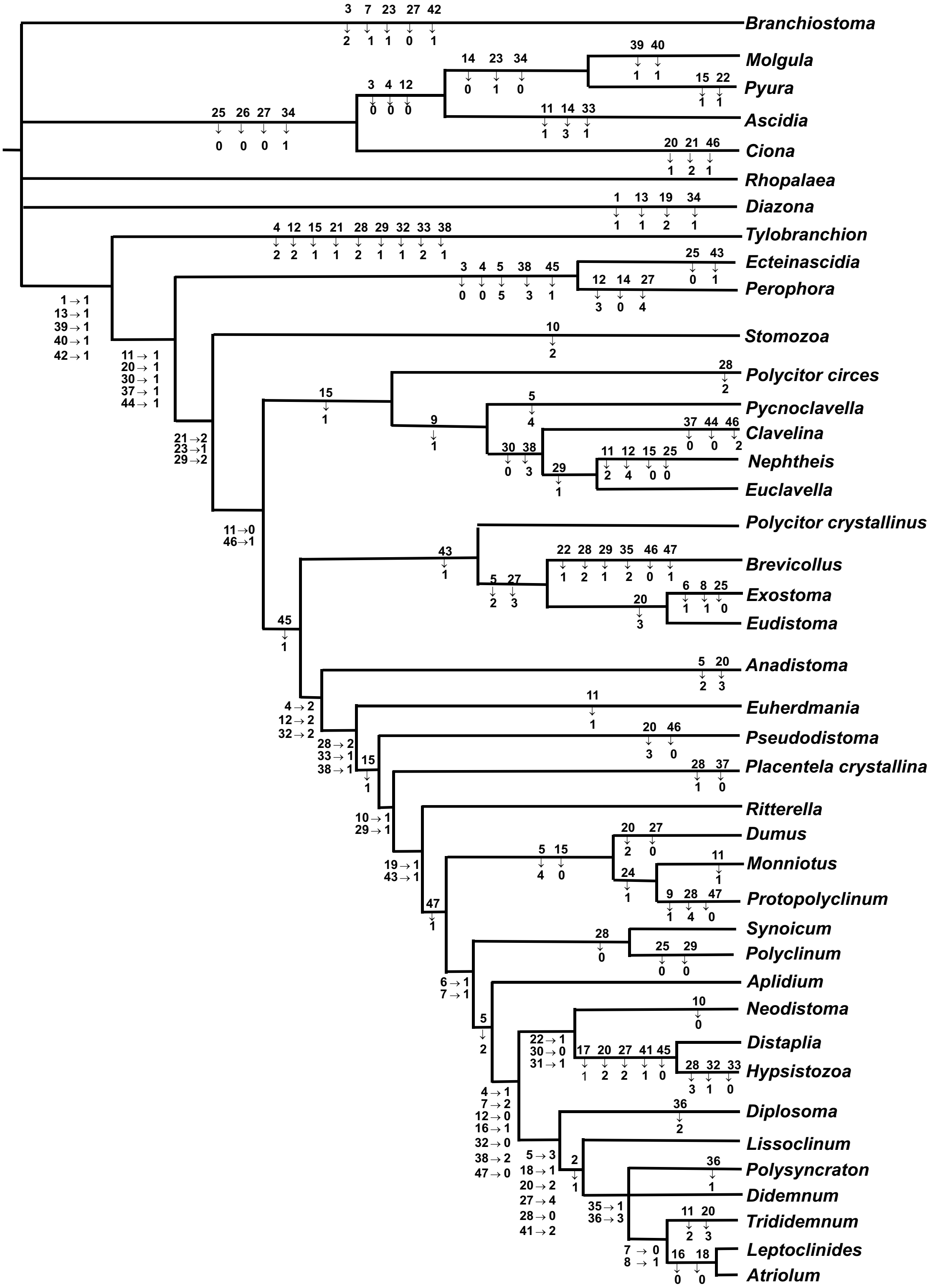


Figure 1. Strict consensus of nine most parsimonious trees (161 steps; CI = 0.5031, IR = 0.7922) for 41 taxa of Aplousobranchia (Tunicata, Ascidiacea) and 47 characters.

Table III. Character matrix, with the 47 morphological characters and states used in the phylogenetic analysis of the Order Aplousobranchia.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Branchiostoma	0	-	2	1	-	-	1	-	0	-	-	-	-	-	0	-	-	-	0	0	0	0	1	0
Pyura	0	0	0	0	-	-	0	-	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0
Molgula	0	0	0	0	-	-	0	-	0	?	0	?	?	0	0	0	0	0	0	?	0	?	1	0
Ascidia	0	0	0	0	-	-	0	-	?	?	1	0	0	3	0	0	0	0	0	0	0	0	0	0
Perophora	1	0	0	0	5	0	0	0	0	0	1	3	1	0	?	0	0	0	0	?	?	0	0	0
Ecteinascidia	1	0	0	0	5	0	0	0	?	?	1	-	?	?	0	0	0	0	0	1	0	0	0	0
Ciona	0	0	1	1	-	-	0	-	0	0	?	1	0	1	0	0	0	0	0	0	0	0	0	0
Rhopalaea	0	0	1	1	-	-	0	-	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0
Diazona	1	0	1	1	0	0	0	0	0	0	0	1	1	2	1	0	0	0	2	0	0	0	0	0
Tylobranchion speciosum	1	0	1	2	0	0	0	0	0	0	0	2	1	1	1	0	0	0	0	0	1	0	0	0
Stomozoa	1	0	1	1	0	0	0	0	0	2	1	1	1	1	0	0	0	0	0	1	2	0	1	0
Polycitor circes	1	0	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	2	0	1	0
Pycnoclavella	1	0	1	1	4	0	0	0	1	-	?	1	1	1	1	0	0	0	0	1	2	0	1	0
Clavelina	1	0	1	1	0	0	0	0	1	-	?	1	1	1	1	0	0	0	0	1	2	0	1	0
Euclavella	1	0	1	1	0	0	0	0	1	-	0	1	1	1	1	0	0	0	?	1	2	0	1	0
Nephtheis	1	0	1	1	0	0	0	0	1	-	2	4	-	-	0	0	0	0	0	1	2	0	1	0
Polycitor crystallinus	1	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	2	0	1	0
Brevicollus	1	0	1	1	2	0	0	0	0	0	?	1	1	?	?	0	0	0	?	1	2	1	1	0
Exostoma	1	0	1	1	2	1	0	1	0	0	0	1	1	1	0	0	0	0	?	3	2	0	1	0
Eudistoma	1	0	1	1	?	0	0	0	0	0	0	1	1	1	0	0	0	0	0	3	2	0	1	0
Anadistoma	1	0	1	2	2	0	0	0	0	0	?	2	1	1	0	0	0	0	?	3	2	0	1	0
Euherdmania	1	0	1	2	?	0	0	0	0	0	1	2	1	1	0	0	0	0	0	1	2	0	1	0
Pseudodistoma	1	0	1	2	?	0	0	0	0	0	?	2	1	1	1	0	0	0	0	3	2	0	1	0
Placentela crystallina	1	0	1	2	0	0	0	0	0	1	0	2	1	1	1	0	0	0	?	1	2	0	1	0
Ritterella	1	0	1	2	?	0	0	0	0	1	0	2	1	1	1	0	0	0	1	1	2	0	1	0
Dumus	1	0	1	2	4	0	0	0	0	1	0	2	1	1	0	0	0	0	?	2	2	0	1	0
Monniotus	1	0	1	2	4	0	0	0	0	1	1	2	1	1	0	0	0	0	1	1	2	0	1	1
Protopolyclinum	1	0	1	2	4	0	0	0	1	-	0	2	1	1	?	0	0	0	?	1	2	0	1	1
Synoicum	1	0	1	2	0	1	1	-	0	1	0	2	1	1	1	0	0	0	1	1	2	0	1	0
Polyclinum	1	0	1	2	?	1	1	-	0	1	0	2	1	1	1	0	0	0	1	1	2	0	1	0
Aplidium	1	0	1	2	2	1	1	-	0	1	0	2	1	1	1	0	0	0	1	1	2	0	1	0
Neodistoma	1	0	1	1	2	1	2	-	0	0	?	0	1	1	1	?	?	0	?	1	2	1	1	0
Distaplia	1	0	1	1	2	1	2	-	0	1	0	0	1	1	1	1	1	0	1	2	2	1	1	0
Hypsistozoa	1	0	1	1	?	1	2	-	0	1	0	0	1	1	1	1	1	0	1	2	2	1	1	0
Diplosoma	1	0	1	1	3	1	2	-	0	1	0	?	?	?	1	1	0	1	1	2	2	0	1	0
Lissoclinum	1	1	1	1	3	1	2	-	0	1	0	0	1	1	1	1	0	?	1	2	2	0	1	0
Polysyncraton	1	1	1	1	3	1	2	-	0	1	0	0	1	1	1	1	0	1	1	2	2	0	1	0
Didemnum	1	1	1	1	3	1	2	-	0	1	0	0	1	1	1	1	0	1	1	2	2	0	1	0
Trididemnum	1	1	1	1	3	1	0	1	0	1	2	0	1	1	1	1	0	1	1	3	2	0	1	0
Leptoclinides	1	1	1	1	3	1	0	1	0	1	0	0	1	1	1	0	0	0	1	2	2	0	1	0
Atriolum	1	1	1	1	3	1	0	1	0	1	0	0	1	1	1	0	0	0	1	2	2	0	1	0

Continue

Table III. Continued.

Taxon	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Branchiostoma	1	-	0	0	0	0	0	-	-	2	-	0	0	-	0	0	-	1	0	-	-	-	-
Pyura	0	0	0	?	0	0	0	-	0	0	0	0	0	-	0	0	-	0	0	0	0	0	0
Molgula	0	?	0	0	0	0	0	-	0	0	0	0	0	-	1	1	0	0	0	-	-	-	0
Ascidia	0	0	0	?	0	0	0	0	1	1	0	0	0	-	0	0	-	0	0	0	0	0	0
Perophora	1	1	4	0	0	1	0	0	0	2	0	?	1	3	1	1	0	1	0	1	1	0	0
Ecteinascidia	0	1	1	?	0	1	0	0	0	2	0	0	1	3	1	1	0	1	1	1	1	0	0
Ciona	0	0	0	?	0	0	0	0	0	1	0	0	0	-	0	0	-	0	0	0	0	0	0
Rhopalaea	1	1	1	1	0	0	0	0	0	2	0	0	0	-	0	0	-	?	?	?	?	?	?
Diazona	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	-	0	0	0	0	0	0
Tylobranchion speciosum	1	1	1	2	1	0	0	1	2	2	0	0	0	1	1	1	0	1	0	0	0	0	0
Stomozoa	1	1	1	0	2	1	0	0	0	2	0	0	1	0	1	1	0	1	?	1	0	0	0
Polycitor circes	1	1	1	2	2	1	0	0	0	2	0	0	1	0	1	1	0	1	0	1	0	1	0
Pycnoclavella	1	1	1	0	2	1	0	0	0	2	0	0	1	?	1	1	0	1	0	1	0	1	0
Clavelina	1	1	1	0	2	0	0	0	0	2	0	0	0	3	1	1	0	1	0	0	0	2	0
Euclavella	?	1	1	0	1	0	0	0	0	2	0	0	1	?	1	1	?	1	0	1	0	-	0
Nephtheis	0	1	1	0	1	0	0	0	0	2	0	0	1	3	1	1	0	1	0	1	0	1	0
Polycitor crystallinus	1	1	1	0	2	1	0	0	0	2	0	0	1	0	1	1	0	1	1	1	1	1	0
Brevicollus	?	?	3	2	1	1	0	0	0	2	2	0	1	?	1	1	0	1	1	1	1	0	1
Exostoma	0	1	3	0	2	1	0	0	0	2	0	0	1	?	1	1	0	1	1	1	1	1	0
Eudistoma	?	1	3	0	2	1	0	0	0	2	0	0	1	0	1	1	0	1	1	1	1	1	0
Anadistoma	?	?	1	0	2	1	0	2	0	2	0	0	?	?	1	?	?	?	?	?	?	?	?
Euherdmania	1	1	1	2	2	1	0	2	1	2	0	0	1	1	1	1	0	1	0	1	-	-	0
Pseudodistoma	1	1	1	?	2	1	0	2	1	2	0	0	1	1	1	1	0	1	0	1	1	0	0
Placentela crystallina	?	1	1	1	1	1	0	2	?	?	0	?	0	1	1	1	0	1	0	1	1	1	0
Ritterella	1	?	1	2	1	1	0	2	1	2	0	0	1	1	1	1	0	1	1	1	1	1	0
Dumus	?	?	0	?	1	1	0	2	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1
Monniotus	?	1	1	?	1	1	0	2	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1
Protopolyclinum	?	1	1	4	1	1	0	2	1	2	0	0	1	1	1	?	?	1	1	?	?	?	0
Synoicum	1	1	1	0	1	1	0	2	1	2	0	0	1	1	1	1	?	1	?	1	1	?	1
Polyclinum	0	1	1	0	0	1	0	2	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1
Aplidium	?	1	1	2	1	1	0	2	1	2	0	0	1	1	1	1	0	1	1	1	1	1	1
Neodistoma	?	?	1	2	1	0	1	0	?	?	?	?	?	?	1	?	?	?	?	?	?	?	?
Distaplia	1	1	2	?	1	0	1	0	?	2	0	0	1	2	1	1	1	1	1	1	0	1	0
Hypsistozoa	1	?	2	3	1	0	1	1	0	2	0	0	1	2	1	1	1	1	1	1	0	1	0
Diplosoma	?	?	4	0	?	1	0	0	1	2	0	2	1	2	1	1	2	1	1	1	1	1	0
Lissoclinum	?	?	4	0	?	1	0	0	1	2	0	?	1	2	1	1	2	1	1	1	1	1	0
Polysyncraton	?	?	4	0	1	1	0	0	1	2	1	1	1	2	1	1	2	1	1	1	1	1	0
Didemnum	?	?	4	0	1	1	0	0	1	2	1	3	1	2	1	1	2	1	1	1	1	1	0
Trididemnum	1	?	4	0	1	1	0	0	1	2	1	3	1	2	1	1	2	1	1	1	1	1	0
Leptoclinides	?	?	4	0	1	1	0	0	1	2	1	3	1	2	1	1	2	1	1	1	1	1	0
Atriolum	1	1	4	0	1	1	0	0	1	2	1	3	1	2	1	1	2	1	1	1	1	1	0

The presence of spicules in the tunic (2.1) is a synapomorphy for *Lissoclinum*+. However, spicule recurrence in some species of *Eudistoma* (*E. spiculiferum* Millar, 1977) and *Cystodytes* Drasche, 1884 indicates that the presence of spicules may be homoplasic.

The position of the intestine and gonads posterior to the pharynx (3.2) is observed in Cephalochordata. The bending of the abdomen with the consequent relocation of the anus towards the oral siphon occurred in Tunicata. This bending is more pronounced in animals with sac-like bodies where the alimentary canal and gonads are next to the pharynx (*Pyura*+ *Molgula*, *Ascidia* and *Perophora*+ *Ecteinascidia*), but *Ciona* has an intermediary condition.

Elongated body is a plesiomorphy shared by Cephalochordata and Aplousobranchia. The *Ciona*+ clade tended towards body length reduction into a sac-like shape (4.0) with the gut next to the pharynx (characteristic of *Pyura*, *Molgula* and *Ascidia*). The body of *Ciona* has been described as undivided, despite its general appearance (VAN NAME 1945, MILLAR 1970, MONNIOT & MONNIOT 1972). However, we suggest that the alimentary canal and gonads in this genus was considered posterior to the pharynx, following KOTT (1969, 1990), thereby dividing the body of *Ciona* into thorax and abdomen, which may be considered as a transition towards the reduction of the later.

In Aplousobranchia, different states evolved: 1. (*Perophora*+ *Ecteinascidia*) bended the alimentary tract in a sac-like body (4.0); 2. further elongation of the body with the appearance of a posterior abdomen enclosing the gonads and the heart. Body division (4), broadly used in the traditional classifications, was modified several times in the evolution of the Order Aplousobranchia. The posterior abdomen (4.2) appears twice in the evolutionary history of the Order: in *Tylobranchion* and in the genera traditionally included in Polyclinidae (*Anadistoma*+), thus body division is clearly a homoplasic character. Reversion of the state 4.2 (body divided into thorax, abdomen and posterior abdomen) to 4.1 (body divided into thorax and abdomen) can be observed in the clade (*Neodistoma*(*Distaplia*+*Hypsistozoa*))+*Diplosoma*+. We coded a true posterior abdomen as present (4.2), when the body extended posterior to the abdomen and when the gonads and heart were situated in this body region. Often, the position of the heart in the most subsequent extremity of the abdominal prolongation has been used to identify the posterior abdomen and consequently to define some families of Ascidiacea; however, this structure is difficult or impossible to identify in conserved specimens, thus we accepted published descriptions for this character.

Taxonomists do not concur on body divisions in some genera, but it is generally accepted that the posterior abdomen is the main apomorphy of the traditional family Polyclinidae. In *Placentela crystallina* Redikorzev, 1913, the heart is clearly located in the abdomen below the intestinal loop and not in the most distal extremity of the abdominal prolongation. *P. crystallina* was first described as not having a posterior abdomen, but subsequent descriptions found gonads and epicardium sacs within the abdominal prolongation, which was therefore considered a posterior abdomen (NISHIKAWA 1984). Here we accepted this view. KOTT (1990) defined a new family comprising the two known species of *Placentela*: *P. crystallina* and *P. translucida* Kott, 1969, but the position of the heart in the latter species was not mentioned. Because doubts remained regarding the presence of a true posterior abdomen as defined above for *P. translucida*, we do not consider this species in the final analysis.

In *Tylobranchion speciosum* Herdman, 1886 the gonads are located below the intestinal loop and the heart is not distant from the alimentary tract; the epicardium extends beyond the pericardial sacs to the most posterior part of the zooid (MILLAR 1960). Therefore, this species was coded having a posterior abdomen in this analysis.

In *Hypsistozoa*, the gonads and the epicardium are placed below the intestinal loop, in the proximal part of the vascular appendix, while the heart is in the abdomen (KOTT 1969, 1990). Thus we considered the body divided into thorax and abdomen.

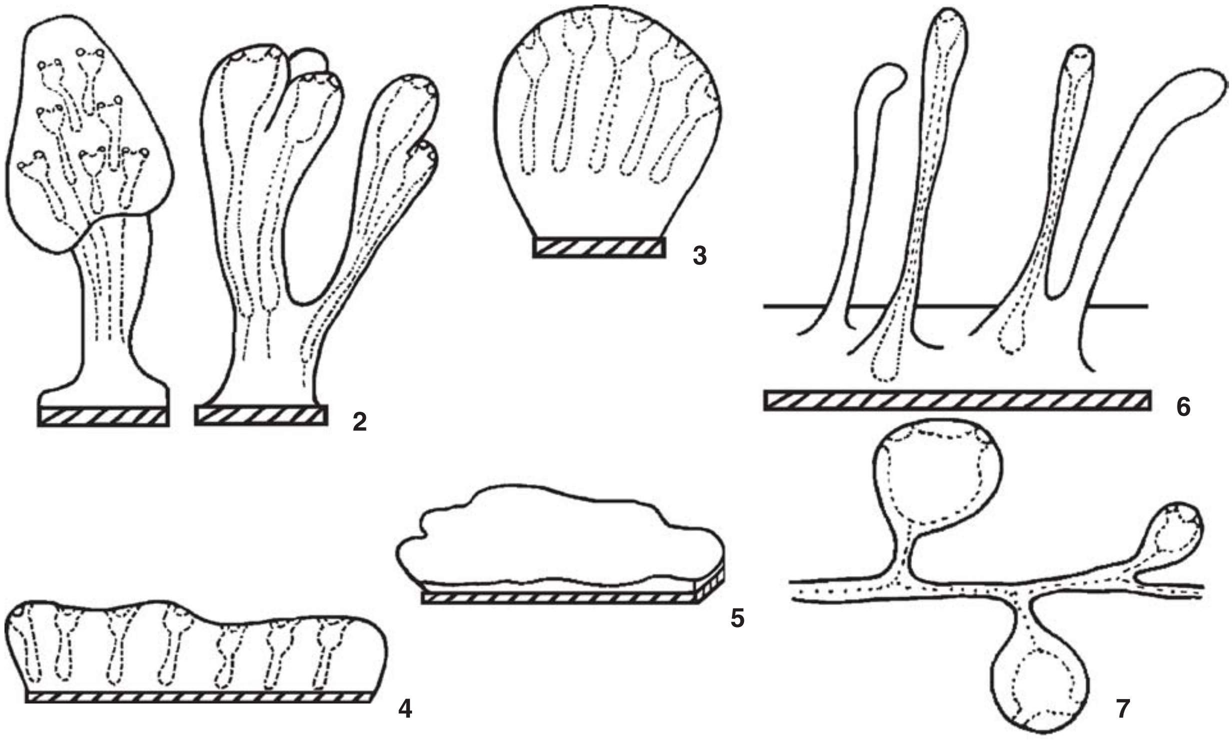
Euherdmania comprises species with and without a posterior abdomen (KOTT 1992). The three species used in this analysis have a posterior abdomen and the genus was among other *taxa* with a posterior abdomen in cladogram retrieved (Fig. 1). A phylogenetic study of this genus is necessary to show whether it is monophyletic (and loss of a posterior abdomen is derived within the genus) or polyphyletic (in which case affinities of the species without the posterior abdomen should be revised).

The more basal colonial genus, *Tylobranchion* to *Stomozoa*, form pedunculated colonies (5.0) (Fig. 2), and this shape modified toward an encrusting form, either maintaining the zooids inside projections (Fig. 6) or not (Figs 3-5). Stoloniferous colonies (5.5) appeared only in Perophoridae (Fig. 7).

In several genera of Aplousobranchia a cloaca is absent and both siphons are tubular and open directly on the colony surface (6.0). In the genera in which zooids are organized into systems, a true cloaca exists and the atrial siphons open into these common cavities and only the oral siphons open directly on the surface (6.1) (Figs 8-10). This condition is synapomorphic for the group (*Synoicum*+*Polyclinum*)+.

In (*Synoicum*+*Polyclinum*)+*Aplidium*+ the atrial aperture is small (7.1) (Fig. 12). The wide atrial opening (7.2) (Fig. 13) is present in ascidians with developed cloacal systems, such as in the clade (*Neodistoma*(*Distaplia*+*Hypsistozoa*))+. It becomes a tubular siphon (7.0) (Fig. 14) in some genera of this last clade: *Trididemnum*(*Leptoclinides*+*Atriolum*). Apical atrial siphons (8.0) are found in the great majority of the internal group, with the exception of *Exostoma* and *Trididemnum*+.

The character 9 (oral siphon margin – Figs 15-18) is polymorphic in *Ascidia*, *Ecteinascidia*, *Hypsistozoa* and *Leptoclinides*. By comparison with functional outgroups, lobed (9.0) is plesiomorphic for *Hypsistozoa* and *Leptoclinides*. The oral siphon



Figures 2-7. Types of colonies: (2) stalked; (3) globular; (4) cushion; (5) sheet; (6) encrusting base; (7) zooids from a stolon.

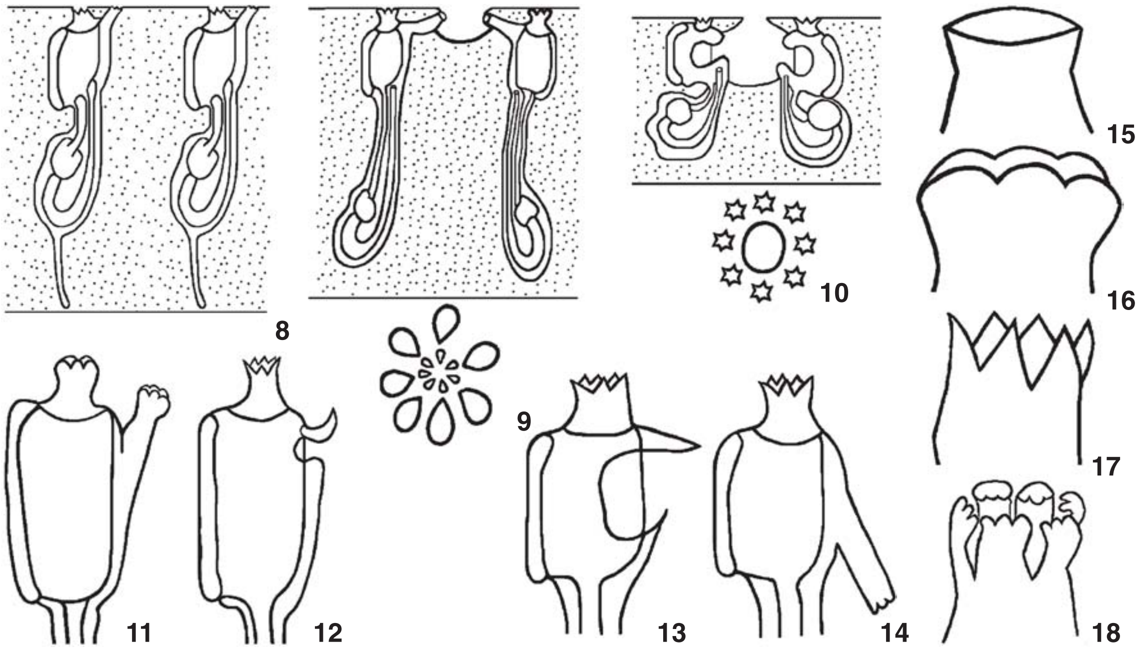
with a smooth margin (9.1) appears independently in *Protopolyclinum* and *Pycnoclavella*(*Clavelina* (*Euclavella*+ *Nephtheis*)). The aspect of the atrial siphon margin is usually similar to the oral siphon margin.

The number of oral tentacles (11) varied, and was polymorphic in *Ciona*, *Pycnoclavella*, *Clavelina*, *Eudistoma*, *Pseudodistoma* and *Aplidium*. In *Eudistoma* and *Aplidium* the plesiomorphic state is "from 11 to 24" (11.0). The number of oral tentacles is unrelated to body size or life habit, since *Pyura*, *Diazona*, *Rhopalaea* (relatively large organisms) have a similar number of tentacles with that found in tiny forms, such as *Diplosoma*+. On the other hand, *Ciona*, a genus of large solitary ascidians has species with both, a reduced number of tentacles (*C. antarctica* Hartmeyer, 1911 – 12 tentacles and *C. pomponiae* Monniot & Monniot, 1989 – 16 tentacles) and species with numerous oral tentacles (*C. intestinalis* – greater than 50). *Perophora* (a colonial ascidian) is another example, with individuals of reduced size, which have around 30 oral tentacles (11.1). The state "few oral tentacles" seems to be plesiomorphic, occurring in *Diazona* and *Rhopalaea*.

Since many descriptive, especially older, studies do not describe the orientation of the muscle bands, in all specimens the muscle was carefully examined (Figs 19-25). Muscle bands in *Pyura* and *Molgula* are oblique in relation to the endostyle, as well as in *Perophora*+*Ecteinascidia*. In some species of *Molgula*, however, muscles are only present in the siphon or extend towards the middle of the thorax (12.3). *Ascidia* have either complete longitudinal muscles, ending at the middle of the thorax or absent. When complete, bands have an irregular distribution (12.3), which is autapomorphic for this genus. "Muscle bands parallel to the endostyle" (12.1) is a homoplasic character and arose independently in *Ciona* and in *Tylobranchion*+. *Rhopalaea* and *Diazona* have a very unique muscle orientation (12.2).

Transverse muscles on the body wall (15) (Fig. 26) found in *Molgula*, *Ascidia* and in *Ciona* differ from those observed in other taxa of the internal group, in that they are irregular and anastomose in the extremities. The thoracic transverse muscles were lost (15.1) independently several times: in *Pyura, Polycitor circes* + and *Pseudodistoma*+. Hence, transverse muscles are present in many basal *taxa*, disappear and appear again supporting the clade *Dumus* (*Monniotus*+*Protopolyclinum*) in the ingroup. In some genera such as *Ascidia*, *Molgula* and *Perophora*, transverse muscles can be absent in one side, usually the left, and therefore only the right side was included in the analysis.

Dorsal longitudinal muscle band (16.1) (Figs 27 and 28) associated with the pharynx was not previously mentioned in the literature for *Distaplia*, *Hypsistozoa* and *Neodistoma*, and was revealed by the study of the genera *Distaplia* and *Hypsistozoa*. In the analysis it supported the position of the clade *Neodistoma* (*Distaplia*+*Hypsistozoa*) as the sister-group of *Diplosoma*+, even though there is no information of this character for *Neodistoma*. The relationship between *Neodistoma* (*Distaplia*+*Hypsistozoa*) and

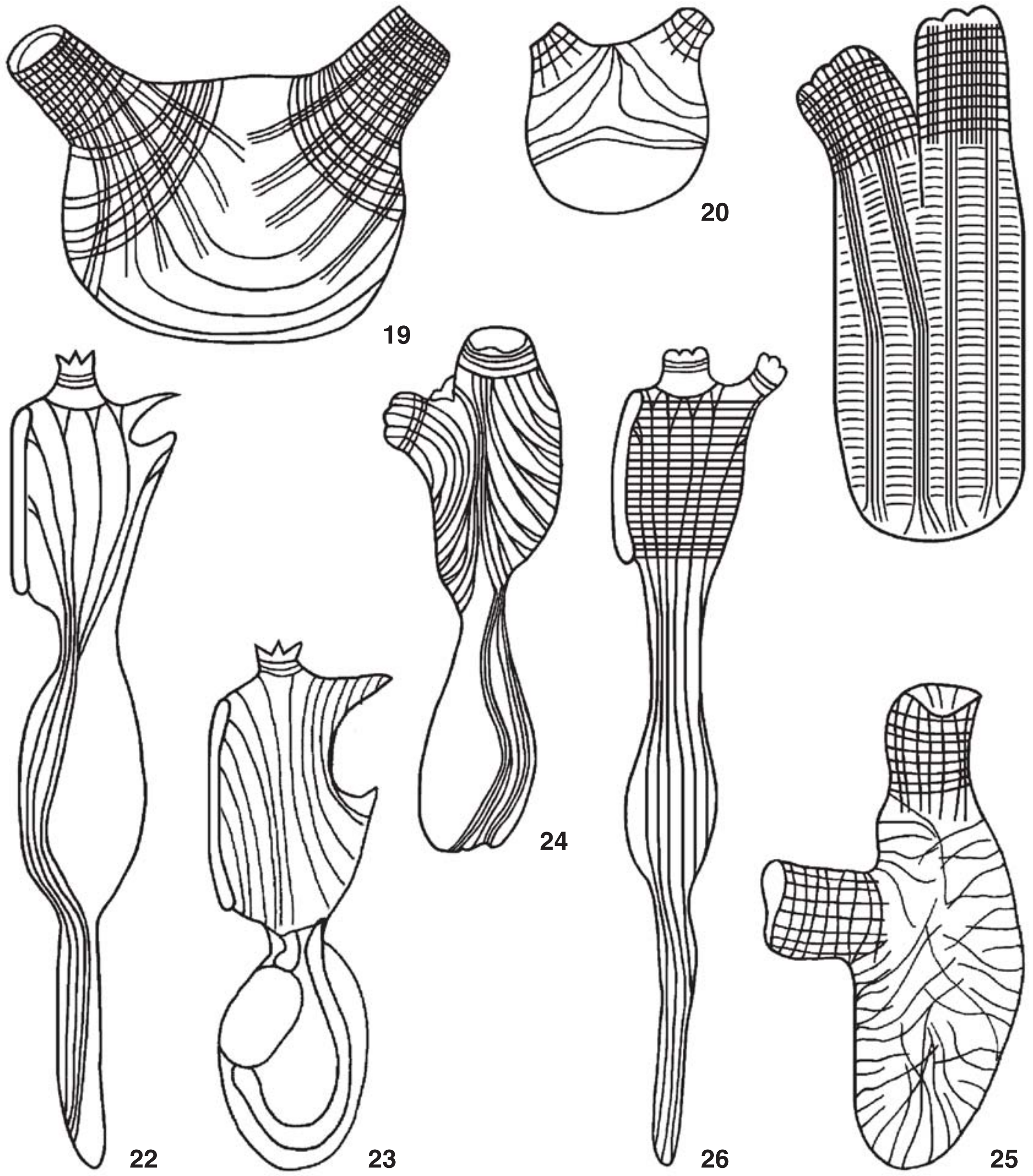


Figures 8-18. (8-10) Openings on the colony surface: (8) both siphons open on colony surface, without cloacal systems, (9) both siphons open on colony surface, the atrial in a depression on the colony surface (rudimentary cloaca) – cross section and view from the colony surface, (10) only oral siphon opens on colony surface: the atrial siphon opens within the tunic in cavities or channels that communicate with the exterior through a cloaca – cross section and view from the colony surface; (11-14) atrial siphon: (11) tubular apical; (12) small and apical; (13) wide; (14) tubular basal; (15-18) oral siphon: (15) margin smooth; (16) margin with rounded lobes; (17) margin with pointed lobes; (18) margin with ramified lobes.

Diplosoma+ was also pointed out by Berrill (1936) and Hirose (2001). This character appears together with the modification of the atrial siphon forming a wide aperture that exposes a large part of the pharynx. In these *taxa*, the body wall is reduced and the appearance of this paired muscle band may have reinforced the pharynx. A secondary loss (16.0) of this character occurs in *Leptoclinides+Atriolum*. Despite further modification of the atrial siphon as a tube in *Trididemnum* (*Leptoclinides+Atriolum*), the dorsal muscle pharyngeal band is still present in *Trididemnum*, but it is lost in the others. The "abdominal prolongation of the dorsal pharyngeal muscle band is present only in *Distaplia+Hypsistozoa* (17.1) (Fig. 27). This character might have appeared in the base of the clade *Neodistoma*+. This dorsal muscle pharyngeal band could be equivalent to the muscular process but a more detailed study is necessary to determine homology. The muscular process (18.1) (Fig. 28) is the posterior continuation of the dorsal pharyngeal muscle band that detaches from the body wall. It is a synapomorphy for *Diplosoma*+, being lost secondarily in *Leptoclinides+Atriolum*, along with the loss of the dorsal pharyngeal muscle band. Polymorphism for this character occurs only in *Lissoclinum*.

Beyond the dorsal longitudinal muscle, some genera also present transverse pharyngeal muscle (19.1) (Figs 29, 34 and 35). It arises in *Ritterella*+, *i.e.*, in ascidians with posterior abdomen, traditionally of Polyclinidae. *Euherdmania* and *Pseudodistoma* had no muscle (19.0) and the character state in *Placentela crystallina* cannot be defined because of the preserved condition of the specimen. "Presence" (19.1) was defined as the plesiomorphic state for *Distaplia*, which is polymorphic. Recently, this character was described in Didemnidae (*Diplosoma*+; Kott 2001). *Dumus* and *Protopolyclinum* must be further studied to determine the presence of transverse pharyngeal muscle bands, which would better define the relationship among the *taxa* of the clade. Transverse muscle bands also occur in *Ciona antarctica* and in *Diazona violacea* Savigny, 1816, yet not on all the transverse vessels. The plesiomorphic state for *Ciona* was defined as "absence" (19.0).

No species were found with between 26 and 74 rows of stigmata, hence we defined the state as 75 rows or more (20.0). This character occurs in genera of simple ascidians (*Ciona*, *Pyura* and *Ascidia*), and in *Rhopalaea*, *Diazona* and in the most basal taxon of the internal group *Tylobranchion*. While "three" and "four" rows of stigmata are congenerically constant, they arise independently in different groups. Three rows (20.3) arose four times: in *Exostoma+Eudistoma*, in *Anadistoma*, in *Pseudodistoma* and in *Trididemnum* and four rows (20.2) arose in *Dumus* and in the clade (*Neodistoma*(*Distaplia+Hypsistozoa*)+. Reversion to many rows occurs in *Neodistoma*. Reduction of four to three rows of stigmata is not obligatory. This character is clearly associated with

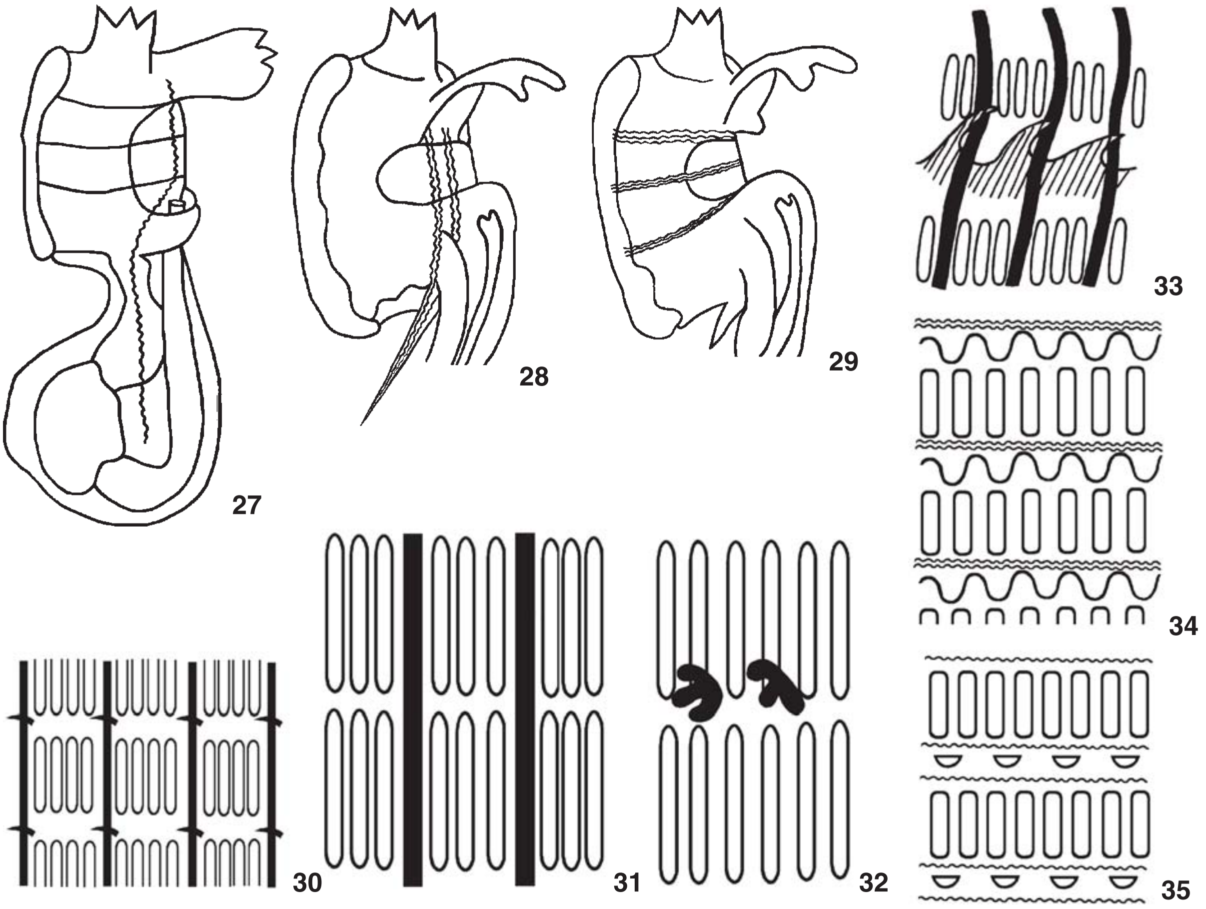


Figures 19-26. Musculature on the body wall: (19-20) oblique to the endostyle; (21-23) parallel to the endostyle; (24) oblique, ramified and anastomosed; (25) irregular; (26) thoracic transversal.

the size of the thorax and the reduction of the number of rows, from state 20.0 (75 rows or more) to 20.1 (5-26 rows) and then to each one of the two other states (20.2 – three rows, or 20.3 – four rows) occurred at different times in the evolution of the group.

The plesiomorphic state "complete longitudinal blood vessels" (21.0) (Figs 30 and 31) is found in *Branchiostoma*, *Ciona*+ and in the most basal *taxa* of the internal group. *Rhopalaea* and *Diazona* occasionally present incomplete vessels, but in the studied specimens, the vessels were apparently ruptured and so were treated as complete vessels. Vessels were incomplete (21.1) (Fig. 32) in *Tylobranchion speciosum* and some species of *Perophora*, whose internal pharyngeal papillae appear in form of "T". Vessels are completely absent (21.2) in the *taxa* beginning with *Stomozoa*+.

Parastigmatic vessels (22.1) is homoplasic and arose independently in the genera: *Pyura*, *Brevicollus*, and supports the



Figures 27-35. (27-29) Pharyngeal muscle: (27) dorsal longitudinal band extending until the abdomen; (28) dorsal longitudinal band originating the muscle process; (29) musculature on the pharyngeal transversal vessels; (30-32) pharyngeal longitudinal vessels: (30-31) complete; (32) incomplete; (33-35) pharyngeal papillae in: (33) *Rhopalaea*; (34) *Polyclinum*; (35) *Monniotus*.

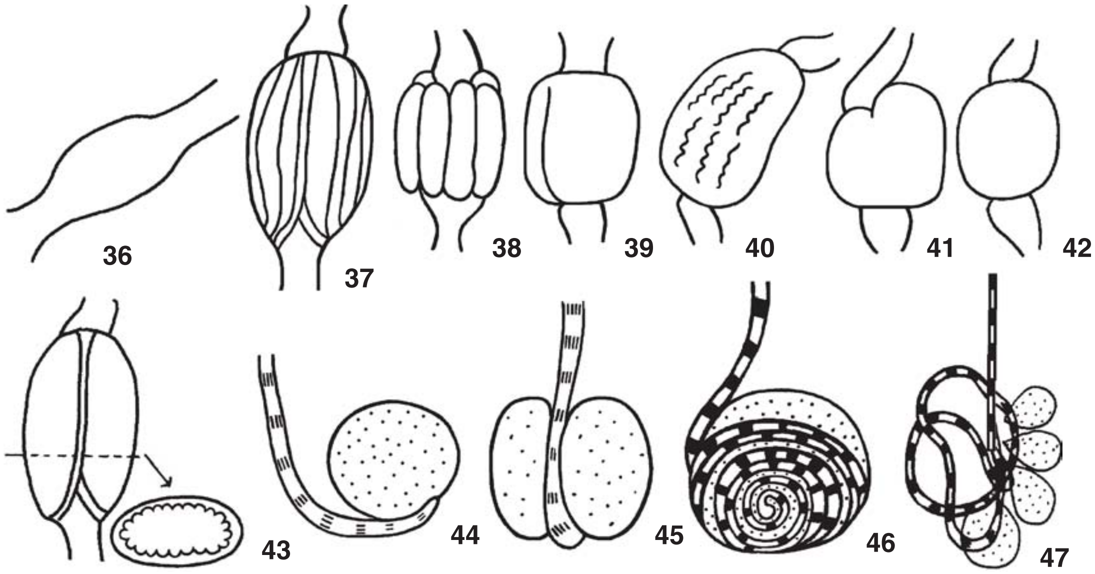
clade *Neodistoma* (*Distaplia+Hypsistozoa*). This character is polymorphic in *Molgula*, *Dumus*, *Monniotus* and *Hypsistozoa*. The plesiomorphic state is "presence" (22.1) in *Hypsistozoa*, in comparison with the external functional group. Conversely, the plesiomorphic state for *Monniotus* and *Dumus* is "absent" (22.0).

Branchial papillae are not present (23.1) (Fig. 33) in *Branchiostoma*, neither in *Pyura+Molgula* or in *Stomozoa+*. Short and rounded branchial papillae (24.1) (Figs 34 and 35) are found in *Monniotus+Protopolyclinum*. This papillae type was also coded for *Protopolyclinum*, even though none of the species was directly studied, and will require confirmation (Millar 1960).

A simple prepharyngeal groove (25.1) appears in *Branchiostoma* and in the basal *taxa* of the ingroup. A double prepharyngeal groove (25.0) was derived many times independently in the *taxa Ciona+*, *Ecteinascidia*, *Nephtheis*, *Exostoma* and *Polyclinum*. Other genera present both states, *e.g.*, *Clavelina*, *Eudistoma* and *Aplidium*.

The shape of the neural gland opening seems to be associated with the size of the organisms, but small and round neural gland opening (26.1) provides a supporting character for the clade *Ciona+*.

The ancestral condition of the digestive tract is elongated with oral and anal extremities distant one from the other, as previously described in the discussion of body shape. A poorly differentiated elongated stomach (27.0) (Fig. 36) occurs in *Branchiostoma* and *Ciona+* and it is also the plesiomorphic state. A short stomach then developed, becoming rectangular or barrel-shaped (27.1) (Figs 37-39), which occurs in most genera of the ingroup. Then other shapes arose: trapezoidal (27.3) (Fig. 41) as a synapomorphy for *Brevicollus* (*Exostoma+Eudistoma*); kidney-shaped (27.2) (Fig. 40) as a synapomorphy of *Distaplia+ Hypsistozoa* and rounded (27.4) (Fig. 42), first in *Perophora* and again in *Diplosoma+*. A reversion to state 27.0 occurred in *Dumus*. While the shape of the stomach is rather constant within the genus, the character "stomach wall" (28) (Figs 36-43) is polymorphic for many of them (*Ciona*, *Ascidia*, *Pyura*,



Figures 36-47. (36-43) Stomach shape: (36) elongated; (37) oval with folded wall; (38) square with longitudinal folds; (39) square and smooth; (40) kidney-shaped and ornamented; (41) trapezoidal and smooth; (42) rounded and smooth; (43) oval with internal folds; (44-47) proximal end of the spermiduct: (44-45) right; (46) spiraled; (47) convolute.

Ecteinascidia, *Euclavella*, *Pseudodistoma*, *Dumus*, *Monniotus* and *Distaplia*) what makes it difficult to interpret. The state (28.4) only appears in *Dumus dumosus* (Monniot, 1987) and *Protopolyclinum pedunculatum* (Millar, 1960).

The esophagus is short (29.0) in the *taxa* considered as out-group and in the more basal genera of the ingroup (plesiomorphic state). With elongation of the intestine in *Stomozoa* + the stomach moved to the posterior half of the abdomen (29.2). In *Placentela crystallina*+ the stomach is in the middle of the proximal branch of the gut (29.1). The middle position arises independently in *Tylobranchion*, *Euclavella*+ *Nephtheis* and in *Brevicollus*. Reversion to state (29.0) occurs in *Polyclinum*.

The most basal *taxa* with a posterior stomach (30.1) are (*Perophora*+*Ecteinascidia*)+, supporting their placement in the internal group. The posterior stomach disappears in the clade *Clavelina* (*Euclavella*+*Nephtheis*) and in *Neodistoma* (*Hypsistozoa*+ *Distaplia*). The gastric reservoir (31.1) is a synapomorphy for the *Neodistoma*(*Distaplia*+ *Hypsistozoa*) clade.

Gonads in the abdomen below the intestinal loop (32.1) arose twice, in *Tylobranchion* and *Hypsistozoa*. In *Tylobranchion speciosum* part of the gonads lies in the intestinal loop, the remainder extends to the posterior abdomen. *Hypsistozoa* has no posterior abdomen, but the gonads are located below the intestinal loop, in the proximal part of the vascular stolon. In both, the state of the character was coded "below intestinal loop" (32.1). Beginning with *Anadistoma*+ the gonads move posteriorly to the posterior abdomen (32.2). Reversion to gonads inside the intestinal loop (32.0) occurs in (*Neodistoma* (*Distaplia*+*Hypsistozoa*))+. A gonadal sac (32.3) was only observed in some species of *Distaplia*. However, by comparison with the outgroup *Neodistoma*, we concluded that the plesiomorphic state of this character is 32.0 for *Distaplia*.

The ovary in the center of the testis lobes (33.0) is the plesiomorphic condition. In *Euherdmania*+ the ovary is anterior to the testis lobes (33.1). A reversion occurs in *Hypsistozoa* (33.0) and in two species of *Distaplia*. The plesiomorphic state for *Polyclinum* was 33.1.

Lobed testes (34.0) are found in *Pyura*+*Molgula,* while *Ciona*, *Ascidia* and *Diazona* have an irregular, ramified and elongated testis (34.1). The rounded or pyriform shape of the lobes (34.2) is found in all other *taxa* studied. Only *Clavelina* is polymorphic, with plesiomorphic state 34.2. All *taxa* have a straight sperm duct (Figs 44 and 45), with spiral proximal sperm duct (35.1) (Fig. 46) being a synapomorphy for (*Polysyncraton*, *Didemnum* (*Trididemnum*+)) while the convoluted duct (35.2) (Fig. 47) for *Brevicollus*. Most *taxa* studied have more than 10 testis follicles (36.0) and there is a tendency to decrease number towards the more apical groups. Genera with more than 10 follicles were often variable. *Distaplia*, for instance, has from 10-20 follicles while in *Aplidium* the variation is even greater: from 10 in *A. lobatum* Savigny, 1816 up to 58 in *A. flavolineatum* Monniot, 1987. That number also varies according to the state of maturation of the gonad. Polymorphisms occur in *Perophora*, *Lissoclinum*, *Leptoclinides* and *Atriolum*.

Numerous small oocytes (37.0) occur in *Branchiostoma*, *Ciona*+ and in the most basal *taxa* of the internal group. A reduction to less than 15 oocytes (37.1) begins with (*Perophora*+ *Ecteinascidia*)+. Only two reversions were observed, one in

Clavelina and other in *Placentela crystallina*. The number of oocytes depends on the size of the ovary; the ovary becomes smaller when asexual reproduction is more developed.

The epicardium, while a difficult character to study in fixed specimens, is an important organ used as regenerative tissue in the reproductive process and formation of colonies in Aplousobranchia (except in Clavelinidae; BERRILL 1936, KOTT 1990). In *Ciona* and other *taxa* of the internal group, the epicardium appears, just after the formation of the heart, as two evaginations of the pharynx (BERRILL 1936, 1950). Only in *Ciona* are these evaginations connected with the pharynx. In most other *taxa*, the epicardium sacs fuse and follow the abdomen – between the heart and the intestinal loop (in *Diazona*, *Clavelina*, *Pycnoclavella*, *Polycitor* and *Eudistoma*) – or the posterior abdomen (in *Polyclinum* and *Aplidium*) (BERRILL 1936). In *Tylobranchion*, the epicardium extends along the vascular stolon and continues towards the base of the colony (KOTT 1969). In *Placentela crystallina*, the epicardium extends into the posterior abdomen (NISHIKAWA 1984), although the heart is not found there. As in *Ciona*, the epicardium sacs remain separate and do not connect to the pharynx in *Euherdmania*. Two reduced epicardium sacs are near the oesophageal stalk of the Didemnidae. *Perophora* retains no trace of the epicardium, neither in its original form nor in the form of a renal vesicle (BERRILL 1936).

Diazona and all the *taxa* of the internal group reproduce asexually. Characters for vegetative reproduction of most genera were obtained from the literature. *Ciona* is a solitary, sexual, ascidian that can regenerate the thoracic area and the epicardium is capable of regenerating all other tissues (KOTT 1981). *Rhopalaea crassa* and *R. neapolitana* also do not bud, but occasionally the thorax degenerates, is absorbed and followed by the formation of a new thorax from the oesophageal region. This process is intermediate between regeneration and true budding through strobilization (KOTT 1981). In *Diazona*, *Polycitor* (KOTT 1990), *Eudistoma* (BERRILL 1950), and possibly in *Stomozoa* (KOTT 1981) budding involves the epicardium and the alimentary canal. Buds form following thorax reabsorption and alimentary reserve accumulation in the abdomen; budding then occurs by strobilization or by epidermal transverse constrictions of the abdomen (38.0) (BERRILL 1950). In *Anadistoma*+ strobilization occurs in the epicardium sac within the posterior abdomen (38.1) (BERRILL 1950, MILLAR 1966, KOTT 1992). In *Tylobranchion speciosum* buds form by dissolution of the thorax and the abdomen, and by constriction of the posterior abdomen (38.1) (BERRILL 1935). In *Neodistoma*, *Distaplia* and *Hypsistozoa* asexual reproduction occurs not in the vascular appendix, but rather by the extension of the epicardium in the vegetative stolon. Bud formation in *Distaplia rosea* Della Valle, 1881 occurs when mature zooids degenerate and the incubatory pouch is isolated and two small masses of tissue (originating in the anterior epicardium) develop near the oesophagus (BERRILL 1935). Highly specialized asexual reproduction occurs in Didemnidae (*Diplosoma*+) in which budding resembles that of *Distaplia* (BERRILL 1936). Buds are formed in the area of the oesophageal stalk, with reduced epicardium sacs. These types of budding were considered homologous (38.2). *Clavelina* and *Perophora* reproduce vegetatively without the involvement of epicardium sacs (38.3). In *Clavelina*, *Nephtheis* and *Perophora*, bud formation occurs in a large vascular stolon (KOTT 1990); here the mesenchyme of the vascular septum is the principal regenerative tissue (KOTT 1981). BERRILL (1950) and TRASON (1963) suggested that *Pycnoclavella* reproduces by strobilization of the abdomen. MILLAR (1966) and KOTT (1990) disagreed and proposed that budding in this *taxon* is through the vascular stolon as in *Clavelina*. Due to the divergence of information this character was classified as absent (?) in the data matrix.

External fertilization (39.0) occurs in *Branchiostoma*, *Ciona*+, *Rhopalaea* and *Diazona* (plesiomorphic state). In *Tylobranchion*, and all other *taxa* of the internal group, fertilization is internal (39.1), accompanied with the incubation of embryos. Internal fertilization appears along with viviparity and has some relationship with colonial life, although internal fertilization also appeared independently in *Molgula*. In *Diazona*, small oocytes leave the zooid before the fertilization and so this genus is considered oviparous (BERRILL 1950, MILLAR 1970, KOTT 1990). We found no embryos in the specimens of *Tylobranchion speciosum* studied here, but BERRILL (1935) and MILLAR (1960) both speak of well-developed larvae incubated in the atrial cavity. A few *taxa* seem to only incubate larvae in the oviduct: *Polycitor circes* Michaelsen, 1930, *Pycnoclavella*, *Monniotus* and *Euherdmania*. TRASON (1957) states that embryos of *Euherdmania claviformis* (Ritter, 1903) pass from the oviduct to the atrial cavity when mature. An incubation pouch (41.1) supports the clade (*Hypsistozoa*+*Distaplia*). Embryos were absent in all of our *Synoicum* specimens, while Kott (1969) describes the incubation of 1-8 embryos in isolated incubation pouches in the tunic of *Synoicum adareanum* (Herdman, 1902).

Most solitary ascidians do not incubate larvae, and so larval characters were included when available in literature. *Ciona*+ and *Diazona* produce simple and tiny larvae (maximum length of 0.2 mm). The character state "small larvae" occurs mostly in solitary ascidians and is associated with oviparity. The larval complexity the appeared in the *taxa* of Aplousobranchia does not occur in the larvae of oviparous species nor in the colonial or solitary viviparous forms of Stolidobranchia. *Diazona*, despite being colonial, is oviparous (BERRILL 1950, MILLAR 1970, KOTT 1990) and the larvae are similar to those of *Ciona*+. No information on larvae from *Rhopalaea* was available. In the remaining ingroup *taxa*, larvae are larger than 0.4 mm (42.1). *Tylobranchion*, the most basal *taxon* of the internal group to present viviparity produces embryos that reach 0.55 mm (MILLAR 1960, 1971) while some genera (*e.g.*, *Stomozoa*, *Synoicum*, *Distaplia* and *Leptoclinides*) produce even larger embryos (> 1.7 mm).

While ectodermal ampullae (Figs 48 and 49) are usually not visible in the larvae of solitary ascidians, in colonial ascidians ampullae are frequently conspicuous processes behind the adhesive papillae being digitiform, capitate, bulbous, or clavate. They grow from the epidermis of the trunk soon after larval fixation, with subsequent retraction (Cloney 1982), thus they are secondary organs of fixation with glandular cells that produce adhesive compounds. Only some species of *Pycnoclavella* (*P. minuta* Millar, 1953b and *P. stanleyi* Berrill & Abbott, 1949) have ectodermal ampullae. *Distaplia*, *Pseudodistoma* and *Stomozoa* were polymorphic for this character. The plesiomorphic states, by comparison with functional outgroups, were 43.0 for *Pycnoclavella* and *Pseudodistoma*, and 43.1 for *Distaplia*. The presence of ectodermal ampullae (43.1) supports the clade *P. crystallinus* Renier, 1804 (*Brevicollus* (*Exostoma*+*Eudistoma*)) and the clade *Ritterella*+. Independent origin for ectodermal ampullae occurs in larvae of *Ecteinascidia*.

Adhesive papillae (44) appeared in the ancestral of ascidians with a loss in larvae of Molgulidae which have no adhesive papillae, but the epidermis of the trunk secretes adhesive compounds serving the same function. While in many colonial ascidians, papillae evert quickly and expose the adhesive compounds, in solitary ascidians and some colonial ones (*e.g.*, *Clavelina*) papillae do not evert but rather simply secrete adhesives (Cloney 1982). Non-everting, conical adhesive papillae (44.0) (Fig. 50) are present in *Ciona*+, *Diazona* and *Tylobranchion*. Everting adhesive papillae (44.1) (Figs 48-50) appear in (*Perophora*+*Ecteinascidia*)+, and only reverting in *Clavelina*. Everting papillae in *Perophora* (Stach & Turbeville 2002) reinforces its position in internal group. This character was not polymorphic in any genus.

The linear row of adhesive papillae (45.1) (Figs 48 and 49) appeared independently two times in the internal group: in *Perophora*+*Ecteinascidia*, and in the clade beginning with (*Polycitor crystallinus* (*Brevicollus*(*Exostoma*+*Eudistoma*)))+. Reversion to the state 45.0 occurs only in *Distaplia*+*Hypsistozoa*. Although modified several times in the internal group, papillar arrangement is very fixed within each genus. Most species included in this analysis have three adhesive papillae, but the studied species of *Euherdmania* all had two adhesive papillae (invaginated tubes), as well as two species of *Pycnoclavella*, which have the invaginated type. Since the type species for *Pycnoclavella* (*P. aurilucens* Garstang, 1891) and *P. stanley* have three papillae, this was considered the plesiomorphic state of this character. The reduction to two adhesive papillae in the larva clearly occurred independently in *Euherdmania* and *Pycnoclavella*. Sessile papillae (46.0) (Fig. 48) are found in the most basal *taxa* of the internal group. The stalk in the adhesive papillae (46.1) (Fig. 49) appeared in (*Polycitor circes* (*Pycnoclavella* (*Clavelina* (*Nephtheis*+*Euclavella*))))+ and while present in most other *taxa* in the internal group, reversions occurred in *Brevicollus* and *Pseudodistoma*. In *Clavelina*, adhesive papillae do not present stalks, however they are supported by a peduncular process (46.2) (Fig. 50). This character was only polymorphic in *Distaplia*, and the plesiomorphic state defined was 46.1.

Vesicles in the larval trunk (47.1) appeared independently in *Brevicollus* and in the clade (*Dumus*(*Monniotus*+*Protopolyclinum*))+, and were lost in the clade (*Neodistoma* (*Distaplia*+*Hypsistozoa*))+*Diplosoma*+. Another reversion occurred in *Protopolyclinum*. Only *Aplidium* was polymorphic for this character, and the plesiomorphic state was defined as 47.1. Vesicles in the trunk of *Aplidium* are derived from evaginations such as the ampullae (Cloney 1982).

Phylogenetic reconstruction

Aplousobranchia *sensu* Lahille (*Stomozoa*+) is a monophyletic group supported by the following characters: loss of internal longitudinal pharyngeal vessels (21.2) and of the branchial papillae (23.1), and the stomach in the posterior half of the proximal branch of the gut (29.2). However, Aplousobranchia *sensu* Kott (1969, 1990) is not monophyletic since, in our analysis, ascidians divided into two branches with *Ciona* in the base of one branch and the other "Aplousobranchia" in the other branch that includes *Perophora*+*Ecteinascidia*. Also the position of *Diazona* and *Rhopalaea* is not resolved, appearing either in each of the branches. *Ciona*, *Perophora*, *Ecteinascidia*, *Rhopalaea*, *Diazona* and *Tylobranchion* have been traditionally considered genera of Phlebobranchia Lahille and not of Aplousobranchia (Van Name 1945, Millar 1966, Tokioka 1967, Monniot & Monniot 1972). The inclusion of some of these genera in Aplousobranchia was proposed by Kott (1969, 1990) and supported by the same oxidation state of vanadium (IV) in *Ciona*, *Rhopalaea*, *Diazona* and *Tylobranchion* and in the *taxa* of Aplousobranchia (Hawkins *et al.* 1983). The position of Cionidae in the base of the other branch suggests that the oxidation state IV of vanadium is the plesiomorphic state. Recent molecular analysis based on partial COI sequences also suggests a close position between *Ciona* and the Aplousobranchia as sister-groups (Turon & López-Legentil 2004). These cladograms also included Diazonidae within Aplousobranchia. Therefore, even though *Ciona* is usually considered a very basal genus within Ascidiacea, its position is still debatable.

Tylobranchion, and *Perophora*+*Ecteinascidia* are basal groups and comprise, together with Aplousobranchia *sensu* Lahille, a natural group, supported by the following characters: colonial life habit (1.1), less than 30 longitudinal thoracic muscle bands (13.1), internal fertilization (39.1) with incubation of embryos (40.1) and length of the larval trunk greater than 0.4 mm (42.1).

The position of *Ecteinascidia*+*Perophora* remains debatable. Here, characters 1.1 (colonialism), 13.1 (few longitudinal muscle bands), 20.1 (up to 30 rows of stigmata), 30.1 (posterior stomach present), 37.1 (few oocytes), 39.1 (internal fertilization), 40.1 (incubation of embryos), 42.1 (large larval trunk) and 44.1 (everting adhesive papillae in the larva), placed these groups within the internal group. The alignment of the adhesive papillae of the larvae in a row (45.1) is also a character that occurs only within Aplousobranchia. On the other hand, molecular data, both from 18S rDNA sequences (Stach & Turbeville 2002) and partial COI sequences (Turon & López-Legentil 2004) placed

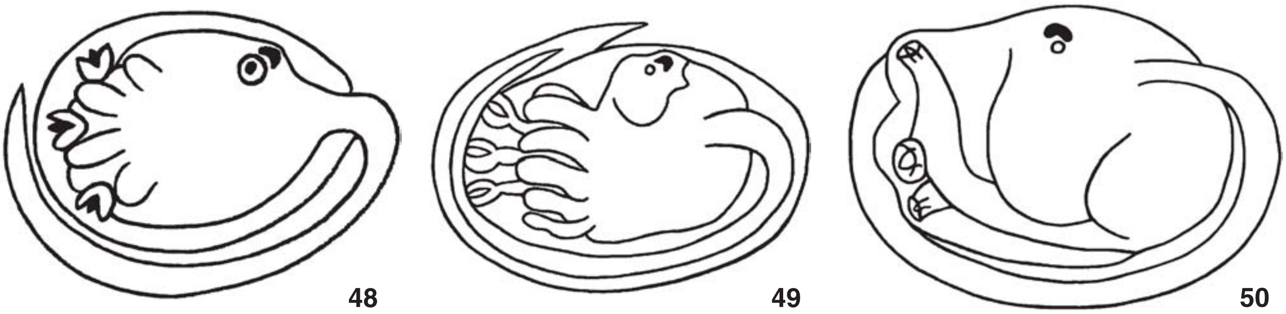


Figure 48-50. Larval adhesive papillae. (48) sessile in a linear arrangement, (49) stalked in linear arrangement, (50) on a peduncular process, in triangle.

Perophoridae close to Ascidiidae outside the Aplousobranchia.

Even though Phlebobranchia was not part of this study and few genera were included in the analysis, the results suggest paraphyly for this *taxon*. Molecular analysis also showed different results with this respect: the analysis with COI did not confirm the Phlebobranchia clade (Turon & López-Legentil 2004) while 18S rRNA analysis indicated monophyly for this *taxon* (Stach & Turbeville 2002).

Our results also indicate paraphyly for the order Enterogona since genera of Phlebobranchia and Aplousobranchia are split into different branches. Nor did analysis based on COI confirm the clade Enterogona (Turon & López-Legentil 2004). This clade apparently was defined on the basis of many plesiomorphic characters either shared by cephalochordates (pharynx not folded with longitudinal vessels) or not (gonads associated with the intestinal tract). Further research with the other genera of Phlebobranchia will be necessary in support of clarifying the evolution of the Ascidiacea.

Evolutionary trends

Colonialism seems to have appeared early in the evolutionary history of Aplousobranchia. Evolution proceeded in the direction of smaller zooids and simplification of the pharynx. The change from solitary to colonial is closely associated with the increase in regenerative ability and the consequent emergence of asexual reproduction. Interruption of individual growth due to a growing investment in asexual reproduction is suggested as the reason for the decrease in zooid size and consequent reduction of structures, especially of the pharynx (Kott 1969).

Although a clear trend towards pharynx simplification occurred, this trend is not clearly correlated with reduction in pharynx size, at least in the basal genera. In these genera, there are large species with or without longitudinal vessels (eg. *Clavelina* without, *Diazona* with), small species with longitudinal vessels (eg. *Perophora*). The large thorax of *Tylobranchion speciosum* has incomplete vessels while some small *Perophora* present complete vessels. A complete loss of longitudinal vessels occurred in *Stomozoa*+. While not previously mentioned for most *taxa*, muscles on the transverse vessels of the pharynx, present in most derived genera support the clade *Ritterella*+, which includes genera from the traditional family Polyclinidae, the Holozoidae (*Neodistoma*(*Distaplia*+*Hypsistozoa*) and Didemnidae (*Diplosoma*+). The dorsal pharyngeal muscle band supports the relationship among the Holozoidae and Didemnidae and its presence seems to be associated with the modification of the atrial siphon into a wide opening directed to the cloacal channels.

All of the characters associated with the pharynx (vessels, papillae, and transverse muscle) seem to have greater phylogenetic value than body division and the position of the heart, gonads and epicardium in the evolution of Aplousobranchia. Dividing the body into two (thorax and abdomen) or three (thorax, abdomen and posterior abdomen) regions was classically proposed as a major driving force in the evolution of Aplousobranchia, reflected in its classification (Van Name 1945, Millar 1966, Monniot & Monniot 1972). In contrast, our analysis shows that *Tylobranchion*, despite its posterior abdomen, is located more basally and distant from the other species with a posterior abdomen (traditional family Polyclinidae). After a second appearance of the posterior abdomen in *Anadistoma*+ it was lost again in (*Neodistoma*(*Distaplia*+*Hypsistozoa*))+*Diplosoma*+.

Characters associated with the musculature of the body wall, not often used to establish relationships among ascidian groups, were phylogenetically useful. The transverse musculature is restricted to some *taxa* and supported the clade *Dumus*(*Monniotus*+ *Protopolyclinum*).

Asexual reproduction triggered the development of colonial systems through different budding mechanisms and apparently colonialism arose only once within Aplousobranchia. Wada *et al*. (1992) stated that asexual reproduction arose many times and independently in Phlebobranchia and Stolidobranchia. However, if the genera *Perophora*, *Ecteinascidia* and *Diazona* are included in Aplousobranchia then asexual reproduction never appeared in Phlebobranchia.

Another evolutionary trend associated with colonial species was the formation of systems, culminating with the organization of specialized cloacal systems in (*Synoicum*+ *Polyclinum*)+. No reversion to the absence of a cloacal system ever occurred.

Again, cloacal systems are present both in species with and without a posterior abdomen, showing that a posterior abdomen is not a valid character for the classification of Aplousobranchia. Associated with the development of the cloacal systems there was a change of the atrial siphon aperture. While in (*Synoicum*+ *Polyclinum*)+*Aplidium*+ the atrial aperture is small, it becomes a wide opening in ascidians with developed cloacal systems, such as in the clade (*Neodistoma*(*Distaplia*+*Hypsistozoa*))+, although it appears as a tubular siphon in some genera of this last clade: *Trididemum*(*Leptoclinides*+*Atriolum*).

Trends associated with the evolution of the larva itself included the change from simple and small larvae, with three conical adhesive papillae in triangle, without ectodermal ampullae or vesicles (*Tylobranchion* or maybe *Diazona*) to larger and quite complex larvae, with everting adhesive papillae of different types in a stalk extremity, ectodermal ampullae and sometimes vesicles. Adhesive papillae in a linear row appeared twice, in *Perophora*+*Ecteinascidia*, and supporting the clade (*Polycitor crystallinus*(*Brevicollus* (*Eudistoma*+*Exostoma*)))+, with reversion only in *Neodistoma*(*Distaplia*+*Hypsistozoa*). The complexity of the larvae produced by Aplousobranchia *taxa* is not found in larvae of oviparous ascidians nor in either colonial or solitary viviparous forms of Stolidobranchia.

Most solitary forms release gametes into the water, where fertilization and development occur. Thence, colonial ascidians became viviparous, producing large eggs in smaller numbers. *Rhopalaea* and *Diazona* do not incubate larvae and produce many oocytes. When more basal *taxa* are viviparous they incubate a greater number of embryos relative to the more derived *taxa*. For example, *Tylobranchion speciosum*, *Clavelina oblonga* and *Placentela crystallina* each incubate more than 20 embryos in the atrial cavity. The increase in larval size is also a trend (MILLAR 1971) in which small larvae (trunk < 0.30 mm) are usually from solitary ascidians, while the largest sizes are from colonial species (trunk > 0.5 mm). Some are quite large, such as *Polycitor circes* and *Hypsistozoa fasmeriana* Michaelsen, 1924, with trunks of 2.5 mm.

Embryo protection developed in a variety of ways: retention within the oviduct, within the atrial cavity, within an incubating pouch, or within the tunic of the colony (MILLAR 1971). Basal *taxa* of the internal group – with large zooids – incubate larvae within the atrial cavity or oviduct. However, in smaller individuals with relatively large larvae (*e.g.*, *Distaplia*+ *Hypsistozoa*), the brood pouches projects from the body wall. Also, the atrial opening is quite wide in *Neodistoma*+ with little space within the atrial cavity for incubation. *Diplosoma*+ are also tiny (average 1 mm) and large larvae are incubated within the tunic, completely independent of the zooid. The most elaborate incubation yet discovered occurs in *Hypsistozoa fasmeriana*, in which the ovary produces a single small egg that grows into a large larva within the incubating pouch, where it receives nourishment through a pair of endodermal tubes. The embryo is incubated over a long time period, resulting in a quite complex larva with many blastozooids (BREWIN 1956).

Larvae leave the colony depending upon where they are incubated. In species that retain embryos in the atrial cavity, larvae escape directly through the atrial siphon or the common cloacal cavity. In *Distaplia*, the zooid dies upon separation from incubating pouch and the larvae are liberated then when the tunic dissolves (BERRILL 1948). A similar process occurs in *Synoicum adareanum* (Kott, 1969). In ascidians with small zooids, one only embryo usually grows and it is liberated by rupture of the body wall. In *Diplosoma*+ extreme specialization occurs in this direction: eggs pass directly from the abdomen to the tunic of the colony, where they are fertilized and grow (MILLAR 1971).

Monophyly of the families within Aplousobranchia

Considering the three traditional families of the order (VAN NAME 1945, MILLAR 1966, MONNIOT & MONNIOT 1972), only Didemnidae is a natural group, and clearly Polyclinidae and Polycitoridae are not monophyletic (Figs 1 and 51). *Ciona* appeared in the resulting cladogram as a more basal *taxon* of the branch *Ciona*(*Ascidia*(*Pyura*+*Molgula*)) out of Aplousobranchia, supporting the valid family Cionidae. Cionidae Lahille, 1887 is considered monogeneric by several authors (VAN NAME 1945, BERRILL 1950, KOTT 1990). MONNIOT & MONNIOT (1972), however, included *Rhopalaea*, *Diazona*, *Tylobranchion*, *Pseudodiazona* Millar, 1963a, *Ciallusia* Van Name, 1918, *Pterygascidia* Sluiter, 1904, *Rhopalopsis* Herdman, 1880, *Corellopsis* Millar, 1970 and *Syndiazona* Oka, 1926 as genera within the Cionidae. Our results indicate that the first three genera belong to other families (the last six genera were not included in this analysis).

Diazonidae Garstang, 1891 traditionally includes the genera *Diazona*, *Rhopalaea*, *Tylobranchion*, *Pseudodiazona* and *Syndiazona* Oka, 1926. Here, *Rhopalaea*, *Diazona* and *Tylobranchion* (*T. speciosum*) did not group together, and Tylobranchionidae **fam. nov.** was created to include *Tylobranchion*. *Rhopalaea* and *Diazona* grouped together in some trees but not in others, thereby further study is required to resolve the position of these and the other genera.

Here, Perophoridae Giard, 1872 including the genera *Perophora* and *Ecteinascidia* was considered valid and well supported by characters intestinal loop and gonads lateral to the pharynx, body undivided, sac like, colony with stolonic form, longitudinal thoracic muscle bands oblique in relation to the endostyle, asexual reproduction by budding of the vascular appendix or vascular stolon and a linear arrangement of adhesive papillae.

The traditional family Polycitoridae is not a monophyletic group. KOTT (1990) proposed the inclusion of *Clavelina*+ *Nephtheis* in Clavelinidae Forbes & Hanley, 1848 and created *Pycnoclavellidae* Kott, 1990 for the genera *Euclavella*+ *Pycnoclavella*. Clavelinidae was characterized by its exclusive form of budding in the terminal portion of the stolonic vase, not involving the epicardium (BERRILL 1936, 1950), the plain margin of the siphon, the many small eggs, the larvae with papil-

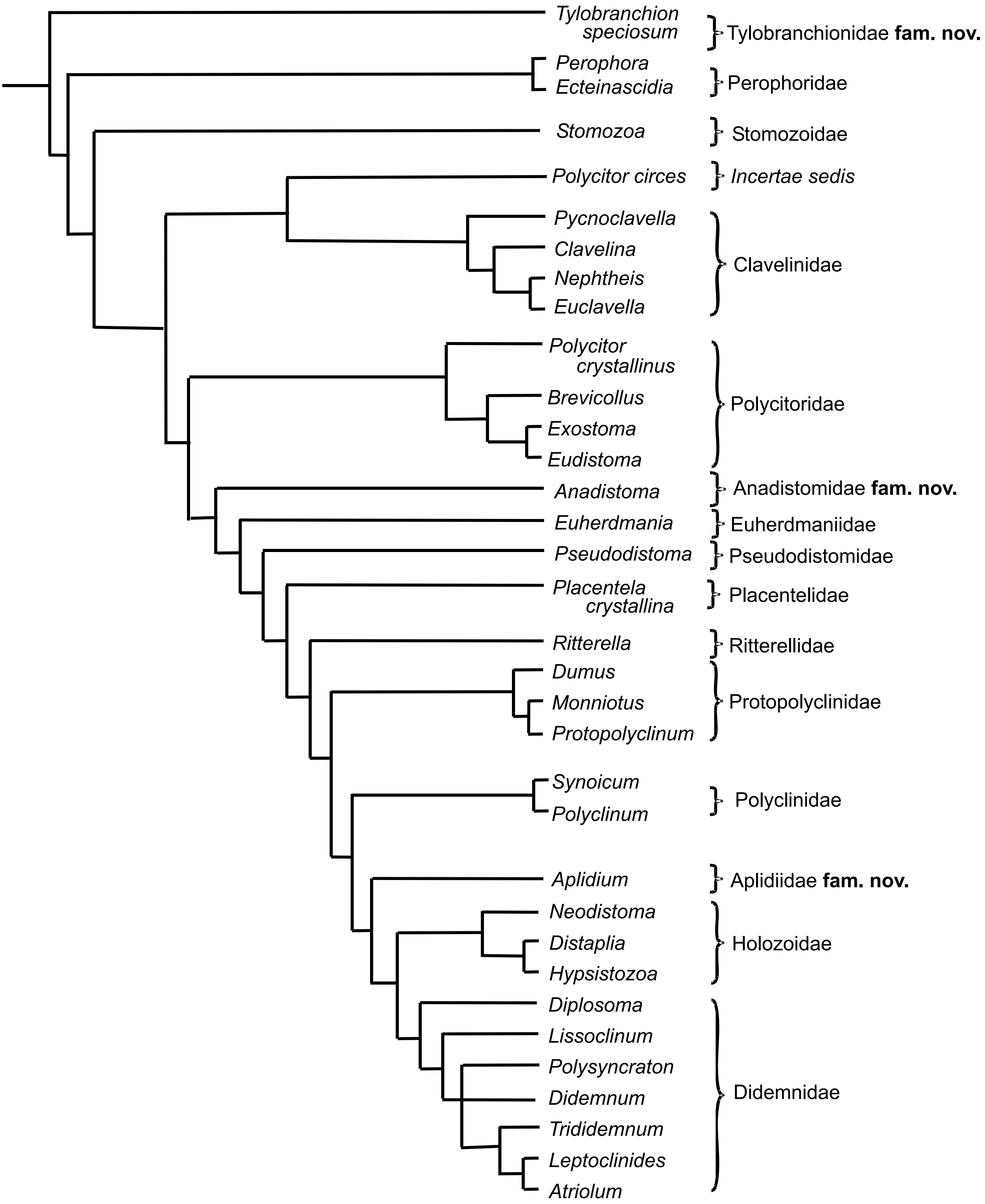


Figure 51. Cladogram indicating the clades which are monophyletic and belong to the category "Family".

lae in triangle and the absence of an epidermal calyx (Kott 1969). In this work, the four genera form a monophyletic group, *Pycnoclavella*(*Clavelina*(*Euclavella*+*Nephtheis*)), supported only by the characters oral siphon with flat margin and asexual reproduction by budding of the vascular appendix or vascular stolon, and represent Clavelinidae Forbes & Hanley, 1848. *Clavelina*(*Euclavella*+*Nephtheis*) is supported by character without posterior stomach. In partial COI sequences, *Clavelina*, *Pycnoclavella* and *Archidistoma* appeared together as a monophyletic group with strong support in all trees retrieved by Turon & López-Legentil (2004), thereby confirming our results.

Stomozoa appears as an isolated branch in the cladogram (Figs 1 and 51) at the base of the clade (*Polycitor circes*(*Pycnoclavella*(*Clavelina*(*Euclavella*+*Nephtheis*))))+. Therefore we follow the proposal of Kott (1990), and put *Stomozoa* as the only genus in Stomozoidae Kott, 1990.

The clade *Exostoma*+*Eudistoma* was supported by character three rows of stigmata, while the clade *Brevicollus*(*Exostoma*+*Eudistoma*) was supported by characters colony in form of cushion and trapezoidal shape of the stomach. *Polycitor crystallinus*(*Brevicollus*(*Exostoma*+*Eudistoma*)) also form a monophyletic group supported by the character presence of ectodermal ampullae in the larva. Kott (1990) included these four genera, together with *Archidistoma* Garstang, 1891, *Polycitorella* Michaelsen, 1924 and *Cystodytes* Drasche, 1883 in Polycitoridae Michaelsen, 1904, which remained valid here.

Polycitor, however, is apparently not monophyletic. *Polycitor circes* appears at the base of the clade (*Pycnoclavella* (*Clavelina*(*Euclavella*+*Nephtheis*))) (Figs 1 and 51), separated from *P. crystallinus* due to the lack of a transverse thoracic muscle in the body wall and the triangular arrangement of the adhesive papillae of the larvae. *Polycitor crystallinus* has many primitive characters (Kott 1969) that indicate a greater proximity with *Diazona* when compared to the other species of the genus. Here, however, *P. circes* is distant from *Diazona* in the cladogram.

Anadistoma is the most basal genus of the clade comprising ascidians with a posterior abdomen (old family Polyclinidae) that share characters associated with the elongation of the body, as the elongation of the longitudinal muscle to the base of the posterior abdomen, gonads in posterior abdomen and asexual reproduction by strobilization of this part of the body. We propose the new family, Anadistomidae **fam. nov.**, to include this genus (Figs 1 and 51).

Kott (1992) proposed Euherdmaniidae Ritter, 1904 for *Euherdmania*, which appears as an isolated branch and is supported here. *Euherdmania* differs from *Pseudodistoma* by transverse muscle in the body wall, by the largest number of rows of stigmata, and only two, invaginated, adhesive papillae in the larva.

Kott (1992) proposed Pseudodistomidae Kott, 1992 to include *Pseudodistoma*, *Anadistoma* and *Citorclinum* Monniot & Millar, 1988, which we consider valid, but with only *Pseudodistoma*. *Pseudodistoma* appears as an isolated branch, with characters absence of transverse thoracic muscle in the body wall and two autapomorphies: three rows of pharyngeal stigmata and stalk absent in the adhesive papillae of the larva. Further studies are needed to define the position of *Citorclinum* in Pseudodistomidae or in Anadistomidae.

Genera belonging to the clade beginning with *Placentela crystallina* differ from *Anadistoma*, *Euherdmania* and *Pseudodistoma* by having siphons with pointed lobes and the stomach in the middle of the proximal branch of the alimentary canal. *Euherdmania* and *Pseudodistoma* do not have muscle bands on the transverse vessels of the pharynx as occurs in the clade *Ritterella*+. Kott (1992) proposed Placentelidae Kott, 1992 to include *Placentela*, which we maintain.

The genera *Ritterella* and *Dumus* comprise Ritterellidae Kott, 1992. In our cladograms, however, *Ritterella* is placed in a separate *taxon* at the base of the clade *Ritterella*+, and supported by the characters presence of transverse pharyngeal muscle and ectodermal ampullae in the larva. Thus, we propose that *Ritterella* only is considered into Ritterellidae. *Dumus* is a sister-group to *Monniotus*+*Protopolyclinum*, forming Protopolyclinidae Kott, 1992 supported by the characters presence of transverse muscles on the body wall and digitiform projections in an encrusting colony with an expanded base). Kott (1992) also included *Condominium* in this family, but our analysis could not confirm this due to the lack of larval information in *Condominium*. Systems without true or rudimentary cloacal cavity, siphons with pointed lobes, presence of muscle on transverse vessels of the pharynx and short oesophageal stalk indicates a probable position of *Condominium* among the genera of the clade beginning with *Ritterella*, possibly as a basal *taxon* in the clade *Dumus* (*Monniotus*+*Protopolyclinum*). However, branchial papillae are lacking, yet present in *Monniotus*+*Protopolyclinum*.

Polyclinidae Milne-Edwards, 1841 *sensu* Kott (1992) is not monophyletic since only *Synoicum*+*Polyclinum* form a clade and *Aplidium* is in an isolated branch in the cladogram. We considered the genera *Synoicum* and *Polyclinum* in Polyclinidae Milne-Edwards, 1841 and *Aplidium* to Aplidiidae **fam. nov.** Polyclinidae should also include the genera *Aplidiopsis* Lahille, 1890, *Sidneioides* Kesteven, 1909 and *Morchellium* Giard, 1872 (Kott 1992).

The clade *Neodistoma*(*Distaplia*+*Hypsistozoa*) appears in all the cladograms and is supported by four characters (Figs 1 and 51) which concur with the inclusion of these genera within Holozoidae as did Kott (1990). Other genera, included in this family are: *Sigillina* Savigny, 1816, *Polydistoma* Kott, 1990, *Hypodistoma* Tokioka, 1967, *Sycozoa* Lesson, 1830 and *Protoholozoa* Kott, 1969, but none of those were included in this analysis.

The monophyly of Didemnidae Giard, 1872 (*Diplosoma*+) is supported by five characters: sheet-like colony, muscle process present, rounded, smooth stomach and larvae incubated in the tunic (Figs 1 and 51). In addition to the seven genera included in this analysis, several other genera are included in the family (*i.e.*, *Botrydemnum* Oka, 1933, *Clitella* Kott, 2001, *Coelocormus* Herdman, 1886, *Didemnopsis* Hartmeyer, 1903 and *Sinecloaca* Michaelsen, 1930). Berrill (1936) suggested a common ancestry for Didemnidae and Holozoinae, while Kott (1992) proposed that similarities between Holozoinae and Didemnidae would be better explained by the independent reduction of zooid size, with subsequent parallel development. Here we confirm the proposal of Berrill (1936) in that the clade *Holozoidae+Didemnidae* was supported by nine characters, referring to modifications in the atrial siphon in function of cloacal systems, number of rows of stigmata, loss of the posterior abdomen, incubation type, absence of the vesicles in the larva and similarity in asexual reproduction (Fig. 1). The intestinal loop and heart of the Didemnidae are also similar to *Distaplia* (Berrill 1936). More recently, Hirose (2001) described bladder cells in the tunic of Holozoidae and Didemnidae; these, however, are also found in Diazonidae and Ascidiidae. Molecular analysis (COI) gave different conclusions, with Didemnidae in a more basal position together with Cionidae (Turon & López-Legentil 2004).

Classification

We recommend a classification based on our hypothesis of phylogenetic reconstruction (Figs 1 and 51). Here, one of the criteria was to minimize modifications of the existing classification. The sequence of the list of *taxa* follows the sequence of the branches in the cladogram (sequential system), because this system allows the classification of large groups using a relatively few categories, hence, the number of redundant names is relatively small and many names and categories of traditional classifications can be conserved (Amorim 1997). The terminal taxa referring to species (generated by problems in the definition of genera), appear in the classification as *incertae sedis* (Wiley *et al.* 1991) (Figs 1 and 51).

Enterogona Perrier, 1898
  Aplousobranchia Lahille, 1887
    Tylobranchionidae **fam. nov.**
      Tylobranchion Herdman, 1886 (*T. speciosum* Herdman, 1886)
    Perophoridae Giard, 1872
      Perophora Wiegmann, 1835; *Ecteinascidia* Herdman, 1880
    Stomozoidae Kott, 1990
      Stomozoa Kott, 1957
      Polycitor circes Michaelsen, 1930 *incertae sedis*
    Clavelinidae Forbes & Hanley, 1848
      Pycnoclavella Garstang, 1891; *Clavelina* Savigny, 1816; *Euclavella* Kott, 1990; *Nephtheis* (Drasche, 1882)
    Polycitoridae Michaelsen, 1904
      Polycitor Renier, 1804 (*P. crystallinus* Renier, 1804); *Brevicollus* Kott, 1990; *Exostoma* Kott, 1990; *Eudistoma* Caullery, 1909
    Anadistomidae **fam. nov.**
      Anadistoma Kott, 1992
    Euherdmaniidae Ritter, 1904
      Euherdmania (Ritter, 1903)
    Pseudodistomidae Kott, 1992
      Pseudodistoma Michaelsen, 1924
    Placentelidae Kott, 1992
      Placentela Redikorzev, 1913 (*P. crystallina* Redikorzev, 1913)
    Ritterellidae Kott, 1992
      Ritterella Harant, 1931
    Protopolyclinidae Kott, 1992
      Dumus Brewin, 1952; *Monniotus* Millar, 1988; *Protopolyclinum* Millar, 1960
    Polyclinidae Milne-Edwards, 1841
      Polyclinum Savigny, 1816; *Synoicum* Phipps, 1774
    Aplidiidae **fam. nov.**
      Aplidium Savigny, 1816
    Holozoidae Berrill, 1950
      Neodistoma Kott, 1990; *Distaplia* Della Valle, 1881; *Hypsistozoa* Brewin, 1953
    Didemnidae Giard, 1872
      Diplosoma MacDonald, 1859; *Lissoclinum* Verrill, 1871; *Polysyncranton* Nott, 1892; *Didemnum* Savigny, 1816; *Trididemnum* Della Valle, 1881; *Leptoclinides* Bjerkan, 1905; *Atriolum* Kott, 1983

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LITERATURE CITED

Amorim, D.S. 1997. **Elementos básicos de Sistemática Filogenética.** Ribeirão Preto, Holos, 2ª ed., 276p.

Berrill, N.J. 1935. Studies in tunicate development. Part IV – asexual reproduction. **Royal Society of London Publications 526 B**: 327-379.

Berrill, N.J. 1936. Studies in tunicate development. Part V – the evolution and classification of ascidians. **Royal Society of London Publication 530 B**: 43-70.

Berrill, N.J. 1948. Budding and the reproductive cycle of *Distaplia*. **Quarterly Journal of Microscopical Science 89**: 253-289.

Berrill, N.J. 1950. **The Tunicata – with an account of the British species.** London, Ray Society, 354p.

Berrill, N.J. & D.P. Abbott 1949. The structure of the ascidian *Pycnoclavella stanleyi* **sp. nov.**, and the nature of its tadpole larva. **Canadian Journal of Research 27**: 43-49.

Bjerkan, P. 1905. Ascidien von dem norwegischen Fischereidampfer Michael Sars in den Jahren 1900-1904 gesammelt. **Bergens Museum Arbog Afhandlingar og Arsberetning 1905 5**: 4-29.

Brewin, I.B. 1952. Ascidians of New Zealand. Part VII – Ascidians of Otago coastal waters. **Transactions and Proceedings of the Royal Society of New Zealand 79**: 452-458.

Brewin, I.B. 1953. Australian ascidians of the sub-family Holozoinae and a review of the sub-family. **Transactions and Proceedings of the Royal Society of New Zealand 81** (1): 53-64.

Brewin, I.B. 1956. The growth and development of a viviparous compound ascidian *Hypsistozoa fasmeriana*. **Quarterly Journal of Microscopical Science 97**: 435-454.

Cameron, C.B.; J.R Garey & B.J.Swalla. 2000. Evolution in the chordate body plan: new insights from phylogenetic analysis of deuterostome phyla. **Proceedings of the National Academy of Science USA 97**: 4469-4474.

Caullery, M. 1909. Récherches sur la famille des Distomidae. **Bulletin Scientifique de la France et de la Belgique 42**: 1-59.

Cloney, R.A. 1982. Ascidian larvae and the events of metamorphosis. **American Zoologist 22**: 817-826.

Della Valle, A. 1881. Nouve contribuzioni alla storia naturalle della ascidie composte del Golfo di Napoli. **Atti della Reale Accademia dei Lincei**, **Series 3**, **Memoir 10** (3): 431-498.

Drasche, R. Von. 1882. *Oxycorynia*, eine neue Synascidien-Gattung. **Verhandlugngen der Zoologisch Botanischen Gesellschaft Wien Österreich 32**: 175-178.

Drasche, R. Von. 1883. Die Synascidien der Bucht von Rovigno (Istrien). *In*: **Ein Beitrag zur Fauna der Adria (Wien)**, 41p.

Drasche, R. Von. 1884. Ueber einige neue und weniger gekannte aussereuropäische einfache Ascidien. **Denkschriften. Akademie Wissenschaftlichen 48**: 369-387.

Fitch, W.M. 1971. Toward defining the couse of evolution: minimum change for a specific tree topology. **Systematic Zoology 20**: 406-416.

Fleming, J. 1822. **The Philosophy of Zoology.** Edinburgh and London, vol. 2, p. 508-518.

Forbes & S.C.T. Hanley. 1848. **A History of British molluscs and their shells.** London, vols 1, 2 and 4.

Gaill, F. 1972. Répartition du genre *Pseudodistoma* (Tuniciers): description de deux espéces nouvelles. **Cahiers de Biologie Marine 13**: 3747.

Garstang, W. 1891. Report on the tunicata of Plymouth. **Journal of the Marine Biological Association**, **New Series 2**: 47-67.

Giard, A.M. 1872. Recherches sur les ascidies composées ou synascidies. **Archives de Zoologie Experiméntale et Générale 1**: 501-704.

Harant, H. 1930. Liste des Ascidies de Banyuls. **Archives de Zoologie Experiméntale et Générale 70** (1): 15-22.

Harant, H. 1931. Contribution à l'histoire naturelle des ascidies et leurs parasites. **Annales de l'Institut Océanographique Monaco 8** (4): 229-389.

Hartmeyer, R. 1903. Die ascidien der Arktis. **Fauna Arctica 3** (2): 93-412.

Hartmeyer, R. 1911. Die ascidien der Deutschen Südpolar-Expedition 1901-1903. **Deutschen Südpolar-Expedition (1905-31) 12** (4): 407-606.

Hartmeyer, R. 1913. Tunicata, p. 125-144. *In*: K. Schultze (Ed.). **Zoologie und Anthropologie Ergebnisse. Forschungsreise in Südafrika Bd 5**, **Lfg 2.** Jena, Denkschriften der Medizinisch-Naturwissenschaftlichen Gesellschaft, 17.

Hartmeyer, R. 1922. Die ascidienfauna des Trondhjemfjords. Meddelese fra Trondhjems Biologiske Station, 14. **Kongelige Norske Videnskabers Selskab Skrifter 1921** (6): 1-47.

Hawkins, C.J.; P. Kott & D.L. Parry. 1983. Vanadium content and oxidation state related to ascidian phylogeny. **Comparative Biochemistry and Physiology 76B**: 555-558.

Heller, C. 1878. Beitrage zur nahern Kenntniss der Tunicaten. **Sitzungsberichte der mathematischnaturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaft Wien 77** (1): 2-28.

Hennig, W. 1966. **Phylogenetic Systematics.** Urbana, University of Illinois Press, vol. 3, 263p.

Herdman, W.A. 1880. Preliminary report on the Tunicata of the Challenger expedition. Ascidiidae. **Proceedings of the Royal Society of Edinburg 10** (1): 458-72.

Herdman, W.A. 1880. Preliminary report on the Tunicata of the Challenger expedition. Part II. **Proceedings of the Royal Society of Edinburg 10**: 714-726.

Herdman, W.A. 1881. Preliminary report on the Tunicata of the Challenger expedition. Molgulidae. **Proceedings of the Royal Society of Edinburg 11** (4): 233-240.

Herdman, W.A. 1886. Report on the Tunicata collected during the voyage of H.M.S. Challenger during the years 1873-1876. Part II – Ascidiae compositae. **Report on the Scientific Results of the Voyage of HMS Challenger 14** (38): 1-399.

Herdman, W.A. 1890. On the genus *Ecteinascidia* and its relations; with descriptions of two new species, and a classification of the family Clavelinidae. **Proceedings and Transactions of the Liverpool Biological Society 5**: 144-163.

Herdman, W.A. 1899. Descriptive catalogue of the Tunicata in the Australian Museum. **Australian Museum**, **Sydney**, **Catalogue 17**: 1-139.

Herdman, W.A. 1902. Tunicata. *In*: **Report on the collections of natural history made in the Antarctic regions during**

the voyage of the "Southern Cross". London, p. 190-200.

Herdman, W.A. 1910. Tunicata. *In*: **National Antarctic Expedition (Discovery) 1901-04** (Natural History), London British Museum, vol. 5, p. 1-26.

Hirose, E. 2001. Acid containers and cellular networks in the ascidian tunic with special remarks on ascidian phylogeny. **Zoological Science 18**: 723-731.

Julin C. 1904. Récherches sur la phylogenèse des tuniciers *Archiascidia neapolitana* nov. gen. **Mitheilungen aus der Zoologischen Station zu Neapel 16**: 489-552.

Kesteven, H.L. 1909. Studies on Tunicata 1. **Proceedings of the Linnean Society of New South Sales 34**: 276-295.

Kornet, D.J. & H.T. Turner. 1999. Coding polymorphism for phylogeny reconstruction. **Systematic Biology 48**: 365-379.

Kott, P. 1957. The ascidians of Australia. II. Aplousobranchiata Lahille: Clavelinidae Forbes & Hanley and Polyclinidae Verrill. **Australian Journal of Marine and Freshwater Research 8**: 64-110.

Kott, P. 1962. The ascidians of Australia. III. Aplousobranchiata Lahille: Didemnidae Giard. **Australian Journal of Marine and Freshwater Research 13**: 265-334.

Kott, P. 1963. The ascidians of Australia. IV. Aplousobranchia Lahille: Polyclinidae Verrill. **Australian Journal of Marine and Freshwater Research 14**: 70-118.

Kott, P. 1969. Antarctic Ascidiacea. A monographic account of the known species based on specimens collected under U.S. Government auspices 1947-1963. **Antarctic Research Series 13**: 1-239.

Kott, P. 1972. The ascidians of South Australia. II. Eastern sector of the Great Australian Bight and investigator strait. **Transactions of the Royal Society of Australia 96**: 165-196.

Kott, P. 1976. The ascidian fauna of Western Port, Victoria, and a comparison with that of Port Phillip Bay. **Memoirs of the National Museum Victoria 37**: 53-96.

Kott, P. 1981. Replication in the Ascidiacea: an adaptive strategy in the coral reef environment. **Proceedings of the Fourth International Coral Reef Symposium 2**: 725-733.

Kott, P. 1983. Two new genera of didemnid ascidians from tropical Australian waters. **Beagle 1**: 13-19.

Kott, P. 1985. The Australian Ascidiacea. Part I Phlebobranchia and Stolidobranchia. **Memoirs of the Queensland Museum 23**: 1-440.

Kott, P. 1990. The Australian Ascidiacea. Part II Aplousobranchia (1). **Memoirs of the Queensland Museum 29**: 1-266.

Kott, P. 1992. The Australian Ascidiacea. Part III Aplousobranchia (2). **Memoirs of the Queensland Museum 32**: 375-620.

Kott, P. 2001. The Australian Ascidiacea. Part IV Aplousobranchia (3), Didemnidae. **Memoirs of the Queensland Museum 47**: 1-410.

Kowalevsky, A. 1874. Ueber die Knospung der Ascidien. **Archiv Mikroscopische Anatomie 10**: 441-470.

Lahille F. 1887. Sur la classification des tuniciers. **Comptes Rendus Hebdomadaires de Séances de L'Academie des Sciences 102**: 1573-1575.

Lahille, F. 1890. **Recherches sur les tuniciers des côtes de France.** Toulouse, Lagarde et Sebille, 330p.

Lafargue, F. 1983. Évolution des ascidies Didemnidae I: cas des espèces françaises. **Vie et Milieu 33**: 1-15.

Lafargue, F. & M. Wahl. 1987. The didemnid ascidian fauna of France. **Annales del'Institut Océanographique 63**: 1-46.

Lesson, R.P. 1830. Zoologie. *In*: **Voyage autour du monde sur La Coquille pendant 1882-1825**, Paris, **2** (1): 256-279, 433-440.

Linnaeus, C. 1767. **Systema Naturae.** Stockholm, 12th ed., vol. 1, part II.

MacDonald, J.D. 1859. On the anatomical characters of a remarkable form of compound Tunicata. **Transaction of the Linnean Society of London (Zoology) 22**: 373-375.

Michaelsen, W. 1904. Revision der compositen Styeliden oder Polyzoinen. **Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten 21** (2): 1-24.

Michaelsen, W. 1924. Ascidiae Krikobranchiae von Neuseeland, den Chatham und den Auckland Inseln. **Videnskabelige Meddelelser fra Dansk naturhistoriske Forening I Kobenhavn 77**: 263-434.

Michaelsen, W. 1930. Ascidiae Krikobranchiae. **Fauna Südwest-Australiens 5** (7): 463-558.

Michaelsen, W. 1934. The ascidians of the Cape Province of South Africa. **Transactions of the Royal Society of South Africa 22** (2): 129-163.

Millar, R.H. 1951. The stolonic vessels of the Didemnidae. **Quarterly Journal of Microscopical Science 92**: 249-254.

Millar, R.H. 1953a. *Ciona*. **L.M.B.C. Memoirs on Typical British Marine Plants and Animals 35**: 1-123.

Millar, R.H. 1953b. On a collection of ascidians from the Gold Coast. **Proceedings of the Zoological Society London 123**: 277-325.

Millar, R.H. 1954. *Pseudodistoma africanum* **sp. nov.**, a new compound ascidian from South Africa. Annals and magazine of Natural History (12) **7**: 128-132.

Millar, R.H. 1955. On a collection of ascidians from South Africa. **Proceedings of the Zoological Society London 125** (1): 169-221.

Millar, R.H. 1960. Ascidiacea. **Discovery Reports 30**: 1-160.

Millar, R.H. 1961. *Euherdmania vitrea*, a new species of ascidian from Brazil. **Annals and Magazine of Natural History 4**: 143-147.

Millar, R.H. 1962. Further description of South African ascidians. **Annals of the South African Museum 46** (7): 113-221.

Millar, R.H. 1963a. Australian ascidians in the British Museum (Natural History). **Proceedings of the Zoological Society London 141**: 689-746.

Millar, R.H. 1963b. The structure and systematic position of the ascidian *Distomus vitreum* Sars. **Annals and Magazine of Natural History 13**: 385-388.

Millar, R.H. 1966. Evolution in ascidians, p. 519-534. *In*: H.

Barnes (Ed.). **Some contemporary studies in marine science.** London, Allen and Unwin, 644p.

Millar, R.H. 1970. **British Ascidians (Tunicata, Ascidiacea).** London, Academic Press, Synopsis of the British Fauna, New Series, n 1, 92p.

Millar, R.H. 1971. The Biology of Ascidians. **Advances in Marine Biology 9**: 1-100.

Millar, R.H. 1977. Ascidians (Tunicata: Ascidiacea) from the Northern and North-eastern Brazilian shelf. **Journal of Natural History 11** (2): 169-223.

Millar, R.H. 1988. Ascidians collected during the International Indian Ocean Expedition. **Journal of Natural History 22**: 823-848.

Milne-Edwards, H. 1841. Observations sur les ascidies composées des côtes de la Manche. **Mémoires de l'Académie des Sciences de l'Institut de France 18**: 217-326.

Molina, G.I. 1782. Animali de Chili. *In*: **Saggio sulla storia naturale de Chili.** Bologna, 2nd ed.

Monniot, C. 1969. Ascidies récoltées par la Thalassa sur la pente du plateau continental du Golfe de Gascogne. **Bulletin du Muséum national d'Histoire naturelle 41**: 1131-1145.

Monniot, C. 1969-70. Campagne de la Calypso au large des côtes Atlantiques de l'Amérique du Sud (1961-1962). Première partie (suite). 17. Ascidies Phlébobranches et Stolidobranches. **Annales del'Institut Océanographique 36**: 3-59.

Monniot, C. 1987. Ascidies de Nouvelle-Calédonie. I: Phlébobranches du lagon. **Bulletin du Muséum national d'Histoire naturelle** (4), **9 A** (1): 3-31.

Monniot, C. 1997. Ascidies phlébobranches du canal du Mozambique. **Zoosystema 19**: 557-571.

Monniot, C. & F. Monniot. 1972. Clé mondiale des genres d'ascidies. **Archives de Zoologie Expérimentale et Générale 113**: 311-367.

Monniot, C. & F. Monniot. 1983. Ascidies antarctiques et subantarctiques: morphologie et biogéographie. **Mémoires du Muséum National d'Histoire Naturelle 125**: 4-168.

Monniot, C. & F. Monniot. 1987a. Some bathyal ascidians from the New Zealand area. **New Zealand Journal of Zoology 14**: 399-407.

Monniot, C. & F. Monniot. 1987b. Les ascidies de Polynésie Française. **Mémoires du Muséum National d'Histoire Naturelle 136**: 1-155.

Monniot, C. & F. Monniot. 1989. Ascidians collected around the Galapagos Islands using the Johnson-Sea-Link research submersible. **Proceedings of the Biological Society of Washington 102** (1): 14-32.

Monniot, C.; Monniot, F. & P. Laboute. 1991. **Coral Reef Ascidians of New Caledonia**. Paris, ORSTOM, Collection Faune Tropicale 30, 247p.

Monniot, F. 1983a. Ascidies littorales de Guadeloupe. I: Didemnidae. **Bulletin du Muséum National d'Histoire Naturelle** (4), **5 A** (1): 5-49.

Monniot, F. 1983b. Ascidies littorales de Guadeloupe. III. Polyclinidae. **Bulletin du Muséum National d'Histoire Naturelle** (4), **5 A** (2): 413-422.

Monniot, F. 1983c. Ascidies littorales de Guadeloupe. V. Polycitoridae. **Bulletin du Muséum National d'Histoire Naturelle** (4), **5 A** (4): 999-1019.

Monniot, F. 1987. Ascidies de Nouvelle-Calédonie. III. Polyclinidae du lagon. **Bulletin du Muséum National d'Histoire Naturelle 9**: 499-535.

Monniot, F. & R.H. Millar. 1988. A new genus and species of an aplousobranchiate ascidian (Tunicata, Ascidiacea) from New Caledonia, of uncertain systematic position. **Indo-Malayan Zoology 5**: 321-327.

Monniot, F. & C. Monniot. 1996. New collections of ascidians from the western Pacific and southeastern Asia. **Micronesica 29**: 133-279.

Monniot, F. & C. Monniot. 2001. Ascidians from the tropical western Pacific. **Zoosystema 23**: 201-388.

Müeller, O.F. 1776. **Zoologiae Danicae**. Copenhagen, Prodromus, p. 224-226.

Nishikawa, T. 1984. Contributions to the Japanese ascidian fauna XXXVIII. Notes on the morphology and the systematic position of *Placentela crystallina* Redikorzev from the North Pacific. **Proceedings of the Japanese Society of Systematic Zoology 29**: 37-56.

Nishikawa, T. 1991. The ascidians of the Japan Sea. II. **Publications of the Seto Marine Biological Laboratory 35**: 25-170.

Nixon, K.C. & J.M. Carpenter. 1993. On outgroups. **Cladistics 9**: 413-426.

Oka, A. 1912. On *Cyathocormus mirabilis* n.gen., **sp. nov.**, the type of a new family of compound ascidians from Japan. **Journal of the College of Sciences Imperial University of Tokyo 32** (12): 1-30.

Oka, A. 1926. On a new genus of compound ascidians (*Syndiazona* nov.gen.). **Proceedings of the Imperial Academy Japan 2**: 133-5.

Oka, A. 1933. Ein Fall von KolonialKnospung bei einer Synascidie. **Proceedings of the Imperial Academy Japan 9**: 436-438.

Nott, J.T. 1892. On the composite ascidians of the Noth Shore Reef. **Transactions New Zealand Institut 24**: 305-334.

Page, R.D.M. 1996. Treeview: An application to display Phylogenetic trees on personal computers. **Computer Applications in the Biosciences 12**: 357-358.

Page, R.D.M. 2000. **NDE 0.4.8 version.** Software.

Pérès, J.M. 1946. Remarques sur le polymorphisme des ascidies. **Compte-rendu de la Société de Biogéographie**, XXI.

Pérès, J.M. 1952. Ascidies de la rôche littorale Corse. **Record du Station Marine d'Endoume 6**: 35-44.

Perrier, J.O.E. 1898. Note sur la classification de Tuniciers. **Comptes Rendus Hebdomadaires de Séances de L'Academie des Sciences 126**: 1758-1762.

Philippi, R.A. 1843. *Rhopalaea* ein neues Genus der einfachen Ascidien. **Archiv für Anatomie und Physiologie 1**: 45-47.

Phipps, C.J. 1774. **Voyage au pôle boréale**. Appendix 194. London.

Redikorzev, V. 1913. Neue Ascidien. **Zoologischer Anzeiger 43**: 204-213.

Redikorzev, V. 1927. Zehn neue ascidien aus dem fernen Osten. **Zoologische Jahrbücher 53**: 373-404.

Renier, S.A. 1804. **Prospetto della classe dei vermi.** p. 15-27 (Padua) *fide* Porro C. 1840, Nota per una Bibliografia Malacologie, Series III Geografica n 1-4, p. 1-3 and numbered columns 27-130.

Ritter, W.E. 1899. A contribution to the knowledge of the tunicates of the Pribilof Islands, p. 511-537. *In*: DS Hirdabm (Ed.). **Fur seals and fur-seal islands of the north Pacific ocean.** Washington, Part 3.

Ritter, W.E. 1903. The structure and affinities of *Herdmania claviformis*, the type of a new genus and family of ascidians from the coast of California, p. 237-261. *In*: **Mark anniversary volume.** New York, Henry Holt.

Ritter, W.E. 1904. *Euherdamania* vs. *Herdmania* preoccupied. **Zoologischer Anzeiger 27**: 650-651.

Ritter, W.E. & R.A. Forsyth. 1917. Ascidians of the littoral zone of southern California. **University of California Publications in Zoology 16**: 439-512.

Ruppert, E.E. 1997. Cephachordata (Acrania), p. 349-504. *In*: F.W. Harrison (Ed.). **Microscopic anatomy of invertebrates.** New York, Wiley-Liss, vol. 15, 504p.

Sars, M. 1851. Beretning om en i Sommeren 1849, foretagen zoologiske Reise i Lofoten og Finmarken. **Nytt Magasin for Naturvidenskapene 6**: 121-211.

Savigny, J.C. 1816. Recherches anatomiques sur les ascidies composées et les ascidies simples. Système de la classe des ascidies. *In*: **Mémoires sur les animaux sans vertèbres.** Paris, G. Dufour, part 2, 239p.

Sluiter, C.P. 1885. Ueber einige einfachen ascidien von der Insel Billiton. **Natuurkundig Tijdschrift voor Nederlandsch Indie 45**: 160-232.

Sluiter, C.P. 1898. Tuniciers récueillis en 1896 par La Chazalie dans la Mers des Antilles. **Mémoires de la Sociéteé Zoologique de France 11**: 5-34.

Sluiter, C.P. 1904. Die tunicaten der Siboga-Expedition. Part I – Die socialen und holosomen ascidien. **Siboga Expedition 56**: 1-126.

Sluiter, C.P. 1909. Die tunicaten der Siboga-Expedition. Part II – Die merosomen ascidien. **Siboga Expedition 56B**: 1-112.

Stach, T. & J.M. Turbeville. 2002. Phylogeny of Tunicata inferred from molecular and morphological characters. **Molecular Phylogenetics and Evolution 25**: 408-428.

Stimpson, W. 1852. Several new ascidians from the coast of the United States. **Proceedings of the Boston Society of Natural History 4**: 228-232.

Stimpson, W. 1855. Descriptions of some new marine invertebrates. **Proceedings of the Academy of Natural Sciences of Philadelphia 7**: 387-388.

Swalla, B.J. 2001. Phylogeny of the Urochordates: implications for Chordate Evolution, p. 219-224. *In*: H. Sawada; H. Yokosawa & C.C. Lambert (Eds). **The Biology of Ascidians.** Tokyo, Springer-Verlag, 470p.

Swalla, B.J.; C.B. Cameron; L.S. Corley & J.R. Garey. 2000. Urochordates are monophyletic within the Deuterostomes. **Systematic Biology 49**: 52-64.

Swofford, D.L. 2002. **PAUP* 4. Beta version 10 for Microsoft Windows.** Smithsonian Institution.

Tokioka, T. 1955. Contributions to Japanese ascidian fauna XI. Sporadic memoranda (2). **Publications of the Seto Marine Biological Laboratory 4** (2, 3): 205-222.

Tokioka, T. 1967. Pacific Tunicata of the United States National Museum. **Bulletin of the United States National Museum 251**: 1-242.

Trason, W.B. 1957. Larval structure and development of the oozooid in the ascidian *Euherdmania claviformis*. **Journal of Morphology 100**: 509-545.

Trason, W.B. 1963. The life cycle and affinities of the colonial ascidian *Pycnoclavella stanleyi*. **University of California Publications in Zoology 65**: 283-326.

Trausteut, M.P.A. 1883. Vestindiske ascidiae simplices. Anden Afdeling. Mogulidae og Cynthiadae. **Videnskabelige Meddelelser fra Dansk naturhistoriske Forening I Kobenhavn 1882**: 108-136.

Turbeville, J.M.; J.R. Schulz & R.A. Ralf. 1994. Deuterostome phylogeny and the sister group of the chordates: Evidence from molecules and morphology. **Molecular Biology and Evolution 11**: 648-655.

Turon X. 1991. Morphology of the adhesive papillae of some ascidian larvae. **Cahiers de Biologie Marine 32**: 295-309.

Turon, X. & López-Legentil S. 2004. Ascidian molecular phylogeny inferred from mtDNA data with emphasis on the Aplousobranchiata. **Molecular Phylogenetics and Evolution 33**: 309-320.

Van Name, WG. 1902. The ascidians of the Bermuda Islands. **Transactions of the Connecticut Academy of Arts and Sciences 11**: 325-412.

Van Name, WG. 1918. Ascidians of the Philippines and adjacent waters. **United States National Museum Bulletin 100** (1): 49-174.

Van Name, WG. 1921. Ascidians of the West Indian region and south eastern United States. **Bulletin of the American Museum of Natural History 44**: 283-494.

Van Name, WG. 1924. Ascidians from Curaçao. Bijdragen tot de kennis der fauna van Curaçao, **Resultaten einer Reis van Dr. C.J. Van der Horst in 1920**, **Bijdragen tot Dierkunde 23**: 23-32.

Van Name, WG. 1945. The North and South American ascidians. **Bulletin of the American Museum of Natural History 84**: 1-476.

Vasseur, P. 1969. Deuxième contribution a l'étude des ascides de Madagascar région de Tuléar. **Bulletin du Muséum National d'Histoire Naturelle, Sér. 2, 40** (5): 912-933.

Verrill, A.E. 1871. Descriptions of some imperfectly known and new ascidians from New England. **American Journal of Science** (3) **1**: 288-294, 443-446.

Wada, H. 1998. Evolutionary history of free-swimming and sessile lifestyles in urochordates as deduced from 18S rDNA molecular phylogeny. **Molecular Biology and Evolution 15**: 1189-1194.

Wada, H.; K.W. Makabe; M. Nakauchi & N. Satoh. 1992. Phylogenetic relationships between solitary and colonial ascidians, as inferred from the sequence of the central region of their respective 18S rDNAs. **Biological Bulletin 183**: 448-455.

Wiegmann, A.F.A. 1835. Tunicata. **Archiv für Naturgeschichte 1** (1): 309.

Wiley, E.O. 1981. **Phylogenetics. The Theory and Practice of Phylogenetic Systematics.** New York, John Wiley, 439p.

Wiley, E.O.; D. Siegel-Causey; D.R. Brooks & V.A. Funk. 1991. The compleat cladist: a primer of phylogenetic procedures. **The University of Kansas Museum of Natural History 19**: 1-158

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Appendix 1. List of species and specimens studied.

Stolidobranchia

Pyura chilensis Molina, 1782 – DZUP PYU 41, Chile, NMNH 16637, Terra do Fogo, Argentina, 02/08/1971; *P. discoveryi* (Herdman, 1910) – NMNH 14300, South Orkney Islands, Antartic, 14/04/1964, DZUP PYU 1, Shetland del Sur, Antartic, 01/1995, IO-USP (Proantar V), 14/02/1987; *P. vittata* (Stimpson, 1852) – NMNH 15056, DZUP PYU 22, Ilha Bela, São Paulo, Brazil, 16/02/1997.

Molgula occidentalis Traustedt, 1883 – NMNH 2734, North Carolina, USA, 20/10/1885. *M. pedunculata* Herdman, 1881 – NMNH 18023, Bransfield Strait, Antartic, 01/04/1983, DZUP MOLG 1, South Shetland, Antartic, 06/1994. IO-USP (Proantar XVI), Summer 1997/1998. *M. phytophila* Monniot, 1969-70 – DZUP MOLG 8, Guaratuba, Paraná, Brazil, 08/08/1998.

Phlebobranchia

Ascidia mentula Müller, 1776 – DZUP ASC 20 (NMNHUVIGO M0203), Ria de Ferrol, Galícia, Spain, 11/07/1988, NMNH 220 Scotland, Strathclyde, 1884; *A. sydneiensis* Stimpson, 1855 – DZUP ASC 11, Penha, Santa Catarina, Brazil, 08/06/1998; *A. interrupta* Heller, 1878 – DZUP ASC 15, São Sebastião, São Paulo, Brazil, 17/11/1994.

Perophora listeri Forbes, 1848 – NMNH 3035, Mediterranean Sea, Napole, Italy, 1893; *P. multiclathrata* (Sluiter, 1904) – DZUP PEROPH 10, Guaratuba, Paraná, Brazil, 20/02/2000; *P. viridis* Verrill, 1871 – DZUP PEROPH 7, Itapema, Santa Catarina, Brazil, 13/10/1996.

Ecteinascidia turbinata Herdman, 1880 – DZUP ECT 01, Marina Puerto Del Sol, Cayo Largo, Cuba, 09/11/1997.

Aplousobranchia

Ciona intestinalis (Linnaeus, 1767) – DZUP CION 02 (UVIGO M0509), Ria de Ferrol, Galícia, Spain, 11/07/1988, CION 03, Mediterranean Sea, France, 07/1992; *C. antarctica* Hartmeyer, 1911 – NMNH 13739, 26/01/1972 e 13740, 26/09/1979, Antartic; *C. pomponiae* Monniot and Monniot, 1989 – NMNH 18247 Holotype, Galapagos Islands, 21/11/1986.

Rhopalaea abdominalis (Sluiter, 1898) – NMNH 20831, Belize, 08/1994; *R. crassa* (Herdman, 1880) – MNHN P1RHO A22 (slide P1-162), Mozambique, 18/11/1996.

Diazona violacea Savigny, 1816 – DZUP DIAZ 02 (UVIGO Fauna II 09.165A), Cabo Peñas, Asturias, N Spain, NMNH 3030, Napoles, Italy, 1893; *D. chinensis* (Tokioka, 1955) – MNHN P1DIA10 (permanent slides P1-122, P1-125), Manado, Sulawesi, Indonesia; *D. textura* Monniot,C. 1987 – MNHN P1DIA13 Holotype (permanent slides P1-98, P1-99), Vauban 376, New Caledonia.

Tylobranchion speciosum Herdman, 1886 – NMNH 018526, South Shetland Island, Antartic, 16/01/1972, IO-USP (Proantar XVI), 11/03/1997, DZUP DIAZ 03 (11/03/1997, Proantar XVI, IO-USP), Antartic.

Stomozoa gigantea (Van Name, 1921) – DZUP STOM 01, Salvador, Bahia, Brazil, 03/08/1999, NMNH 15684.

Pycnoclavella stanleyi Berrill and Abbott, 1949 – NMNH 17616, 24/05/1986 and 17617, 25/05/1986 California, USA.

Clavelina lepadiformis Müller, 1776 – DZUP POLY 50 (UVIGO I2212), Ria de Ferrol, Galícia, Spain, 30/07/1988; *C. oblonga* Herdman, 1880 – NMNH 7148, Florida, USA, 03/04/1887, DZUP POLY 25, Arraial do Cabo, Rio de Janeiro, Brazil, 13/04/1993; *C. steenbrasensis* Millar, 1955 – SAM A25948 (UPE 98-136), Port Elizabeth, Algoa Bay, South Africa, 04/10/1998, SAM A25949 (EL 99-030), East London, Harbour, South Africa, 09/02/1999.

Nephtheis fascicularis (Drasche, 1882) – MNHN A3OXY2, New Caledonia, permanent slides: A3-606 New Caledonia and A3-626 Indonesia, NMNH 20137, Vietnam, Nha Trang Bay, South China Sea, 16/07/1990.

Polycitor crystallinus Renier, 1804 – MNHN A3POL A 16, New Caledonia, 1987, permanent slide A3 558; *P. circes* Michaelsen, 1930 – MNHN A3POL A 7, Sulawesi, Manado, Indonesia, permanent slides A3-644, A3-646; *P. vitreus* (Sars, 1851) – NMNH 854, Newfoundland, Canada, 22/08/1886.

Exostoma ianthinum (Sluiter, 1909) – MNHN A3EXO1, permanent slide A3-801, Palau, 22/06/1993, NMNH 6028, 6029 Filipinas, Sulu Archipelago, 14/02/1908.

Eudistoma olivaceum (Van Name, 1902) – MNHN A3EUD16, Guadeloupe, permanent slide A3-328; *E. recifense* Millar, 1977 – DZUP POLY 52 (PALB 48PE-RE), Recife, Pernambuco, Brazil; *E. vannamei* Millar, 1977 – DZUP POLY 51 (PALB 46AL-MA), Marechal Deodoro, Alagoas, Brazil; *Eudistoma* sp. – DZUP POLY 53 Mont Serrat, Salvador, Bahia, BRAZIL 05/08/1999.

Euherdmania claviformis (Ritter, 1903) – NMNH 17600, California, USA, 24/05/1986, MNHN A1EUH10, New Caledonia (permanent slide A1-1148 WA 137NCZO); *E. vitrea* Millar, 1961 – DZUP POCL 19, Ilha do Arvoredo, Santa Catarina, Brazil, 14/11/1998; *Euherdmania* sp. – DZUP POCL 21, Ilha do Arvoredo, Santa Catarina, Brazil, 28/11/1998.

Pseudodistoma cereum Michaelsen, 1924 – MNHN A1PSEU02 (permanent slides A1 223, A1 224), Senegal, 1950; *P. crucigaster* Gaill, 1972 – NMNH 1006176, NMNH 1006179, Balearic Islands, Mediterranean, 10/1999; *P. cyrnusense* Pérès, 1952 – NMNH 1006180, Balearic Islands, Mediterranean, 10/1999; *P. africanum* Millar, 1954 – SAM A25950 (UPE 98-054), Port Elizabeth, Algoa Bay, South Africa, 01/10/1998.

Placentela crystallina Redikorzev, 1913 – NMNH 20354, Mar de Okhotsh, Russia, 30/07/1991; *P. translucida* Kott, 1969 – NMNH 11978 paratype, Adelaide Island, Antartic Peninsula, 26/10/1962.

Ritterella aequalisiphonis (Ritter & Forsyth, 1917) – NMNH 17599, California, USA, 25/05/1986; *R.. mirifica* Monniot and Monniot, 1983 – Holotype NMNH 14492, 15/03/1964 Holotype, 15/03/1964, Antartic, Joinville Island, MNHN A1RIT05 permanent slide A1 890, Eltanin; *R. dispar* Kott, 1957 – MNHN A1RIT01 (permanent slide A1 505), Mozambique.

Monniotus ramosus Millar, 1988 – NMNH 18471, paratype, Mozambique, 19/08/1964.

Synoicum adareanum (Herdman, 1902) – NMNH 17817, IO-USP (Proantar V), 14/02/1987, Antartic; *S. jordani* (Ritter, 1899) – NMNH 18625, Alaska, USA; *S. partitionis* Monniot, 1987 – MNHN A1 1154, Canal Woodin, New Caledonia.

Polyclinum constellatum Savigny, 1816 – NMNH 17380, México, 12/04/1960, DZUP POCL 23, Penha, Santa Catarina, Brazil, 21/05/1999, (permanent slide 1.13, Praia Grande, São Paulo, BRAZIL, 18/11/1991); *P. aurantium* Milne-Edwards, 1841 – DZUP POCL 26 (UVIGO I1003), Ria de Ferrol, Galícia, Spain, 20/02/1988; *P. pute* Monniot and Monniot, 1987b – MNHN A1POL B 34 (permanent slides A1-1034 tipo), Tikelau Polynesie.

Aplidium lobatum Savigny, 1816 – MNHN A1APL B284 (permanent slides A1 1138, A1 1174), New Caledonia; *A. accarense* (Millar, 1953b) – DZUP POCL 24, Penha, Santa Catarina, Brazil, 19/03/1999; *A. flavolineatum* Monniot, 1987 – SAM A25951 (UPE 2000-003), Port Elizabeth, Algoa Bay, South Africa, 14/02/2000.

Distaplia cylindrica Lesson, 1830 – NMNH 18879, South Georgia Island, Antartic, 05/12/1986, DZUP POLY 60 (Proantar VIII, IO-USP), Antartic; *D. bermudensis* Van Name, 1902 – NMNH 6945, Florida, USA, 02/1887, DZUP POLY 28, São Sebastião, São Paulo, Brazil, 12/02/1997; *D. skoogi* Michaelsen, 1934 – SAM A25952 (UPE 98-063), Port Elizabeth, Algoa Bay, South Africa, 03/10/1998.

Hypsistozoa fasmeriana Michaelsen, 1924 – NMNH 17205, Balleny Islands, Antartic.

Diplosoma listerianum (Milne-Edwards, 1841) – DZUP DID 88, Penha, Santa Catarina, Brazil, 16/01/1998; *D. macdonaldi* (Herdman, 1886) – EJ-66-439, Baía de Tampa, Florida, USA, 1966 (Hourglass Cruises); *D. glandulosum* Monniot, F 1983a – NMNH 15396, Belize, 14/03/1983.

Lissoclinum aureum Verrill, 1871 – NMNH 4754, Massachusetts, USA, 17/09/1878. *L. fragile* Van Name, 1902 – NMNH 7251, Virgin Islands, Caribbean Sea; *L. perforatum* Van Name, 1902 – DZUP DID 110, Ilha do Arvoredo, Santa Catarina, Brazil, 28/11/1998.

Polysyncraton chondrilla (Michaelsen, 1924) – NMNH 12223, S. Shetland Islands, Antartic; *P. amethysteum* Van Name, 1902 – NMNH 20035, São Sebastião, São Paulo, Brazil, 12/03/1986.

Didemnum candidum Savigny, 1816 – NMNH 7385, Sulu Archipelago, Philippines, 24/02/1908; *D. fusiferum* Van Name, 1921 – NMNH 6954, Florida, USA, 02/1881, EJ-66, Tampa Bay, Florida, USA, 1966 (Hourglass Cruises); *D. vanderhorsti* Van Name, 1924 – DZUP DID 107, Ilha do Arvoredo, Santa Catarina, Brazil, 15/11/1998.

Trididemnum orbiculatum (Van Name, 1902) – permanent slides DZUP DID 2.2, Itapema, Santa Catarina, Brazil, 22/10/1994, DID 2.4, São Sebastião, São Paulo, Brazil, 04/11/1991; *T. cerebriforme* Hartmeyer, 1913 – SAM A25953 (SAF 96-052), 24/04/1996, SAM A25954 (PLET 2000-009), 23/03/2000, South Africa; *T. hians* Monniot, F 1983a – EJ-67, Tampa Bay, Florida, USA, 1966 (Hourglass Cruises).

Leptoclinides faeroensis Bjerkan, 1905 – NMNH 3750, Nova Scotia, Canada; *L. hawaiiensis* Tokioka, 1967 – NMNH 11793 paratype, Auau Channel, between Maui e Lanai, Hawaii, USA; *L. rufus* (Sluiter, 1909) – NMNH 17231, 12/12/1962, Terra do Fogo, Argentina.

Atriolum quadratum Monniot and Monniot, 1996 – MNHN OCDN 1442-T, holotype (permanent slides I2-1583, A2-1582), Chuuk Island; *A. marinense* Kott, 2001 – NMNH 1845, 15/10/1964, Mozambique, Madagascar, SAM A25955 (UPE 2000-080), Port Elizabeth, Algoa Bay, South Africa, 15/03/2000.

Tylobranchion nordgaardi (Hartmeyer, 1922) – MNHN P1 TYL 2, Golfo de Gascogne, Thalassa, France, 1968, permanent slide of the pharynx (T 451-PI-9, Thalassa, 1967).

Tetrazona porrecta Millar, 1962 – SAM A25956 (UPE 99-012), 17/02/1999, SAM A25957 (UPE 2000-075), 14/03/2000, Port Elizabeth, Algoa Bay, South Africa.

Species evaluated using only literature information

Branchiostoma lanceolatum (Pallas, 1774): Ruppert (1997)

Ecteinascidia diaphanis Sluiter, 1885: Kott (1985)

Stomozoa australiensis Kott, 1990: Kott (1990); *S. bellissima* Kott, 1990: Kott (1990)

Pycnoclavella aurilucens Garstang, 1891: Garstang (1891), Millar (1970); *P. minuta* Millar, 1953b: Millar (1953b); *P. diminuta* (Kott, 1957): Kott (1957, 1990), Millar (1963)

Euclavella claviformis (Herdman, 1899): Kott (1990)

Brevicollus tuberatus Kott, 1990: Kott (1990)

Anadistoma attenuatum Kott, 1992: Kott (1992)

Dumus areniferus Brewin, 1952: Brewin (1952), Kott (1976, 1992), Monniot (1987); *D. dumosus* Monniot, 1987: Monniot (1987)

Monniotus australis (Kott, 1957): Kott (1957, 1992); *M. radiatus* Kott, 1992: Kott (1992)

Protopolyclinum pedunculatum Millar, 1960: Millar (1960)

Neodistoma mammillatum Kott, 1990: Kott (1990)

Hypsistozoa obscura Kott, 1969: Kott (1969); *H. distomoides* (Herdman, 1899): Kott (1990)

Polysyncraton millepore Vasseur, 1969: Kott (2001)

Atriolum robustum Kott, 1983: Kott (1983, 2001)