

Colonial life-history: a major evolutionary transition, involving modularization of multicellular individuals and heterochrony (miniaturization and adultation)

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Abstract

The diversification of life-histories is mediated by cooperation, innovations of biological information, modularity, and heterochrony in developmental processes. These processes are defined, contextualized, and exemplified, studying the evolution of coloniality (i.e. life-history involving modularization of the multicellular individual) in the family of benthic tunicates Styelidae. This study proposes that in these colonial tunicates there is an inter-generational division of labor, where one generation is feeding, a second is developing by morphogenetic processes, and a third is aging by programmed cell death and phagocytosis. The communication system developed in these colonies is mediated, by changes in proportion, location, and gene expression of specialized blood cells. Colonial life-history in animals is related to the reduction of individual size; development of extra-corporeal tissues to interconnect zooids; the inter-generational division of labor; and the reduction of zooid's individuality. Processes analogous with the widely accepted major evolutionary transitions (METs), suggesting that coloniality could be studied as a MET. The understanding of colonial life-histories could provide information about key mechanisms for life diversification.

Keywords: colonial life-history, major evolutionary transition (MET), cooperation, modularity, biological information, heterochrony, tunicate.

Glossary:

-Biological unit: Entity which is systematically the target of natural selection in different levels of biological complexity. For example, the cell is the biological unit at the cellular level; the multicellular individual is the biological unit at multicellular level.

-Biological hierarchies: or “levels of organizations” are categories used to characterized biological units using spatio-temporal magnitudes. For example, biological units composed just by one cell are

categorized at the cellular level.

-Cooperation: Process of mutual working between biological units, in function of a communal benefit, sometimes reducing selfish benefits.

-Individuality: Particular character or qualities, which distinguishes a biological unit from another.

-Module: Functional part of a biological system. Modules are in constant communication, by feedback loops to maintain the homeostasis. In this study the module is a zooid (multicellular individual), the functional part of a colonial animal. Therefore, modularity refers to morphological modularity, the morphological modules that composed the colonies.

1. Introduction

Life in our planet has evolved to a great diversity of life-histories, reaching an estimated of 2 billion species (Larsen et al., 2017). One way to organize such diversity of life-histories is in levels of biological organization. Spatio-temporal magnitudes are the main parameters used to define these levels of biological organization (e.g molecular, cellular, multicellular, populations), classifying the specific type of biological unit in each level (Sadava et al., 2014; Wimsatt, 1980). In biology we use these categories to understand life diversity, however, life has evolved in many spectacular ways, making generalizations difficult. The integration and cooperation of biological units can results in the evolution of new biological systems (Grosber & Strathman, 2007; Wimsatt, 1980). Therefore, the biological unit becomes a component of a larger system, maintaining their own structural and functional identity (i.e. module). The resulting new system can be a new biological unit in another level of organization (Fig. 1) (Jablonka, 1994a; Laubichler, 2003; Wimsatt, 1980).

This study presents four key concepts in evolution of life-histories: cooperation, biological information, modularity and heterochrony. These concepts are contextualized, and exemplified, by the study of colonial life-history evolution in Styelidae (a family of benthic tunicates).

1.2 Evolution of novel biological hierarchies involves innovation in biological information and cooperation strategies

The functionality of biological systems requires compatibility, and harmony between their modules to form a cohesive whole. This cohesion is regulated by information exchange and cooperation (Fig. 1) (Michod, 2006; Szathmáry, 2015). Novelties in the use and transmission of information, and cooperation between biological units were decisive in the evolution of new biological systems such as (a) the auto-replication machinery of organic molecules: RNA, DNA and proteins; (b) the compartmentalization of molecules, and the use of DNA as the genetic code for cellular replication; (c) the consolidation of symbiotic cells, generating the eukaryotic cells with chloroplasts, and mitochondria; (d) the evolution of multicellular organisms from unicellular ancestors; and (e) the establishment of colonies composed by discrete multicellular clonal individuals (Grosberg & Strathmann, 2007; Jablonka & Lamb, 2006; Michod, 1996; Szathmáry, 2015). These transitions towards novel levels of cooperation were highlighted by John Maynard Smith and Eörs Szathmáry in their seminal book *The Major Transitions in Evolution*. The authors went forward to name these events as Major Evolutionary Transitions (METs) (Maynard Smith & Szathmáry, 1995).

In each of the METs changes in the type of information, and mechanisms of communication facilitated the origin of new biological systems. Known examples of this process are (a) the use of

DNA, RNA and proteins, as the fundamental elements of the genetic code, and replication conditioned the origin of cells; (b) inter-cellular communication by feedback mechanisms using diffusible proteins (e.g. FGF, Hedgehog, TGF- β), and signal transduction pathways to establish tissues and multicellular organisms; (c) the mechanism of communication, and coordination of the clonal individuals in animal colonies; and (d) the use of symbolic and sound language, and their transmission in the formation of animal societies (Jablonka 1994b; Maynard 1999, 2000; Godfrey-Smith, 2000; Gatenby & Frieden, 2007; Szathmáry, 2015). The transmission of biological information is fundamental in the functionality, heredity and replication of biological systems (Fig. 1C).

When a new form of communication emerges between biological units, a more complex level of cooperation is made available. This level of cooperation implies an increase of fitness, for the biological system as a whole, while individual fitness of components may decrease. Thus, altruist behaviors are required in the components to maintain the cohesion, and functionality of the whole system (Michod, 1998; Michod & Nedelcu, 2003). For example, in *Volvox* colonies, cells with the same genotype exhibit cooperation by the division of labor: somatic cells are in charge of viability functions, and germ cells are specialized in reproduction.

The harmony of biological systems requires a constant cooperation between the modules that make up the system. As a part of this equilibrium, there are mechanisms in place to mitigate competition, and conflict between components of the same biological system (Fig. 1B) . Examples of this are: (a) clonal replication to preserve same genotype in the units of the system (Michod & Nedelcu, 2003; Michod, 2006); (b) DNA replication, and programmed cell death (PCD) to regulate cell number and prevent emergence of defector lineages (e.g. mutational process in cancer) (Umansky, 1982; Ameisen, 1996; Blackstone & Kirkwood, 2003); and (c) kinship recognition systems to associate with

relatives, and prevent the invasions of foreigners genotypes (e.g parasitic infections) (Michod, 1996; Michod & Nedelcu, 2003). Cooperation is essential to maintain cohesion and functionality in biological systems. The diversification of life-histories is related with the innovation in cooperation strategies.

1.3 Heterochronic mechanisms orchestrated development during the evolution of new life-histories

Heterochrony is the temporal displacement of characters in development, relative to the ancestor. The processes that produce heterochrony are acceleration and retardation, during the development of somatic tissues, or in the sexual maturation (Gould, 1977; Smith, 2003). The main morphological consequences of heterochrony are recapitulation, and paedomorphosis (Table. 1). Recapitulation is the appearance of adult features, earlier in descendants than in ancestors. On the other hand, paedomorphosis is the retardation of characters development, during the lifetime of descendants. Paedomorphosis usually happens by retardation of the development of somatic tissues. This retardation generates the retention of juvenile characters (i.e. neoteny), such as the gills of the axolotl (Fig. 2A). (Gould, 1977; McNamara, 1986). In contrast, recapitulation can be the result of retardation in sexual maturation. Retarded maturation with proportional increase of growth, produces gigantism. Moreover, retarded maturation without body size increase, involves disproportionated increment in size of a body part (i.e positive allometry) (Table.1). This process is called hypermorphosis, like the positive allometry of antlers in deers (Fig. 2B) (Gould, 1977; McNamara, 2012).

Feature displacement by acceleration, or retardation in somatic development, or sexual maturation produces morphological diversity. Heterochronic effects such as neoteny are hypothesized

to be a motor for evolutionary transitions: *“it (neoteny) supplies one of the very few Darwinian justifications for large and rapid evolutionary transitions, by permitting major changes in morphology without extensive genetic reorganization”* (from Ontogeny and Phylogeny, pg 285, Gould, 1977). Moreover, neoteny may produce individuals with a mixture of juvenile and adult characters. As a result, neotenic descendants can enter in new adaptive zones promoting ecological plasticity (Gould, 1977).

Retardation or acceleration of sexual maturation (i.e. hypermorphosis and progenesis) are also involved in the emergence of morphological diversity, and evolution of new life-histories. The adaptive radiation of mammals is an example of morphological (e.g. skulls, teeth and antlers) and life-history (e.g. carnivores, herbivores and frugivores) diversification, produced by heterochrony in sexual maturation and allometry (Kohler & Moya-Sola, 2009; Kolb et al., 2015; Klevezal, 2018). The acceleration of sexual maturation usually involves body size reduction. Precocious reproduction and smaller body size are related with the diversification of life-histories in some insects groups, such as parthenogenetic aphids and mites (Gould, 1977). Displacement in sexual maturation has ecological implications for the increase in progeny, resource exploitations, and competition. Therefore, heterochronic processes have been related with r-k ecological strategies. Acceleration in sexual maturation, reduction in body size, and faster growth are related with r strategies. In contrast, retardation in sexual maturation, increase in body size and brooding are related with k strategies (Gould, 1977; McNamara, 2012). Heterochronic mechanisms are essential in evolution promoting ecological plasticity, morphological innovations, rapid evolutionary transitions, and new life-histories.

Modularity and cooperation are strategies associated to the functionality of biological systems. These strategies are associated with the appearance of novel “biological tools” in evolution.

Specifically, new types of biological information and communication, related with innovations in cooperation strategies and modularity. In addition, heterochrony is the main developmental mechanism that generates morphological variety in evolution. The interaction between modularity, cooperation, biological information, and heterochrony is key for understanding evolution.

2. Colonial life-histories as evolutionary transitions in benthic ecosystems

In colonial life-histories, the former multicellular individual becomes a module of a new biological hierarchy. As a result, the multicellular modules are components of the colonial architecture maintaining physical cohesion (Jackson & Coates, 1986). These modules are formed by clonal replication maintaining identical genotype and physiological cooperation (including division of labor) (Hughes, 1989). Coloniality evolved by convergence in some animal phyla, involving a large diversification of life-histories (Fig.3) (Jackson & Coates, 1986; Wake, 2003) :

-In cnidarians, coloniality evolved in scleractinian corals and octocorals (Hexacorallia and Alcyonaria). Pelagic larvae settles and metamorphoses into a sessile polyp, forming a colony by asexual reproduction. These colonial animals are keystone species, forming coral reefs in marine ecosystems (Kaiser et al., 2010). In Hydrozoans, the colonial state is alternated between polyp generation and solitary jellyfish. Also, nested within hydrozoans, are the siphonophores: an exceptional floating colony of polymorphic polyps (e.g The Portuguese man of war) (Scrutton, 2015).

-Entoprocta evolved a diversity of budding strategies, in colonial and solitary species. In

entoprocts colonies, the budding mechanism is the formation of buds from the base of the stalk (Fuchs et al., 2010).

-Bryozoans compose a completely colonial phylum of filtering marine sessile animals. Colonies are formed by the development of new zooids, from bud primordia in adult zooids (Wood, 2014). The bryozoan colonies are polymorphic with a clear division of labor (i.e. Morphological differentiation and functional specialization between zooids) (Schack & Ryan, 2018).

-Hemichordates are marine animals with a worm-like body shape. Solitary and colonial hemichordates form an external tubular structure. The colonies are composed by tubular chambers with clusters of zooids attached to basal discs, in which the buds are formed (Lester, 1985).

-In Tunicates, coloniality evolved multiple times in pelagic and benthic clades. The Doliolids (class Thaliacea) are pelagic colonies with alternation of sexual and asexual generations in the life cycle. The asexual generation of doliolid colonies are composed by a buoyant zooid and feeding zooids to nourish the colony. In contrast, the sexual generations of these animals are formed by the buoyant zooid and reproductive zooids (Bone, 2003; Holland, 2016). In the orders Stolidobranchia, Aplousobranchia and Phlebobranchia of the class Ascidiacea, coloniality evolved multiple times. In ascidians, colonial life-history is related to a large diversity of budding strategies (Holland, 2016; Kocot et al., 2018).

Colonial animals are mostly marine organisms, and make up a considerable proportion of the diversity and biomass in the benthic and pelagic ecosystems (Jackson & Coates, 1986). Among colonies, sessile animals are essential components of benthic communities in tropical, and subtropical regions (e.g. coral reefs) (Jackson, 1977). There are several characteristics of the colonial life-history that may confer adaptive advantages for settlement, and growth on marine substrata: (a) the compact size of zooids allows faster development and shorter generations; (b) increased survival rates after weather disturbance and predation, by the dispersion of their genotype in the replaceable modules (i.e budding and regeneration); (c) and higher metabolic efficiency for the occurrence of different metabolic processes at the same time, by functional specialization and division of labor between modules of the colonies (Coates & Jackson, 1985; Davidson et al., 2004; Greene et al., 1983).

2.1 The higher proportion of colonial species in tropical areas is the result of colonial animals resilience to environmental disturbance

Colonial animals are more predominant and diverse in tropical and subtropical benthos. This diversity decreases along the latitudinal increment, whereas solitary forms are dominant in the temperate marine substrata (Hiebert et al., 2019; Jackson, 1977). Tropical oceans are oligotrophic, which results in most of the biodiversity being concentrated in some ecosystems. This is the case of coral reefs, rocky substrates, mangroves and sea grasses (Kaiser et al., 2010). Competition and disturbances, like predation or desiccation, are critical in these environments (Jackson & Hughes, 1985).

Colonial animals are formed by multicellular modules (i.e zooids), which are replaceable. This

characteristic confers to colonies the ability to overgrow and occupy substrates. Also, increasing their survival in case of environmental disturbance or predation (Jackson & Hughes, 1985). The resilience related with colonial life-histories can explain the preponderance of colonial animals in tropical benthos, and could be key for making sense of the convergent evolution of this life-history in multiple phyla.

3. Coloniality in styelids resulted from the evolution of complex characters through heterochrony

Styelidae is a tunicate family in which colonial life-history evolved multiple times (Fig. 4) (Alié et al., 2018). Morphological and molecular evidence suggest that the ancestor of this family was a solitary life-form. (Alié et al., 2018; Kott, 2005; Zeng et al., 2006) (Fig. 4B). In Styelids, the evolution of coloniality is linked to (a) the reduction in the size of the modular units that compose the colonies (colonial zooids range in size from 2 mm to 20 mm, whereas solitary adult individuals range in size from 15 mm to 10 cm); (b) the development of tissues and structures to maintain cohesion and communication between the colonial modules, such as extra-corporeal vascular systems or stolons; (c) the development of asexual reproduction strategies; (d) the development of brooding organs inside mother colonies; (e) an increase in larval size and adulation of the larvae; (f) and diversification of blood cell types to orchestrate the modular development of colonies (Alié et al., 2018; Ballarin et al., 2008; Davidson et al., 2004; Kott, 2005; Pérez-Portela et al., 2009; Zeng et al., 2006). These characteristics in colonial descendants often come from modifications in function of characters already present in solitary forms (Fig. 4A).

Heterochronic processes are related to the evolution of coloniality in the Styelids (Berrill,

1935; Davidson et al., 2004). For instance, the time period of embryonic development from egg fertilization to larval hatching is longer in colonies in comparison with solitary species. This lapse is twice as long in the more derived colonies than in solitary species (Fig. 5A). This retardation brings as likely consequences the larval adultation, and an increase of larval size (e.g. larva in *Botryllids* hatch with an oozoid and bud primordia) (Fig, 5A). Retardation during embryogenesis in colonies is also probably related to the egg size increase and brooding in colonies (Berrill, 1935).

Another heterochronic phenomenon is the accelerated growth stage after metamorphosis in colonial styelids. The growth stage in colonies is significantly faster than in solitary forms (Fig. 5A). Colonial zooids have limited time to grow, resulting in the miniaturization of modules, the main characteristic of colonial animals. Heterochronic mechanisms (e.g. adultation, miniaturization) are probably involved in evolution of colonial life-history in Styelidae.

3.1 Complex characters in colonies resulted from heterochrony and phenotypic plasticity

The formation of a colony in the clade *Symplegma+Botryllids* involves embryonic development, larval settlement, metamorphosis, and the formation of clonal zooids by non-embryonic development (i.e budding) (Berrill, 1940, 1941; Sabbadin, 1955a). This non-embryonic development is the main innovation of colonial animals, which provides resilience to external disturbances (Jackson, 1986). The formation of a clonal zooid through non-embryonic development is a comparable process to the formation of the body of a solitary individual in a related species. In both of these processes, a functional body plan is laid out from undifferentiated cellular prime material (Ballarin et al., 2008; Franchi et al., 2016). In *Symplegma+Botryllids*, the first stage of budding comprises evagination of

epithelia, followed by cell movements and tissue reorganization (analogous as a gastrulation in embryogenesis). Afterward, organs are formed, the zooid increases its size, and finally the fully differentiated adult begins feeding (Fig. 5B) (Gutierrez & Brown, 2017; Kawamura & Nakauchi, 1986). In non-embryonic development, the first stages are slower in comparison with embryogenesis. However, individual growth in solitary forms is a longer process, congruently with the general miniaturization of individual body size in colonies (Fig. 5B). Colonial life-history is associated to the modularization of developmental processes, such as the ability to bud. A budding zooid undergoes cell movements and reorganization of tissues with a comparable function to the gastrulation of an embryo. Eventually, the bud also undergoes organogenesis and achieves functional maturity. These developmental processes happen simultaneously in colonies with multiple, renewing modules. This is an innovation in comparison with solitary individuals, in which these processes are temporarily separated.

The clonal zooids formed by non-embryonic development are interconnected by a extracorporeal blood vessel system that is formed during metamorphosis and diversifies with the increment of colonial area (Mukai et al., 1978). The formation of blood vessel systems is probably related with the longer time required for metamorphosis in colonial animals (Fig. 5A).

The development of colonial morphological characteristics (e.g. larval adulation, body size miniaturization, and extracorporeal vessels) often involves heterochronic processes. In Styelidae, solitary species have similarities with colonial ones during their early adult life. For example, juveniles of *Styela canopus* develop extracorporeal blood vessels after metamorphosis. These vessels are shorter compared with the vessels in colonial Styelids. However, it has been hypothesized that these structures can be involved in a gregarious strategy (Jiménez-Merino et al., 2019). These observations suggest that

phenotypic plasticity and heterochrony in Styelidae are involved in the development of complex characters in colonies, such as non-embryonic development and an extracorporeal blood vessels system.

4. The evolution of coloniality is mediated by novel systems of cooperation between multicellular modules

Space is the main limiting resource in the tropical benthos (Barnes & Hughes, 1982; Coates & Jackson, 1985). Consequently, space competition is one of the critical environmental pressures in benthic ecosystems (Jackson, 1977). As a result, benthic organisms develop strategies to maintain local coexistence or exclusion (Crowley et al., 2005).

Cooperation between organisms is maintained through strategies such as chimerism, philopatry and inbreeding. In contrast, there are mechanisms in the benthic ecosystem used for space competition, such as mechanical and chemical defenses (e.g. spicules, strong tunics, calcified skeletons, secondary metabolites); high rates of overgrowth; young sexual maturity; and dispersion by fragmentation (Barnes & Hughes, 1982; Kaiser et al., 2010). The coexistence in benthic communities of cooperative interactions, and competitive exclusion may promote the development of novel communication ways between organisms. Evolution of new cooperation systems as ways of communication is a mechanism proposed to be involved in METs (Jablonka, 1994a; Szathmáry, 2015).

4.1 The trade-off between competitive exclusion and cooperation, may influence the evolution of life-histories in benthic ecosystems

The maintenance of a constant communication between organisms requires an interactive network. In colonial animals, local interactions between modules generate a cohesion that arguably characterizes the formation of a new biological system (Fig.1) (McShea, 1996; Miller & Page, 2007). For instance, this process was observed in the green alga *Chlamydomonas reinhardtii*. When a predator was introduced in the environment of the alga, multicellular colonies were formed from unicellular ancestors after several generations (Herron et al., 2019). The algae exposed to predators generated an extracellular matrix (ECM), which served as an attachment site for new daughter cells. Then, clusters of cells released multicellular propagules. Finally, after some generations, the unicellular green algae had evolved a multicellular life-history in response to predation. The process followed by this green alga has many parallels with the evolution of colonial animals in benthic ecosystems. For example, the evolution of colonial animals can be promoted by environmental pressures (e.g. space competition, predation). In response to these pressures, solitary organisms form conglomerates (Jackson & Hughes, 1985). Formation of conglomerates involves constant coexistence and cooperation between individuals. Usually, these conglomerates are formed by relatives (Jackson & Coates, 1986; Jackson, 2002). The interaction of proximally related solitary individuals facilitates the emergence of a communication nets between individuals. Moreover, the capacity to form extracorporeal structures to maintain cohesion of individuals may be involved in evolution of colonies, as was the case for *Chlamydomonas*. Solitary species in Styelidae have the capacity to form extra-corporeal vessels during a brief period following metamorphosis of the larva (Jiménez-Merino et al., 2019). Structures such as these vessels could be instrumental to the physical cohesion of individuals during evolution of colonial life-histories.

The capacity of individuals to produce extracorporeal structures, that could be adapted to serve in cooperative interactions is probably involved in important evolutionary transitions. This capacity is frequently linked to phenotypic plasticity, which allows the development of different phenotypic characters in response of environmental pressures (West-Eberhard, 2003). In metazoa and related clades, phenotypic plasticity is associated to a cellular reaction to variable environmental conditions. This could be involved in the cellular diversification in metazoa (Sogabe et al., 2019). The recurrent evolution of coloniality in animals, could be the result of the interaction between phenotypic plasticity, and a competitive variable environment.

Colonial life-histories have evolved multiple times and have also evolved back into a solitary state. These shifts between solitary and colonial forms could be linked to the plasticity of animals, and to the exposition to different environmental pressures. Marine animals are exposed to changing conditions such as temperature, pH, salinity, predation and competition (Barnes & Hughes, 1982). In addition, marine ecosystems are heterogeneous, forming micro-environments with a large variety of physical and biotic conditions. This environmental heterogeneity in presence of animal plasticity is propitious to evolution of complex life-histories such as coloniality (McShea, 1996; Yaeger, 2009).

Cohesive tissues in colonial animals are diverse in structure and function. These tissues are usually extensions of epithelia and extracellular matrices, such as tunics in tunicates and calcified tissues in cnidarians and bryozoans (Mackie, 1986; Shack et al., 2018). In these animals, coordination and communication by cohesive tissues is mediated by nervous fibers, muscles or vessels with corporal fluids (Coate & Jackson, 1985; Mackie, 1986; Schack et al., 2018). The basic physiological functions of these tissues are the transport of nutrients, oxygen and wastes; and movement of corporal fluid through the colony (Hughes, 1989). Moreover, cohesive tissues allow the communication and

coordination between zooids (Mackie, 1986). For example, zooids in cnidarians are interconnected by a communal gastric cavity. In this cavity, nutrients and corporal fluids are transported. In pelagic colonies of siphonophores, neurons with long axons interconnect the zooids. These nerve fibers are involved in zooid communication to coordinate movements after external stimulus (Mackie, 1986). Bryozoan colonies have a diversity of pores for communication between zooids, allowing transmission of corporal fluids and nutrients (Shack et al., 2019). Zooids in salps are also interconnected by epithelia and nerves. Salp zooids are attached to each other by a pair of attachment plaques. The neurons are distributed around the plaques, interconnecting zooids and transmitting nervous signals. This system, composed by neurons and epithelia, allows the coordination required for swimming in salps (Macki & Bone, 1977). In sessile colonies of tunicates, zooids are interconnected by the tunic and epithelia. In Styelid ascidians, zooids are interconnected by extracorporeal vessels. Within this family, highly aggregate colonies –such as the *Botryllids* and *Symplegma*– have a vascular vessel system with blood cells in constant circulation (Mukai et al., 1978a). This diversity in cohesive tissues suggests that colonial life-histories evolved different ways to maintain the cohesion and communication between zooids.

The appearance of novel communication elements, is crucial in the evolution and functionality of animal colonies. In the same way an extracellular matrix was a crucial factor, in the evolution of multicellular organisms from unicellular ancestors (Herron et al., 2009, 2019). The evolution of colonial life-histories in animals, shows a similar pattern of innovation of cooperation mechanisms, and biological communication with major evolutionary transitions, as defined by Szathmáry (1995, 2015) (Fig.7). Thus, this study proposes that evolution of colonial life-history in animals could be studied as a major evolutionary transition.

4.2 Evolution of coloniality in the *Symplegma* + *Botryllids* clade involved an increase in cooperation and reduction of individuality in multicellular modules

In the Styelid *Symplegma* + *Botryllids* clade, colonies are conformed by interconnected zooids with a communal tunic and extra-corporeal blood vessels (Berril, 1940, 1941). Around the edge of the colony, the vascular system forms bulbous projections, called ampullae, which exhibit contractile movements, facilitate growth and have a role in substrate recognition (Fig. 4A-6A,B) (Mukai et al., 1978; Sabbadin, 1955a). The cohesive tissues in *Symplegma* + *Botryllids* clade developed the function to maintain homeostasis in case of external disturbances (Rodriguez et al., 2017). This system is essential during tissue repair, as it transports factors and cells required for regeneration (Brown et al., 2009a, Lauzon et al., 2002). Additionally, in case of adverse climatic conditions, colonies enter a dormancy stage through the resorption of all zooids, leaving only blood vessels (Hyams et al., 2017).

The blood vessel system in *Symplegma* + *Botryllids* colonies maintains physiological cohesion and allows communication between zooids. A diversity of blood cells and presumably diffusible factors flow through this system (Fig. 6C) (Ballarin et al., 2008; Franchi et al., 2016; Lauzon et al., 2002; Menin et al., 2005). Blood cell types in this group are diverse in morphology and function. Some known functions of blood cells include: (a) phagocytosis and programmed cell death (PCD) by macrophage-like cells (MLCs); (b) budding and regeneration by undifferentiated cells (UCs) with characteristics of stem cells; (c) immune reactions by morula cells (MCs) and (d) storage and excretion by nephrocytes and pigment cells (Ballarin et al., 1995; Cima et al., 2001; Brown et al., 2009; Franchi et al., 2016). Moreover, these migratory blood cells are related to the modulation of the non-embryonic budding process characterized in *Symplegma* + *Botryllids*. In these colonies, while some buds are in morphogenesis, some zooids are in a programmed senescent process. UCs are more predominant at the

stage of morphogenesis and MLCs and MCs are more predominant at the senescent stage (Fig. 6 C) (Ballarin et al., 2008; Franchi et al., 2016; Gutierrez, 2020). The communication between zooids probably involved specialization of blood cells to modulate the non-embryonic development.

In *Botryllids*, three non-embryonic stages are simultaneous and cyclic: A parental zooid (fully developed in charge of filtering), a primary bud (going through organogenesis) and a secondary bud (tissues folding, analogous to gastrulation in embryogenesis) (Fig. 6C). These three zooid generations are highly interdependent: bud removal induces early senescence of zooids, and zooid removal reduces the size of zooids in the next generation (Lauzon et al., 2002; Lauzon et al., 2007). Blood cells in *Botryllids* have differential proportion and gene expression during budding. Undifferentiated cells are more predominant and expressing the inhibitor of apoptosis IAP7 during bud formation (middle of the cycle, Fig. 6C-Stg.B), while phagocytes and morula cells are more predominant and expressing the pro-apoptotic factors AIF1, PARP1, and the modulator of apoptosis BsBAX, during the senescent process (Take over stage Fig. 6C-Stg.D) (Campagna et al., 2016; Franchi et al., 2016). Therefore, cyclic budding in *Botryllus* is regulated by the change in proportion and gene expression of the specialized blood cells.

In contrast to *Botryllids*, *Symplesma* colonies have asynchronous budding and more independence between zooids. Colonial development is unaltered with removal of mature individuals (bud removal induces an accelerated development of new buds), and blood cell proportions are constantly maintained at normal conditions. The proportion of phagocytes and precursor cells increase in case of injuries, which is probably related with inflammation and regeneration of affected tissues (Gutierrez & Brown, 2017). This suggest that the control of zooids development at the colonial level is higher, in more integrate colonies, with higher interdependence between zooids (i.e *Botryllids*) than in

the colonies with more independent zooids. Thus, the individuality of units (i.e zooids) is reduced with the increase of control by the higher biological hierarchy (i.e colony). Coloniality in this group illustrates the appearance of a major evolutionary transition (MET), involving the cooperation and a reduction of individuality of modules, that integrate the new biological system (Jablonka, 1994a; Michod, 2006) (Fig. 7).

5. Conclusions

Modularity, cooperation, biological information and heterochrony are useful concepts in the comprehension of life diversification. These concepts have been observed and studied in evolutionary transitions, such as auto-replication of organic molecules, evolution of eukaryotic cells, and multicellular organisms from unicellular ancestors (Herron et al., 2009; Jablonka & Lamb, 2006; Michod, 2003; Szathmáry, 2015).

In the diversification of metazoans, evolved animals with a colonial life-history. An animal colony is a biological hierarchy, composed by multicellular modules (i.e. multicellular individual became a module of colonial biological level). The cohesion and functionality of colonies involved new communication ways, and heterochrony during development. Therefore, the evolution of coloniality in animals could be consider as a major evolutionary transition. (MET) This idea is congruent with the discussions about colonial animals proposed by several authors, and the relevance about the study of this life strategy in the understanding of life diversification, specially in marine environments(Davidson et al., 2004; Jackson & Coates, 1986; Schack et al., 2019). Moreover, the study of colonial animals could provided new relevant information about developmental mechanisms and evolution in general, such as mentioned by Gould, 1977 in his synthesis about heterochrony in

Graptolites.

The colonial strategy evolved in the tunicate clade *Symplegma* + *Botryllids* is an example of colonial life-history evolved from a solitary ancestor (Alié et al., 2018; Kott, 2005; Zeng et al., 2006). In these colonies evolved a cellular communication system, where signals are transmitted between modules by migratory cells, coordinating biological colony-wide processes (Ballarin et al., 2008; Lauzon et al., 2007). This would imply that colonies act as self-regulating or autonomous higher-level systems that can respond to perturbations by altering the development of their individual modules. Congruently, it is possible to hypothesize that in more integrated colonies the individuality of zooids is reduced to increase the benefit of the colony as a whole. This could involve division of metabolic and physiological processes between generations: one generation is feeding the colony (i.e. zooids); a second generation undergoing morphogenetic and inductive processes (i.e. buds); and a third generation undergoing programmed cell death and phagocytosis (i.e. aging zooids).

Multicellular individuals became a module of a higher-level of biological organization (i.e. colony), by the cooperation and harmonic interaction with another modules. During the development of colonies adult zooids are in charge of feed the entirely colony, while buds develop, promoting growing of whole colony. Aging mechanisms such apoptosis and phagocytosis are involved in the recycling of old zooids tissues. In this way these recycled material is used in the formation of new buds, and colonial development.

In the study of major evolutionary transitions, biological concepts such as cooperation, and evolution of new types of information are compared with some process in human society (Jablonka &

Lamb, 2006; Szathmáry, 2015). Marine colonial animals have been living thousand of years in our planet, showing that the resilience in case of disturbances (e.g environmental catastrophes, diseases, predation) is dependent of the equilibrium between cooperation and individuality. Therefore a consideration about these concepts could give interesting tools to the development of our modern human societies.

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7. Tables

Table 1. Categories of heterochronic mechanisms (Edited from Gould, 1977, pp229)

Timing			
Somatic features	Reproductive organs	Heterochronic process	Morphological result
Accelerated	--	Acceleration	Recapitulation (by acceleration)
--	Accelerated	Progenesis	Paedomorphosis (by truncation)
Retarded	--	Neoteny	Paedomorphosis (by retardation)
--	Retarded	Hypermorphosis	Recapitulation (by prolongation)

8. Figures

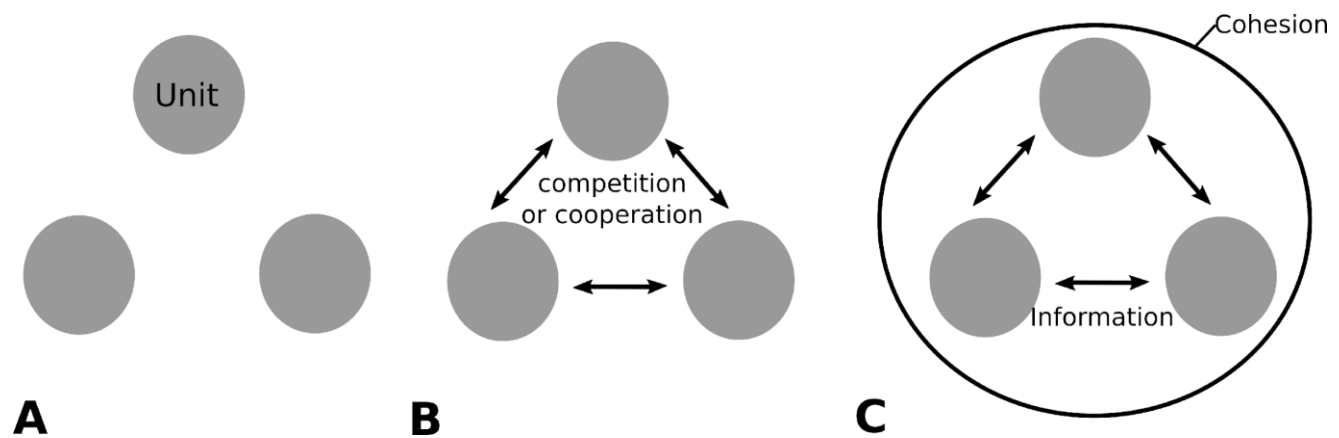
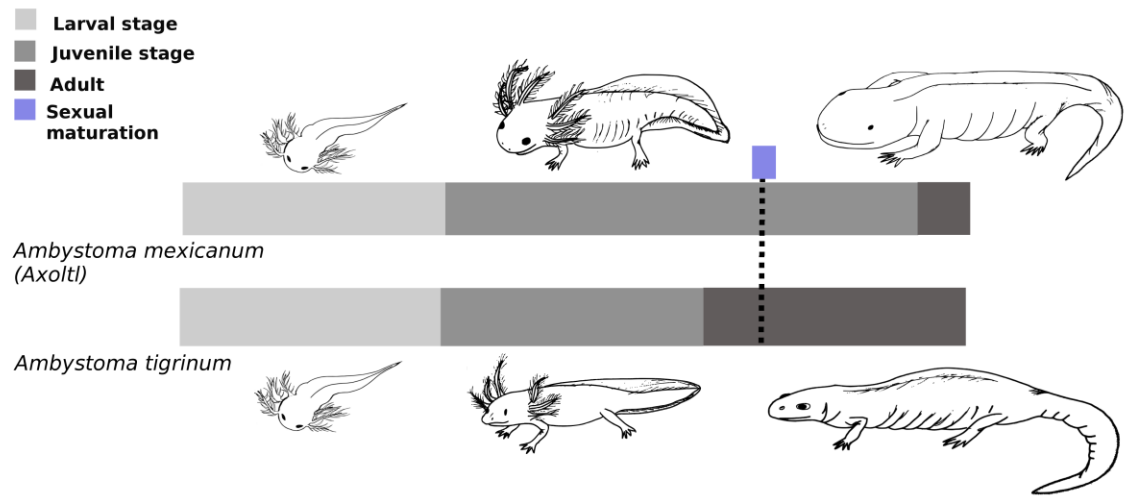
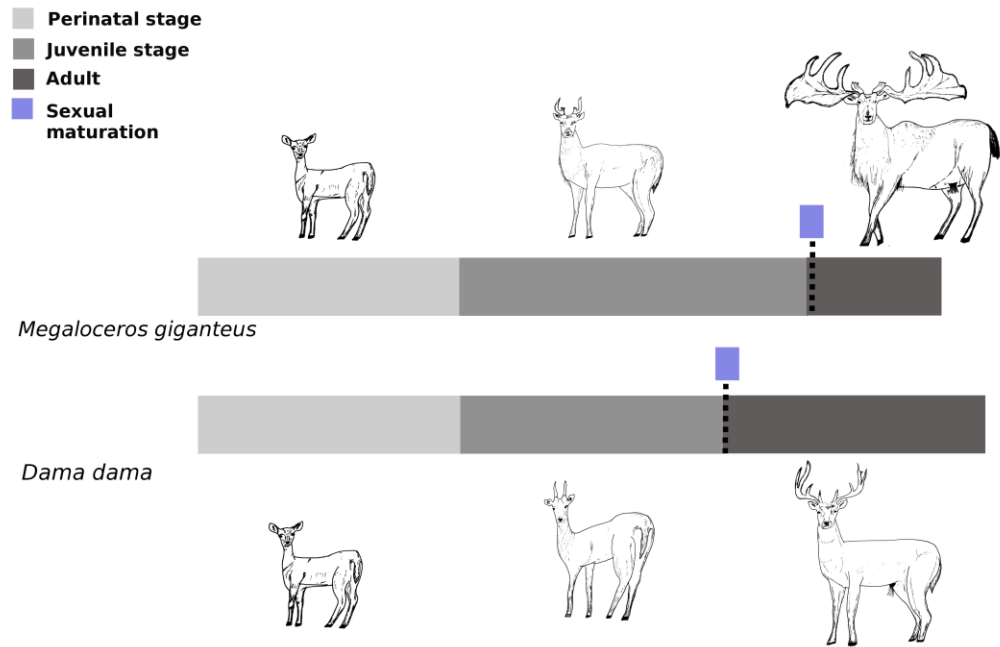


Figure 1. Cooperation, modularity and biological information in evolutionary transitions. (A) Biological units coexist sharing the same spatio-temporal context. (B) Coexistence between biological units results in interactions, such as competition or cooperation. (C) These interactions are regulated during time, generating new networks of interactions and conglomerates that share information. The cohesion of biological systems, is based in the harmonic interactions between the components, by constant transmission of information between these components



Heterochronic effect: Retardation of somatic tissues development
Morphological result: Paedomorphosis / neoteny

A



Heterochronic effect: Retardation of sexual maturation
Morphological result: Recapitulation / Hypermorphosis

B

Figure 2. Heterochronic mechanisms.

Examples of retardation and acceleration are shown. These heterochronic mechanisms can occur during

somatic development or sexual maturation, resulting in morphological alterations in descendants. (A) Retardation of somatic development in the axolotl (*Ambystoma mexicanum*), resulting in a retention of juveniles characters or neoteny (Paedomorphosis). (B) Retardation of sexual maturation, resulting in the increase of growth stage and gigantism, like the giant deer (*Megaloceur giganteus*).

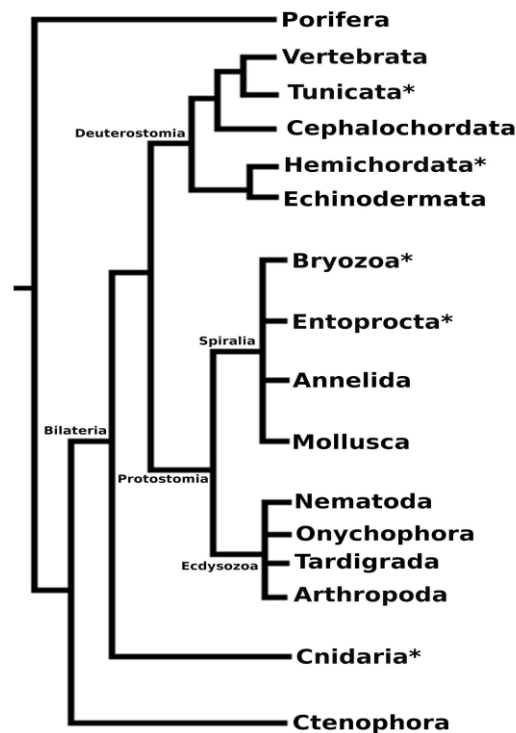


Figure 3. Phylogeny of metazoans, phyla with colonial clades are highlighted with an asterisk.

During evolution of animals, the colonial life-history evolved multiple times. Cnidaria has a large diversity of colonial strategies, pelagic and benthic colonies. The Entoproct and Hemichordate evolution of coloniality is related with a body size reduction and maintenance of similar body shape as solitary sister groups. Bryozoa is an entire colonial phylum with specialization and division of labor. Tunicates evolved coloniality multiple times. Like in cnidarians, pelagic and benthic colonies evolved in tunicates. Phylogeny based on Davidson et al., 2004; Dunn et al., 2014 and Simion et al., 2017.

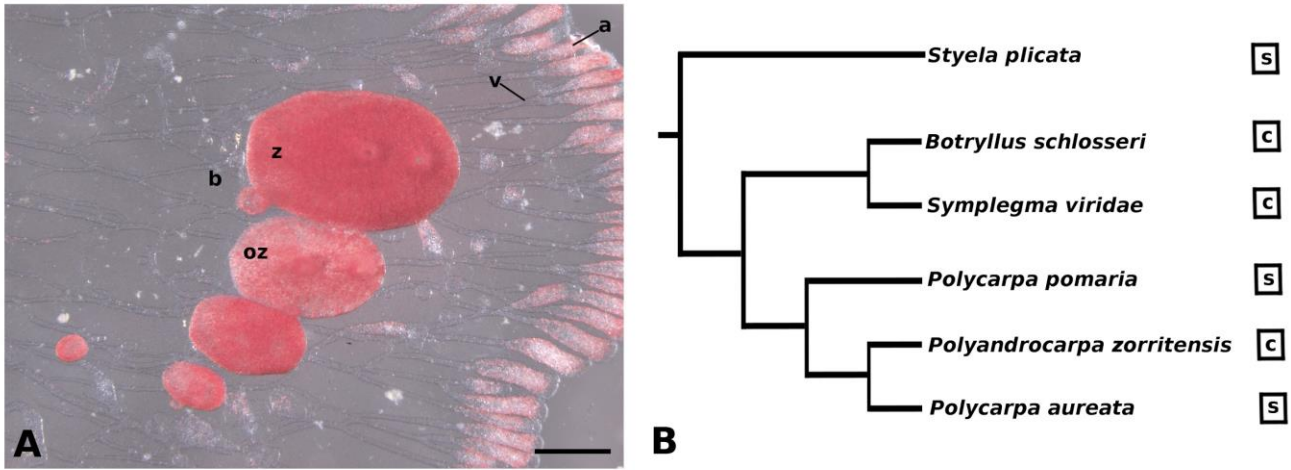


Figure. 4 Colonial life-history in Styelids tunicates. (A) *Symplegma rubra* colony, the. ampullae are shown in the periphery, at the edge of blood vessels. Blood vessel system can be seen connecting buds, zooids and senescent zooids. (B) Topology summarizing the phylogeny of the solitary and colonial species in Styelidae, based in Alié et al., 2018; Hiebert et al., 2019 and Zeng et al., 2006. *a:ampulla; b:bud; oz: old zooid; v: blood vessel; z: zooid*. Scale bar is 1,5 mm.

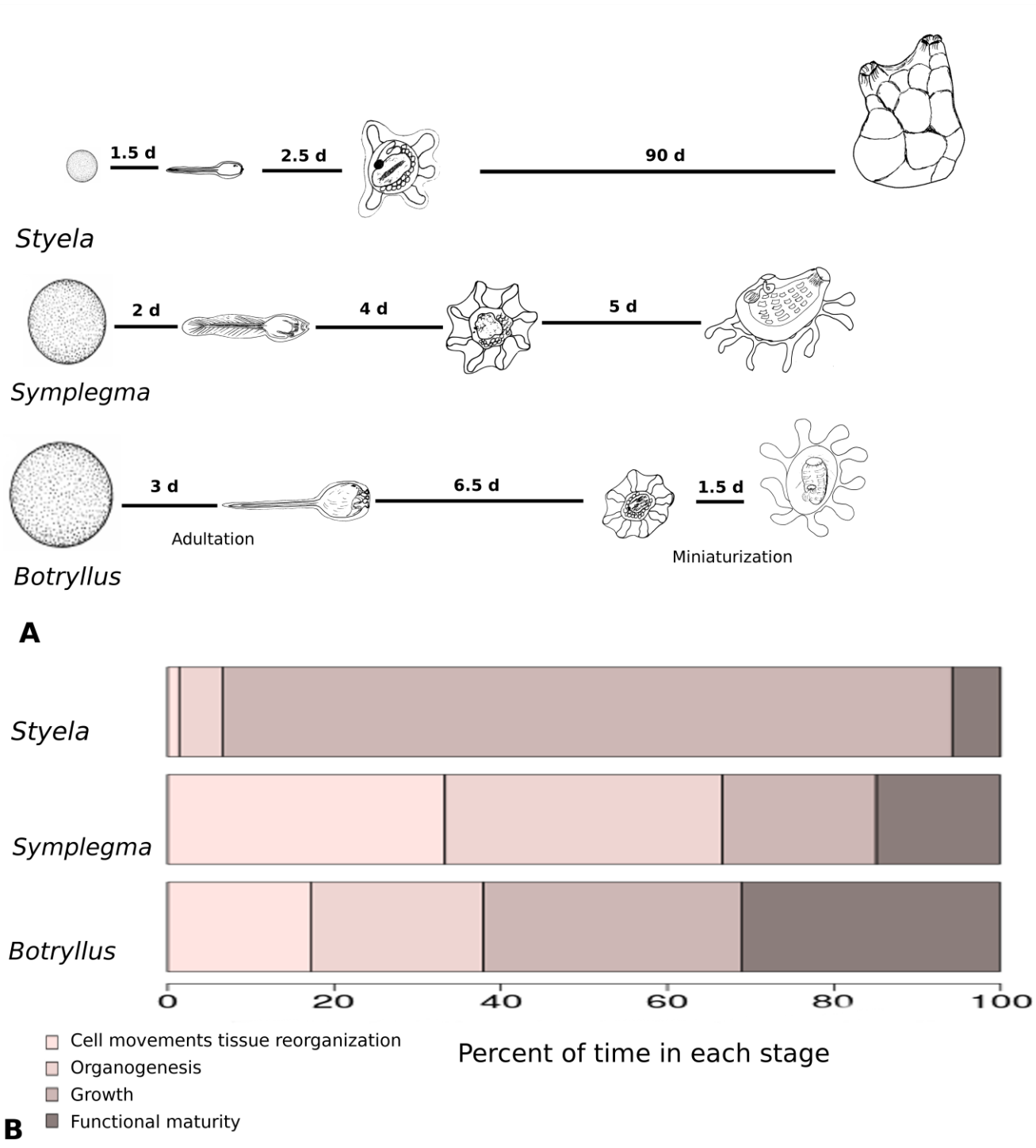
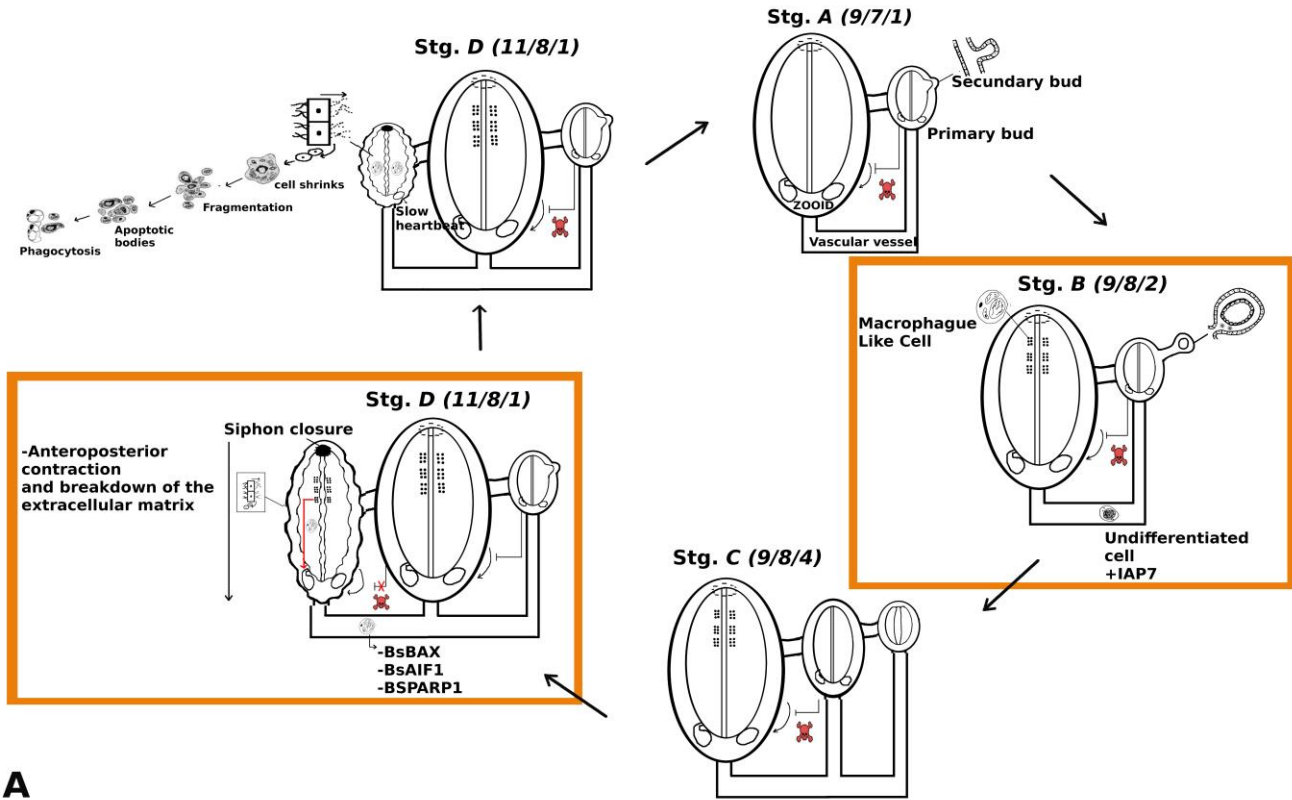
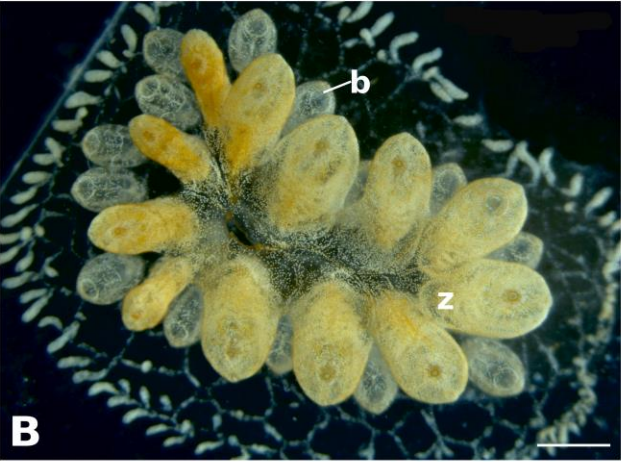


Figure 5. Heterochrony in evolution of coloniality in Styelidae. Figure. (A) Average time in days of the embryogenesis, metamorphosis and growth in *Styela plicata*, *Symplegma sp* and *Botryllus sp*, based in our observations and Berrill, 1935. Numbers represent duration in days between developmental

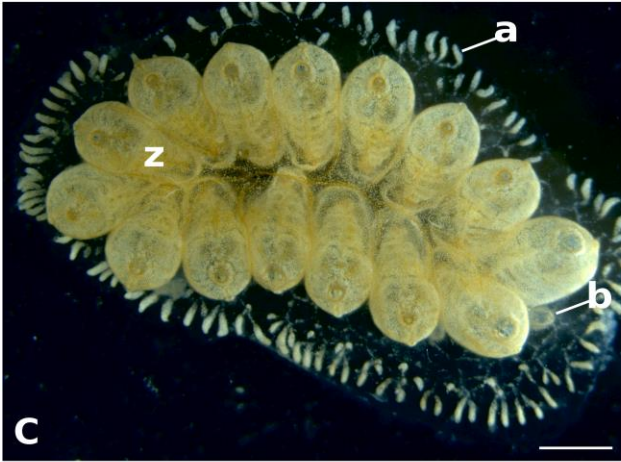
stages: embryogenesis, larval development, metamorphosis, and fully differentiated individual (B) Comparison of body plan formation and organogenesis in an individual of *Styela plicata* and zooid buds of *Symplegma braknhielmi* and *Botryllus schlosseri*. Based in Gutierrez and Brown, 2017; Jimenez, 2018; and Berrill, 1945.



A



B



C

Figure. 6 *Botryllus* model of budding mechanisms. (A) The budding in *Botryllus* is a cyclic process, buds are forming from zooid epitheliums. Budding is synchronized, all the buds are forming and developed at the same time. The stage nomenclature use letters from A to D, and a three numerical convention, where each number indicates the developmental stage of one of three generations of buds/zooids (Sabbadin, 1955). The middle of the budding cycle is stage B or 11/8/1, and represents a state where an aging zooid (stg. 11), a zooid finishing organogenesis (stg.8) and the primordium of a new bud (stg. 1) coexist. The middle of the cycle (stg. B= 9/8/2) and the final of the cycle or take-over (stg. D= 11/8/1) (Lauzon et al., 2002), are stages in which blood cell types are changing proportions (These stages are highlighted with orange squares). At the middle of the cycle (stg. B= 9/8/2) undifferentiated cell (UC) are more predominant. Otherwise at take-over stage (stg. D= 11/8/1) macrophage-like cells increase the proportion (Ballarin et al., 2008). Proportion in blood cell types at budding stages is also related with differences in gene expression. UCs express anti-apoptotic marker IAP7 at the middle of the cycle, MLCs express pro-apoptotic markers BsBAX, BSAIF1 and BsPARP1 at take-over (Franchi et al., 2016). This blood cell type modulation is probably related with the control of zooids lifespan. Development and presence of early buds in the colony arrests PCD of adult zooids. (B) *Botryllus tabori* colony at the middle of the budding cycle (stg. B= 9/8/2). Fully differentiated parental zooid, primary bud during organogenesis, secondary bud during morphogenesis. (C) *Botryllus tabori* colony at the take-over stage of the budding cycle (stg. D= 11/8/1). Parental zooid enter in a programmed cell death process, the primary bud is a fully differentiated zooid and the secondary bud is in organogenesis. Scale bar is 2 mm.

