



## Commentary

## The tail of the underwater phoenix

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The legendary Phoenix is an ancient Egyptian mythical grand and colorful bird that lived in the desert for five centuries. Then, once ignited itself with a single clap of its wings, the fire completely consumed it, but it later rose from its own ashes utterly renewed, and so on in endless cycles. Mystical birds akin to the Phoenix are known from old Greek and Roman myths, from Judaism, Christianity, Chinese, Japanese, Russian and Arabic folklore, all tie the myth of the Phoenix with the yearnings for immortality and resurrection. Whereas the Phoenix symbolizes the ideas of renewal and rejuvenation in the most imaginary way, a query may be raised whether we can find an authentic correspondence from the Animalia, which demonstrates the trait of recurrent deaths and regrowth by a single biological entity? The first retort would be a complete rebuff of such an idea, just from the point of view of simple energy resource limitation, among others. From the ecological and evolutionary perspectives, animals cannot afford the luxury of losing and rebuilding their bodily parts over and over again.

To amaze us, the phenomenon of cyclical death and rebirth does exist in nature, and is portrayed as a major developmental biology trait in the globally distributed group of botryllid ascidians (shallow water colonial tunicates of the family Styelidae, commonly associated with hard bottom biological assemblages) that includes two genera, *Botryllus* and *Botrylloides*, with almost 50 valid species. It is thus of prime scientific interest to read the recent publication by Manni et al. (2018), a review that focuses on the worldwide occurring and the model species *Botryllus schlosseri*, summarizing 60 years of research on the Phoenix-like astogeny characteristic of this group of organisms.

Colonial organisms represent structural patterns that consist of repeated sub-units, the basic modules (the zooids in botryllid ascidians). Upon accomplishing ontogeny, the first established basic modules then commence astogeny (building of a colony), where similarly sized modules are continuously added, in a process also known as asexual reproduction. The tessellation of colonial modules in different shapes and wide range of pattern formations leads to the creation of simple to highly complex colonial structures, with basic modules that are arranged in a hierarchical subdivision. In *Botryllus schlosseri*, like in all other botryllid ascidians, this continuous developmental process of colonial growth is repeatedly interrupted by the Phoenix-like death and rebirth cycles.

While the Manni et al. (2018) review concentrates on results obtained from the last six decades, it should be noted that this cosmopolitan distributed organism (Ben-Shlomo et al., 2006; Reem et al., 2017) is

known for more than 460 years. It was initially documented and illustrated in a book by Rondelet (1555, p. 130) that described and sketched for the first time its typical star-like structure (Fig. 1a in Manni et al., 2018). Each *B. schlosseri* colony is composed of few, and to up to thousands of genetically identical and functional modules (each called a zooid), all embedded in the translucent-gelatinous matrix, the tunic, and grouped in star-like shapes referred to as systems, each contains up to almost 20 zooids around common cloacal (exhalant) apertures, the siphons (Fig. 1a). Each one of the zooids carries its own inhalant siphon that allows the intake of food and oxygen, as well as the entry of sperm released from other gravid colonies. All zooids within the systems, and between systems, are connected to each other's via a ramified vasculature (Fig. 1b), possessing a number of circulating blood cells. The blood vessels terminate in the periphery of the colony in pear shape structures called vascular ampullae, which are also scattered between the systems and near the upper surface of the colony (Fig. 1b). Three successive generations of modules contemporaneously reside in an adult *B. schlosseri* colony at any given time throughout the colony's lifespan. They include the mature filtering zooids and two cohorts of buds, the primary buds that are connected to the zooids and secondary buds (budlets), emerging from the primary buds (zo, pb and sb in Fig. 1b). The differentiation and the lifespan of the modules within a colony are genetically controlled and synchronized, so that all modules of a certain cohort are exactly at the same differentiating state (Milkman, 1967).

The Phoenix-like astogeny in *B. schlosseri* is manifested by week-long repeating cycles of life and death, development and regression (each cycle is called blastogenesis), where in the last 24–36 h of each blastogenic cycle, all mature zooids die in a stepwise, anterior-to-posterior, synchronized wave of massive apoptosis and phagocytosis processes (the takeover stage; Fig. 1b). In concert with this systemic death ('the Phoenix consumed by fire'), all primary buds swiftly progress and transform into functional zooids ('the Phoenix reborn from its own ashes'), while the budlets convert into primary buds and a new generation of up to 4 budlets per zooid are formed (Lauzon et al., 2002; Ballarin et al., 2010). Reproductive processes, such as oogenesis, spermatogenesis and embryogenesis, are also synchronized with the blastogenesis so that ovulated eggs are ready the moment a new cohort of zooids opens their siphons, while tadpole larvae escape the colony just before the onset of the takeover phase.

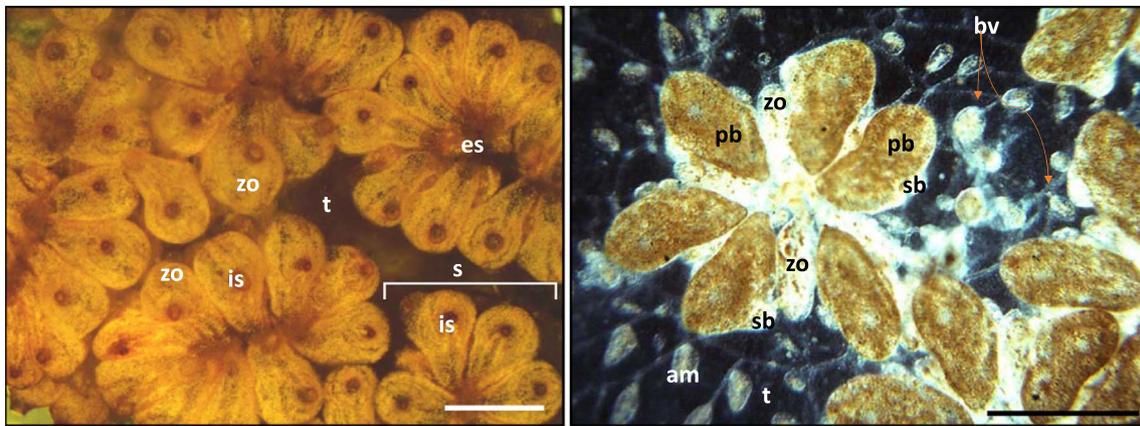
In the present review Manni et al. (2018) discusses further the use of *B. schlosseri* as a model system in various biological features and

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**Fig. 1.** *Botryllus schlosseri* – The Underwater Phoenix-like organism. (a) A general view on an orange colored colony from Nelson city marina, New Zealand. The colony is composed of several to many similar units, each called a zooid (1–3 mm in length), which are embedded within the translucent-gelatinous matrix, the tunic. Zooids within a colony usually form star-shaped clusters (called systems) around common cloacal apertures, the exhalant siphons, while each zooid in the colony bears a private inhalant siphon. (b) A colony from the Monterey marina, CA, USA, at the take-over stage. The old generation of zooids are in the process of morphological resorption and are replaced by the new generation of primary buds. The small secondary buds are also seen. A ramifying network of blood vessels (interconnects all zooids within the colony). This common blood system is embedded within the tunic and bears sausage-like enlargements called vascular ampullae found along the periphery of the colony and are scattered between the systems and near the upper surface of the colony. Abbreviations: am = ampulla; bv = blood vessel; es = exhalant siphon; is = inhalant siphon; pb = primary bud; s = system; sb = secondary bud; t = tunic; zo = zooid. Bars = 2 mm.

biological traits that emerged during the last 60 years, and are associated with the Phoenix-like blastogenesis. These include numerous developmental features, the cross-talk between consecutive blastogenic generations, vascular budding, immunity, the impacts of colonial chimerism, the stem cells and their niches in blastogenesis, genes associated with blastogenesis, in vitro approaches, homeostasis and more. To that we may add the various patterns of senescence and aging processes associated with botryllid ascidians blastogenesis (Rinkevich, 2017; Ben-Hamo et al., 2018). Clearly this Phoenix-like behavior is a central phenomenon in the biology of botryllid ascidians.

Still, there is very little information regarding the development of this Phoenix-like phenomenon, or the ecological and the evolutionary drivers leading to the emergence of blastogenesis. From a pure evolutionary standpoint, it is a waste of energy to invest in the formation of these complex modular colonial structures (each zooid possesses all internal organs of an adult organism, including the heart, stomach, intestine and the endostyle) that operate for just a single week during the longer life span of the organism (colonies in the field survive for several months, at least). These ephemeral zooids are then cast off and consumed, replaced by the next cohort of transient ‘Phoenix bird units’. Even the suggested re-use of energetic resources for bud development through the successful phagocytosis and digestion of the old generation of zooids (Lauzon et al., 2002), cannot compensate for the major loss of energy throughout the recurring blastogenic cycles. Moreover, while the maximal size of each individual zooid within a colony is fixed and constrained, the size of the entire colony may escape any intrinsic size restriction, further illuminating the superfluous energetic investment in this type of colonial growth.

One aspect of botryllid developmental biology worthy of further study is whether the transient ‘Phoenix bird units’ in botryllid ascidians are linked to the disposable soma tenet (sensu Kirkwood and Holliday, 1979), further pointing to the evolutionary trade-off between growth, reproduction and DNA repair maintenance with regards to animal senescence. According to the disposable soma concept, natural selection favors the protection of germ cell immortality at the expense of guarding the somatic senescence of organisms. This leads to reduced repair capacities of somatic cells (as recorded in botryllid ascidians; Svanfeldt et al., 2014) and requires easing of the boundaries between germ/somatic cell lines, including the existence of immortal stem cell lineages (Rosner et al., 2009; both issues detailed in Manni et al., 2018). The different aging processes associated with botryllid ascidians’ blastogenesis (Rinkevich, 2017; Ben-Hamo et al., 2018) as the phe-

nomenon of programmed lifespan (Rinkevich et al., 1992) provide an inkling about the connection between the death/rebirth blastogenic cycles and the disposable soma tenet. Time is ripe therefore for capturing the evolutionary traits of this marine colonial group, the underwater ‘Phoenix birds’.

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