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A multi-locus analysis of phylogenetic relationships within cheilostome bryozoans supports multiple origins of ascophoran frontal shields

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ABSTRACT

Phylogenetic relationships within the bryozoan order Cheilostomata are currently uncertain, with many morphological hypotheses proposed but scarcely tested by independent means of molecular analysis. This research uses DNA sequence data across five loci of both mitochondrial and nuclear origin from 91 species of cheilostome Bryozoa (34 species newly sequenced). This vastly improved the taxonomic coverage and number of loci used in a molecular analysis of this order and allowed a more in-depth look into the evolutionary history of Cheilostomata. Maximum likelihood and Bayesian analyses of individual loci were carried out along with a partitioned multi-locus approach, plus a range of topology tests based on morphological hypotheses. Together, these provide a comprehensive set of phylogenetic analyses of the order Cheilostomata. From these results inferences are made about the evolutionary history of this order and proposed morphological hypotheses are discussed in light of the independent evidence gained from the molecular data.

Infraorder Ascophorina was demonstrated to be non-monophyletic, and there appears to be multiple origins of the ascus and associated structures involved in lophophore extension. This was further supported by the lack of monophyly within each of the four ascophoran grades (acanthostegomorph/spinocystal, hippothoomorph/gymnocystal, umbonulomorph/umbonuloid, lepraliomorph/lepralioid) defined by frontal-shield morphology. *Chorizopora*, currently classified in the ascophoran grade Hippothoomorpha, is phylogenetically distinct from Hippothoidae, providing strong evidence for multiple origins of the gymnocystal frontal shield type. Further evidence is produced to support the morphological hypothesis of multiple umbonuloid origins of lepralioid frontal shields, using a step-wise set of topological hypothesis tests combined with examination of multi-locus phylogenies.

1. Introduction

The phylum Bryozoa consists of aquatic, colonial invertebrates predominantly found in the marine environment. They have a global distribution with roughly 6000 known living species and a further 15,000 fossilised species, currently classified entirely on morphological characteristics. Despite non-skeletal characteristics such as tentacle number, behavioural and reproductive attributes and colony colour being reported to vary among species, these are often poorly described and skeletal characteristics have become the focus for classification (McKinney and Jackson, 1989). This is partly because soft-part characters in cheilostomes are best determined in living or histologically prepared specimens, and are typically poorly preserved in ship-collected material that is bulk-fixed or dried. Also skeletal characteristics are the only reliable ones detectable in fossil specimens and are thus useful for secular clade comparisons (McKinney and Jackson, 1989). Further, there are generally enough skeletal characters in most cheilostome clades to allow cladistic analysis (e.g. Gordon et al., 2002); however determining which morphological characteristics are taxonomically informative is often difficult. Variation in zooidal morphology has been observed in single colonies, which, in some species, may be induced by different environmental conditions or threats of predation, adding to the difficulties of classification via traditional taxonomy (Jackson and Cheetham, 1990; Schwaninger, 1999; Yagunova and Ostrovsky, 2008). In a recent study on the taxonomy of the cheilostome genus *Macropora*, difficulties were encountered from having only a few differences in morphological characteristics compared to species number, making it difficult to obtain a phylogeny that was not susceptible to small changes in the characteristics used (Gordon and Taylor, 2008). This raises questions about how much variation and what kinds of variation are important in

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species identification and inference of evolutionary relationships, and indicates a clear need for analysis independent of morphological characteristics.

One approach is to use DNA sequencing technologies to analyse the molecular characteristics of different species. While analyses of this type would be greatly beneficial in untangling the evolutionary relationships within this phylum, problems are also encountered using these techniques. The cheilostome radiation was rapid, beginning in the mid-Cretaceous and resulting in this order now being the most abundant and diverse of the bryozoan orders (Jablonski et al., 1997). When radiations occur within a short time period, there is little time for lineage sorting to take place before a subsequent divergence. What mutations do occur along the short internodes may then be masked by substitution saturation along the long terminal branches, making it difficult to untangle the true order of divergences leading to the extant lineages (Whitfield and Lockhart, 2007). By combining the strengths from both morphological and molecular analyses, a clearer picture of cheilostome evolution can be uncovered.

Phylum Bryozoa nominally occurs in the late Cambrian (Landing et al. 2010), but the evidence presented is equivocal. It is known with certainty from the earliest Ordovician (several orders). There are three recognised classes – Phylactolaemata, Stenolaemata and Gymnolaemata – with the focus of our study being on the Gymnolaemata (see Fig. 1 for the current levels of classification). Stenolaemata, with all members calcified, is the earliest recorded class of bryozoans and dominated the bryozoan fauna until the Permian–Triassic boundary when almost all Stenolaemata groups were rendered extinct; survivors of four orders continued into the Triassic (Schäfer and Fois, 1987), with only the Cyclostomata persisting to the present-day (Ryland, 1970; McKinney and Jackson, 1989; Fuchs et al., 2009). Gymnolaemates dominate modern bryozoan faunas and mostly comprise marine, skeletized forms. The class is currently generally divided into two orders – the Ctenostomata, comprising uncalcified species, and Cheilostomata, with calcified species. This arrangement has been questioned on both morphological (Silén, 1942; Jebram, 1992) and molecular grounds (Jebram, 1992; Fuchs et al., 2009). The Cheilostomata appears nested in Ctenostomata, with the first cheilostomes appearing in the latest Jurassic, having ctenostome ancestry (Taylor, 1990, 1994; Todd, 2000).

Early cheilostomes were of anascan grade (i.e. lacking an ascus) with a non-calcified frontal membrane (McKinney and Jackson, 1989; Jablonski et al., 1997; Dick et al., 2009). Emerging subsequently, ascophoran bryozoans are defined by the presence of a flexible sac called an ascus which is located beneath the calcareous frontal shield of the zooid and fills and empties with water, acting as a hydrostatic system for extending the lophophore (Dick et al., 2009). The development of the ascus allowed for constraints on the calcification of the frontal wall to be overcome, resulting in the development of (in order of appearance in the fossil record) spinocystal, umbonuloid, gymnocystal and lepralioid frontal shields (Gordon and Voigt, 1996; Dick et al., 2009). These frontal shield types are what define the four grades within Ascophorina.

It is now thought that frontal shields observed within Ascophorina may have arisen on multiple occasions from different ancestors, suggesting that these groups, including Ascophorina itself, are not monophyletic (Gordon and Voigt, 1996; Gordon, 2000). Multiple models of frontal wall or shield evolution in cheilostome bryozoans have been inferred from morphological investigations (Gordon, 2000); however these are yet to be suitably tested by independent means of molecular analysis. One of these models includes the hypothesis that lepralioid frontal shields arose multiple times from umbonuloid-shielded ancestors (Gordon, 2000); umbonuloid and lepralioid shields have a similar frontal appearance in fully differentiated zooids and are often confused on initial examination (Gordon and Voigt, 1996). This hypothesis suggests that reduction and subsequent loss of the umbonuloid-shield component and invagination of the ascus could allow lepralioid shields to be relatively easily derived (Gordon, 2000). There is preliminary molecular evidence supporting this hypothesis, based on the 18S rRNA gene, with the placement of the lepraliomorphs *Celleporina* and *Schizomavella* with the umbonulomorphs *Escharoides* and *Umbonula* (Tsyganov-Bodounov et al., 2009), although further investigation with larger taxon sampling and across a wider range of loci is required to verify these findings.

Molecular analyses investigating the internal relationships within Bryozoa are only in their preliminary stages but are becoming more common (e.g. Fuchs et al., 2009; Tsyganov-Bodounov et al., 2009); however, these are limited in their taxonomic coverage when the age and diversity of Bryozoa are taken into account, and few studies have focussed specifically on diversification within the order Cheilostomata. A study of the Cheilostomata using 16S rRNA gene sequences (Dick et al., 2000) revealed that the group Ascophora (infraorder Ascophorina) appears to be polyphyletic, as suggested by Gordon (2000) from morphological data, with the genus *Celleporella* being placed amongst anascans. Subsequent phylogenies constructed from the 16S rRNA gene for 40 cheilostome species from Australia, China and the United States of America have indicated discrepancies with the morphological classifications at higher taxonomic levels (Hao et al., 2005).

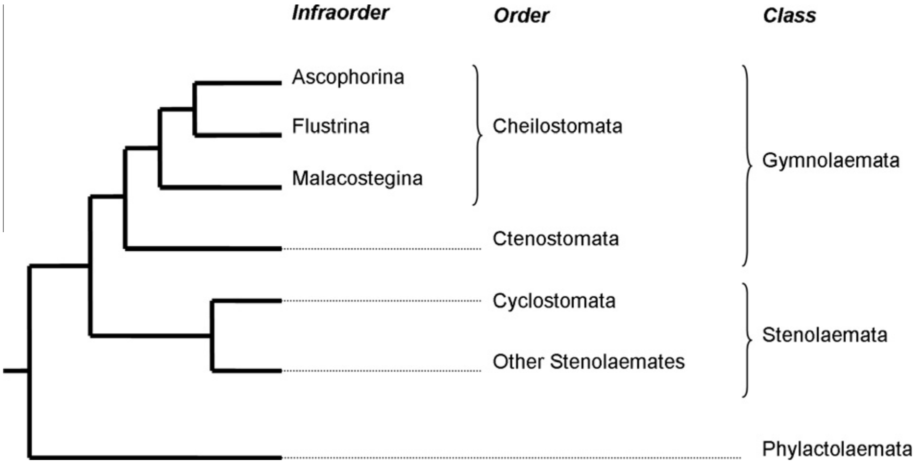

Fig. 1. A simplified representation of the hypothesised relationships within Bryozoa (Hausdorf et al., 2010).