Molecular Phylogenetics and Evolution 81 (2014) 147–158

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny, systematics and morphological evolution of the acorn barnacles (Thoracica: Sessilia: Balanomorpha)

Marcos Pérez-Losada [a,b,c,*], Jens T. Høeg [d], Noa Simon-Blecher [e], Yair Achituv [e], Diana Jones [f], Keith A. Crandall [b,c]

[a] CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Rua Padre Armando Quintas 7, Universidade do Porto, Campus Agrário de Vairão, Vairão 4485-661, Portugal
[b] Computational Biology Institute, George Washington University, Ashburn, VA 20147, USA
[c] Department of Invertebrate Zoology, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA
[d] Marine Biology Section, Department of Biology, University of Copenhagen, Universitetsparken 4, DK-2100 Copenhagen, Denmark
[e] The Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, 52900 Ramat Gan, Israel
[f] Perth Museums & Collections Western Australian Museum, 49 Kew Street, Welshpool, Western Australia 6106, Australia

ARTICLE INFO

Article history:
Received 10 March 2014
Revised 5 August 2014
Accepted 12 September 2014
Available online 27 September 2014

Keywords:
Balanomorpha
Barnacle
DNA
Morphology
Phylogeny
Systematics

ABSTRACT

The Balanomorpha are the largest group of barnacles and rank among the most diverse, commonly encountered and ecologically important marine crustaceans in the world. Paradoxically, despite their relevance and extensive study for over 150 years, their evolutionary relationships are still unresolved. Classical morphological systematics was often based on non-cladistic approaches, while modern phylogenetic studies suffer from severe undersampling of taxa and characters (both molecular and morphological). Here we present a phylogenetic analysis of the familial relationships within the Balanomorpha. We estimate divergence times and examine morphological diversity based on five genes, 156 specimens, 10 fossil calibrations, and six key morphological characters. Two balanomorphan superfamilies, eight families and twelve genera were identified as polyphyletic. Chthamaloids, chionelasmatoid and pachylasmatoids split first from the pedunculated ancestors followed by a clade of tetraclitoids and coronuloids, and most of the balanoids. The Balanomorpha split from the Verrucidae (outgroup) in the Lower Cretaceous (139.6 Mya) with all the main lineages, except Pachylasmatoidea, having emerged by the Paleocene (60.9 Mya). Various degrees of convergence were observed in all the assessed morphological characters except the maxillipeds, which suggests that classical interpretations of balanomorphan morphological evolution need to be revised and reinterpreted.

1. Introduction

Within cirripedes the suborder Balanomorpha, or acorn barnacles, is by far the most species rich and important group in many marine communities. They get their vernacular name from lacking the fleshy stalk of their pedunculated relatives and from being encased in a more or less cone shaped receptacle of shell plates with its base firmly cemented to the substratum (Anderson, 1994). They are best known from their dominating presence in rocky intertidal habitats, although some species also inhabit the deep sea, while others (epibionts) attach to a variety of plants (tropical mangroves), animals and artificial structures. Epibiont barnacles are found on vertebrates (e.g., sea turtles, sea snakes and whales) and invertebrates (e.g., mollusks, gorgonians, crustaceans, sponges and corals). None of these specialized barnacles are true parasites, but it is suspected that they have various deleterious effects on their host, including the survival and growth of threatened mangrove habitats (Satumanatpan and Keough, 1999). Balanomorphans are also known as primary foulers of man-made structures in the sea with enormous economic repercussions to human society, particularly to shipping and cooling systems of power and desalination plants (Schultz et al., 2011).

Acorn barnacles are very diverse in their morphology (Anderson, 1994; Newman, 1987; Newman and Ross, 1976). Structurally, most species are volcano shaped, but the number of wall plates encircling the body can vary (8, 6, 4 plates), and in extreme cases the wall is concrescent with no evidence of separation into individual plates. There is also extensive variation in other hard characters associated with the wall plates, like the basis of the shell, which can be calcareous or membranous, or the interlocking

* Corresponding author at: Computational Biology Institute, George Washington University, Ashburn, VA 20147, USA.
E-mail address: mlosada323@gmail.com (M. Pérez-Losada).

http://dx.doi.org/10.1016/j.ympev.2014.09.013
1055-7903/

between the wall plates and their connection to the opercular plates. Soft structures like the feeding appendages are also diverse in their morphology. Only their reproductive biology, which varies extensively in pedunculated cirripedes, seems rather conservative in the Balanomorpha inasmuch as the overwhelming majority of the species are hermaphrodites (Høeg and Møller, 2006).

The large biological and structural diversity exhibited by the acorn barnacles makes them ideal models in studies of ecology and evolution. For example, the zonation of different acorn barnacle species and the recruitment of attached juveniles from larvae in the plankton are standard research areas in marine ecology (Høeg and Møller, 2006). Their specializations in adult structures, growth and feeding biology, on the other hand, have been the focus of intense research on the evolution of adaptation, including the seminal studies of Charles Darwin (Anderson, 1994; Crisp, 1983; Darwin, 1854, 1855; Newman, 1987; Schram and Høeg, 1995). Tremendous effort and scientific literature have also been devoted to the prevention of barnacle fouling. For example, the cement used in barnacle attachment has been intensively studied both for trying to emulate it technologically (underwater superglue) and for finding a means of preventing fouling (Kamino, 2013). Furthermore, the importance of the balanomorphans in all these areas has made the cyprid (the last larval stage before adulthood) the preferred model in studying settlement factors in marine larvae (Aldred and Clare, 2008; Chen et al., 2011; Kamino, 2013).

Considering the biological and economic importance of acorn barnacles, a robust phylogenetic framework to study barnacle ecology and assess antifouling models is crucial. Similarly, a comprehensive phylogeny of the Balanomorpha would aid our understanding of how and when acorn barnacles evolved to exhibit their current diversity and would guide the development of a robust taxonomy and classification based on evolutionary relatedness. Unfortunately, the relationships among the main balanomorphan groups are still not well understood and their taxonomy has not been revised in nearly 40 years (Newman and Ross, 1976). Historically, morphology-based systematic studies have relied on non-cladistic approaches where taxa were not defined in terms of apomorphies and character evolution was inferred from general ontogenetic patterns and fossil series (Buckeridge, 1995; Newman, 1987, 1996; Newman and Ross, 1976; Newman et al., 1969). This has resulted in para- and polyphyletic assemblages within the Balanomorpha, as identified in previous phylogenetic analysis (Pérez-Losada et al., 2008, 2012). The characters used in classical studies (mostly hard parts) are undoubtedly important, but to adequately resolve thoracican and in particular balanomorphan relationships, they must be re-evaluated and formally coded (e.g., Glenner et al., 1995), while new ones must be developed (e.g., Pitombo, 1999, 2004). Molecular characters, on the other hand, are straightforward and have greatly advanced thoracican systematics, providing consistent results across multiple studies (Linse et al., 2013; Pérez-Losada et al., 2008, 2004; Rees et al., 2014). But for the Balanomorpha, molecular phylogenies have until now either been limited on their taxonomic coverage of the suborder (Linse et al., 2013; Pérez-Losada et al., 2008, 2004; Rees et al., 2014), or confined to balanomorphan subgroups such as the coral barnacles (Malay and Michonneau, 2014; Simon-Blecher et al., 2007; Tsang et al., 2014), coronuloids (Hayashi et al., 2013) or chathamaloids (Fisher et al., 2004; Pérez-Losada et al., 2012; Wares et al., 2009). Consequently, a comprehensive and robust hypothesis of the evolutionary relationships of the Balanomorpha and estimates of divergence times for major clades within the suborder is still missing.

Here we present an extensive phylogenetic analysis of the Balanomorpha based on five genetic loci and 156 specimens representing all of the twelve extant families and nine outgroups (*Lithotrya* and Verrucidae). We then combined our phylogeny with fossil and morphological information to estimate divergence times across the Balanomorpha and reconstruct the evolutionary history of some structurally and ecologically important characters. Additionally, we discuss the implications of our results for interpreting barnacle morphological evolution.

2. Methods

2.1. Molecular analysis

Newman and Ross (1976) recognized three superfamilies within the Balanomorpha, viz., the Chthamaloidea, the Balanomorphoidea (Bathylasmatidae + Tetraclitidae + Coronulidae), and the Balanoidea. But Newman (1996) split the two former resulting in six superfamilies, viz., the Chionelasmatoidea, Pachylasmatoidea, Chthamaloidea, Coronuloidea, Tetraclitoidea, and Balanoidea. These are the ones adopted here and also in Martin and Davis (2001) and, with minor differences, in the Worms Register of Marine Species (2014). Our sampling included 147 taxa representing at least 124 species from all of the six balanomorphan superfamilies and their twelve described families. Additionally, we used two pedunculates of the genus *Lithotrya* and seven verrucids as the outgroup (Supplementary Table 1 and Fig. 1). Our outgroup choice is supported by both molecular and morphological evidence (Pérez-Losada et al., 2008, 2004). Specimens were preserved in 70% EtOH and are housed at the Smithsonian National Museum of Natural History, the Zoological Museum of the Hebrew University of Jerusalem, and the Mina and Everard Goodman Faculty of Life Sciences (Bar Ilan University). Barnacle DNA extraction, amplification, and sequencing were performed as described in Pérez-Losada et al. (2004). The 18S rRNA (1822 bp), 28S rRNA (1742 bp), 12S (345 bp) and 16S (527 bp) genes, and the COI (670 bp) gene were sequenced using primers in Pérez-Losada et al. (2004) and Folmer et al. (1994), respectively. We generated 155 new sequences (GenBank Accession numbers: KM217412 to KM217565).

2.2. Phylogenetic analyses

Nucleotide sequences from each gene region were aligned using MAFFT v6 (Katoh, 2008) under the global (G-INS-i) algorithm and default settings. Phylogenetic congruence among gene regions was assessed using Wiens' (1998) protocol. No areas of strongly supported incongruence were observed among gene trees. All gene regions were analyzed as separate partitions (COI was subdivided into 1st + 2nd and 3rd codon positions) under the best-fit model of evolution selected by JModelTest v1.0.1 (Posada, 2009). The general time reversible model of evolution with proportion of invariable sites and gamma distribution was selected for each data partition (GTR + G + I). Maximum likelihood analysis of the concatenated partitions was performed in RAxML v7.2.0 (Stamatakis et al., 2008) using 1000 searches and 100 runs. Clade support was assessed using the non-parametric bootstrap procedure with 5000 bootstrap replicates run on the CIPRES Science Gateway portal (Miller et al., 2010). A likelihood topological test was conducted using the Shimodaira and Hasegawa (1999) test as implemented in RAxML.

We also performed a Bayesian–Markov chain Monte Carlo (BMCMC) analysis of the concatenated partitions in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Three independent BMCMC analyses were run in CIPRES with each consisting of four chains. Each Markov chain was started from a random tree and run for 10^7 cycles, sampling every 1000th generation. Model parameters were unlinked and treated as unknown variables with uniform default priors and they were estimated as part of the analysis. Convergence and mixing were monitored using Tracer v1.5