SYMPOSIUM

Deep Phylogeny and Character Evolution in Thecostraca (Crustacea: Maxillopoda)

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Synopsis The thecostracans include the Facetotecta, Ascothoracida, and Cirripedia and show great diversity in both morphology and biology. This makes them ideal models for studying evolutionary adaptations of the larval and adult body-plan, lifestyle, and reproduction. Surprisingly, despite all the work published since Darwin’s seminal monographs, few studies have tested evolutionary hypotheses about Thecostraca within a phylogenetic context. In this review, we combine a Bayesian phylogenetic method and multilocus sequence data to reconstruct the evolutionary history of 12 key thecostracan phenotypic traits associated with their lifecycle, larval biology, reproduction, and adult morphology. Our analyses show that thecostracan biological diversity resulted both from unique innovations and from events of convergence. This provides an opportunity to reevaluate previous classifications of the Thecostraca and the theories relating to the origin and diversification of this taxon.

Introduction

The Thecostraca (Maxillopoda) rank among the most commonly encountered marine crustaceans in the world. They deviate from almost all other Crustacea in that only the larvae are free-living, while the adults are permanently sessile as either suspension feeders or as various types of parasites. Sessile life starts from a cypridoid larva that follows after the naupliar stage and has natatory thoracopods and prehensile antennules that it uses for attachment (Grygier 1987a; Høeg et al. 2004). The Thecostraca sensu Grygier (1987a) form a monophyletic group containing Facetotecta, Ascothoracida, and Cirripedia (Martin and Davis 2001). The Facetotecta (<ca. 10 species, but many undescribed) are one of the most enigmatic groups in all Crustacea. They occur as y-larvae in marine plankton throughout the world, but the adult stage has never been found in the wild (Grygier 1996). Glenner et al. (2008) was able to induce the y-cyprids to metamorphose, in vitro, into a slug-shaped stage (ypsigon) very similar to the endoparasitic stage found in the Rhizocephala (vermigon), which strongly supports previous suspicions that the adult facetotectans are endoparasites. The Ascothoracida represent a relatively small group of approximately 90 species which are ecto- or endo-parasites in either cnidian (Anthozoa) or echinoderm hosts. The highly diversified Cirripedia are comprised of the boring Acrothoracica (approximately 80 species), the parasitic Rhizocephala (approximately 250 species), and the stalked and sessile Thoracica (approximately 1000 species).

The Thecostraca are highly variable in morphology and biology (Høeg and Møller 2006; Ruppert et al. 2003), which renders them prime models for studying evolutionary adaptations both in morphology and in reproductive systems (Charnov 1987). In fact, their specializations in lifecycle, adult structures, growth, feeding biology, and sexual strategies prompted Darwin first (Darwin 1851, 1852, 1854, 1855) and many others later (Crisp 1983; Grygier 1987a, b; Newman 1987; Anderson 1994; Høeg 1995; Schram and Høeg 1995) to study cirripedes,
resulting in one of the first “model organisms” of evolutionary adaptation. Accounts of barnacles evolution, however, have surprisingly lagged behind theoretical developments elsewhere in biology (Schram and Høeg 1995) and were until recently still presented as “bubble diagrams” or “ancestor-descendant” relationships (Newman 1987; Anderson 1994). Only a few modern studies were framed as testable hypotheses within a rigid phylogenetic framework (Høeg 1992, 1995; Høeg and Kolbasov 2002; Pérez-Losada et al. 2008; Pérez-Losada et al. 2009; Yusa et al. 2011).

Phylogenetic analysis of molecular data is a powerful tool that allows us not only to reconstruct the evolutionary history of taxa, but also that of traits of interest for characterizing those taxa (e.g., morphological, behavioral, ecological, and reproductive). Traditional methods of character inference (e.g., maximum parsimony and maximum likelihood) rely on implicit or explicit mapping of those characters onto a single phylogenetic tree, thereby, ignoring alternative reconstructions of the same character (mapping uncertainty) and alternative phylogenies (phylogenetic uncertainty; Ronquist 2004; Drummond et al. 2006). Ignoring either of these sources of error is potentially misleading. Bayesian phylogenetic and character inference, however, can handle both types of uncertainties through the estimation of posterior probabilities for both alternative reconstructions and phylogenetic trees. Here, we apply such a Bayesian approach to multilocus sequences previously generated by our group to explore the evolution of 12 key thecostracan phenotypic characters. Phylogenetic trees and ancestral states with their posterior probabilities were estimated for each character using the Bayesian approach implemented in BEAST v1.7 (beta version; Drummond and Rambaut 2007). All three gene regions were analyzed separately using the GTR+Γ+I model of nucleotide substitution (Tavaré 1984) and the relaxed lognormal model of rate of substitution (Drummond et al. 2006). A symmetric substitution model and an exponential prior (mean = 1) of substitution rate were implemented for each phenotypic character (Lemey et al. 2009). Other models and priors were tested, but no significant differences in character-state posterior probabilities were observed. A Yule speciation prior was used for the tree prior as recommended by the authors. Two independent runs 2 × 10⁷ generations long were performed and then combined in LogCombiner v1.6.1 (part of the BEAST package). Convergence and mixing was evaluated in Tracer v1.5 (http://tree.bio.ed.ac.uk/software/). Phenotypic characters were annotated in TreeAnnotator v1.6.1 (part of the BEAST package) and visualized in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/).

**Materials and methods**

**Thecostracan phylogeny and character evolution**

To study the evolution of thecostracan phenotypic features, we used the most comprehensive and well-supported phylogenetic hypothesis of their relationships published to date (Pérez-Losada et al. 2009). That phylogeny includes 79 species from the three infraclasses of Thecostraca, the Facetotecta (seven species), Ascothoracida (five spp.), and Cirripedia (67 spp.), the last represented by three superorders, the Acrothoracica (three spp.), Rhizocephala (25 spp.) and Thoracica (39 spp.). Twelve species of Malacostraca and 10 of Copepoda were used as out-group taxa. Evolutionary relationships were estimated using three nuclear genes [18S rRNA (2305 bp), 28S rRNA (2402 bp), and histone H3 (328 bp)] and 41 larval characters, which were analyzed separately or combined using maximum parsimony, maximum likelihood (ML), and Bayesian approaches of phylogenetic inference. Both DNA ML and DNA-morphological Bayesian methods produced the same basic tree. In this new study, we used the DNA data set aligned by Pérez-Losada et al. (2009) to explore the evolution of 12 key thecostracan phenotypic characters. Phylogenetic trees and ancestral states with their posterior probabilities were estimated for each character using the Bayesian approach implemented in BEAST v1.7 (beta version; Drummond and Rambaut 2007). All three gene regions were analyzed separately using the GTR+Γ+I model of nucleotide substitution (Tavaré 1984) and the relaxed lognormal model of rate of substitution (Drummond et al. 2006). A symmetric substitution model and an exponential prior (mean = 1) of substitution rate were implemented for each phenotypic character (Lemey et al. 2009). Other models and priors were tested, but no significant differences in character-state posterior probabilities were observed. A Yule speciation prior was used for the tree prior as recommended by the authors. Two independent runs 2 × 10⁷ generations long were performed and then combined in LogCombiner v1.6.1 (part of the BEAST package). Convergence and mixing was evaluated in Tracer v1.5 (http://tree.bio.ed.ac.uk/software/). Phenotypic characters were annotated in TreeAnnotator v1.6.1 (part of the BEAST package) and visualized in FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/).

**Thecostracan phenotypic characters**

A large list of characters were used by Pérez-Losada et al. (2008, 2009) and Høeg et al. (2009b) to study thecostracan evolutionary relationships. Here, we present a subset of those, adapted to illustrate major facets of thecostracan evolution with respect to lifestyle, larval biology, reproduction, and adult morphology (Table 1). Some scorings are based on the authors’ unpublished database of thecostracan larval morphology.

**Lifestyle and metamorphosis**

1. **Advanced metamorphosis: presence versus absence**

In some Thecostraca, the larva passes through a very advanced metamorphosis after attachment,
Table 1 Morphological character matrix for the Thecosatraca, Malacostraca, and Copepoda.

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Table 1 Continued

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(continued)
involving the formation of an extremely simplified cuticle-covered stage, which is motile, but unsegmented and lacks appendages, gut, and sensory organs. In the Rhizocephala, this stage (vermigon) is known to initiate an endoparasitic phase and the same is strongly suspected for the very similar stage (ypsigon) in the Facetotecta. The remaining Therostraca either have almost no metamorphosis (some Ascothoracica) or the stages involved in metamorphosis, even if rather profound, retain segmentation, appendages, and sensory organs throughout the process (Hoeg et al. 2012).

Larval biology

2. c-cypris larva: presence versus absence

All thecostracan species terminate larval development as a cypridoid larva that attaches and metamorphoses into the juvenile stage. The true cypris larva (c-cypris) coded here is characterized by a

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Character 1: advanced metamorphosis (adv = advanced/not adv = not advanced); 2: c-cyprid (cyp = present/nocyp = absent); 3: abdomen (abd = present/noab = absent); 4: frontal horns or glands (horn = present/nohorn = absent); 5: compound eyes (ceyes = present/noceye = absent); 6: free male sex (male = present/nomale = absent); 7: female or hermaphrodite (fem = female/herm = hermaphrodite); 8: dwarf male (dwm = dwarf male/nodwm = no dwarf male); 9: plate number (0, 4, 5, 6, 8, >8); 10: adductor muscle (pre = preoral/post = postoral); 11: peduncle (ped = present/noped = absent); 12: symmetry of shell plates (sym = symmetrical/asym = asymmetrical).
multitude of additional specializations, as listed by Høeg et al. (2009a, 2004).

3. The abdomen of cypridoid larva: presence versus absence

In cypridoid larvae, the abdomen can be well developed and about as wide as the thorax, much narrower and rudimentary, or absent altogether. We code the abdomen as present, whether it is well developed or only rudimentary (Kolbasov and Høeg 2007).

4. Frontal horns or glands in larva: presence versus absence

Frontal horns are complex glandular and sensory structures that project antero-laterally from the naupliar head shield (Semmler et al. 2009; Walker 1992). The glands and pores can be retained in the ensuing cypridoid larva. Coding for the presence of these frontal glands in either of the larval phases allows us to score for this character also in species that lack nauplii and hatch as cyprids.

5. Compound eye: presence versus absence

This concerns the presence of compound eyes at any stage of the lifecycle, whether larva or adult. In most major thecostracan taxa, the cypridoid larva carries a pair of image-forming compound eyes located within the mantle cavity near the base of the antennules. The compound eyes are lost during metamorphosis in all Cirripedia, but they may be retained and perhaps functional in at least some adult Ascothoracica. We code as “present” also when the compound eyes are present in a reduced state, as in most Ascothoracica, and “absent” only if there is no trace of these organs, such as in most rhizocephalan and some thoracican cyprids.

Reproductive biology

The Thecostraca display a variety of sexual systems coded here as characters 6–8. Thecostracans can have pure hermaphroditism, dieocy (males and females), or androdioecy (males and hermaphrodites). When present, males can be either of about similar size as their female partners (as in most out-group taxa), or they can be much smaller and permanently attached to a much larger partner, which can be either a female (dieocy) or a hermaphrodite (androdioecy). Instead of coding the sexual system as a triple state character (hermaphroditism/androdioecy/dieocy), we score it as three separate characters. This allows us to better trace the evolution of the male and female functions.

6. Separate male sex: presence versus absence

7. Hermaphroditism: presence of hermaphrodite versus presence of female

8. Dwarf male: presence versus absence

9. Plate number: 0 (naked), 4, 5, 6, 8, >8

Here, we have greatly simplified these characters into merely scoring the number of shell plates to test the hypothesis of a gradual increase during cirripede evolution.

10. Adductor muscle: postoral versus preoral

Crustaceans with a more-or-less bivalved carapace (head shield) can have an adductor muscle that is situated either postorally or preorally. In cypridoid larvae, the adductor muscle always has a postoral position, but in adult Thecostraca, it can be either postoral or have moved to a preoral position (Klepář 1985; Glenner and Høeg 1998).

11. Peduncle: presence versus absence

We do not score this character for the highly modified Rhizocephala, although the possibility of a homology between the “stalk” of their external parasitic body and the peduncle in the Thoracica does exists (Glenner and Høeg 2002).

12. Shell plate asymmetry: asymmetry versus symmetry

This involves an asymmetric disposition of shell plates as seen in the Verrucidae (Verruca, Metaverruca, Rostratoverruca) and Neoverrucidae (Neoverruca).

Results and discussion

The BEAST Bayesian phylogeny was very similar in topology and support to the MrBayes Bayesian tree in Pérez-Losada et al. (2009). In both analyses, all deep nodes were supported by posterior probabilities ≥0.95. Differences were found only in the less-supported clades. We refer the reader to the phylogenetic trees in Pérez-Losada et al. (2009) for a more detailed view of thecostracan evolutionary relationships.

Character evolution

Applying a Bayesian approach of phylogenetic and character inference, in the next sections, we discuss
the evolution of 12 thecostracan characters related to lifestyle, larval development, reproductive biology, and adult morphology.

**Lifestyle (Character 1)**

*Metamorphosis and lifestyle* (Fig. 1-1): Parasitism occurs frequently both in the out-groups (Copepoda) and within the Thecostraca (Rohde 2005), but a very remarkable similarity exists between the Facetotecta and Rhizocephala, in which the attached cypridoid larva metamorphoses into a highly reduced stage, called vermigon in the rhizocephalans and ypsigon in the facetotectans (Pérez-Losada et al. 2009). In rhizocephalans, the vermigon is injected into a crustacean host and initiates the endoparasitic part of the lifecycle. It is strongly suspected that the ypsigon also

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**Fig. 1** The evolution of 12 thecostracan phenotypic characters (Fig. 1-1 to 1-12). Character-state posterior probabilities (P) are indicated for nodes of interest and nodes with \( P < 0.95 \) for both states. All the other nodes showed \( P \geq 0.95 \) for the state indicated by the color of its branches. For the character “plate number” only \( P \) for the most likely state is shown.
initiates an endoparasitic phase in the facetotectan lifecycle. These highly simplified, unsegmented, and vermiform instars (Glenner 2001; Glenner et al. 2000, 2008) are unique within Crustacea, which a priori suggests a close relationship between the Facetotecta and the Rhizocephala. Since our analysis failed to find such a relation, we must conclude that the ypsi- gon and the vermigon evolved by a remarkable process of convergence (Pérez-Losada et al. 2009). This underscores both the evolutionary flexibility inherent in thecostracan development and the degree to which this taxon can become specialized when evolving into advanced parasitism.

Larval development (Characters 2–5)

Cypris larva (Fig. 1-2): All Thecostraca have a cypri- doid larva; it is called y-cyprid in the Facetotecta and a-cyprid in the Ascothoracida. The highly specialized c-cypris larva (or simply cyprid) is an autapomorphy for the Cirripedia, in which it occurs in all species (Hoeg et al. 2004). The cyprid first disperses by swimming. It then locates a settlement site by...
walking over the substratum on its specialized antennules until it finally cements itself and initiates metamorphosis (Walker 1992; Lagersson and Høeg 2002; Maruzzo et al. 2011). The cyprid (c-cyprid) is remarkably similar throughout all cirripedes (Høeg and Møller 2006) and probably contributed significantly to the successful radiation into numerous habitats and sessile lifestyles, spanning from the deep sea to the intertidal and from extreme parasitism to suspension feeding.

Abdomen in the cypris larva (Fig. 1-3): The cyprid (c-cyprid) is characterized by at best having a highly reduced abdomen, opposed to a well-developed one in a-cyprids (Ascothoracidace) and y-cyprids (Faceto-tecta). Within the Cirripedia, Kolbasov and Høeg (2007) argued that the sister-group relationship between the Rhizocephala and the Thoracica, first argued from molecular evidence alone (Spears et al. 1994; Pérez-Losada et al. 2002) receives support by the complete lack of an abdomen in their cyprids,
whereas a reduced one is present in the Acrothoracica. Our analysis is based on a much wider taxon sample and shows that the picture is less clear. The cyprids also have a reduced abdomen in several pedunculated thoracicans, although this body part is completely absent in the basally situated genus *Ibla* and in all Balanomorpha and Rhizocephala. Such convergent loss is probably to be expected in structures already under reduction.

*Frontal horns and glands in larva* (Fig. 1-4): The presence of frontal horns and their associated glands is an autapomorphy for the Cirripedia. All cirripede nauplii possess the horns/glands and they occur nowhere else in the Thecostraca or in Crustacea in general. For species that hatch as cyprids, the glands are absent only in the highly advanced rhizocephalan Akentrogonida. This near omnipresence of frontal horns/glands in cirripede nauplii and/or cyprids suggests that they play a very critical, but still unknown, role (Walker 1992; Høeg and Møller 2006).

*Compound eye* (Fig. 1-5): In our analysis, the compound eyes were lost in a few thoracicans, most likely because these species are inhabitants of deep water. Much more surprisingly, the eyes were also lost at the base of the parasitic Rhizocephala, but then reappeared twice deep within that taxon. As for a similar situation in Ostracoda (Oakley 2003; Oakley et al. 2007), a more detailed analysis of eye morphology and evolution is needed for deciding whether eyes actually re-evolved in these advanced parasites or were lost multiple times during rhizocephalan evolution. Interestingly, the cypridoid larvae of all Facetotecta (strongly suspected to be parasitic) and some Ascothoracica (known to be parasitic) have retained compound eyes, although for the ascothoracidans in a reduced state. From the presence of compound eyes in these two taxa and in some advanced Rhizocephala, we conclude that there is no clear correlation between loss of these visual organs in the larvae and a parasitic mode of life in the adults.

Reproductive biology (Characters 6–8)

Since Darwin (1851, 1854) cirripeds have been models for studying the evolution of reproductive systems (Ghiselin 1969a, b; Charnov 1982, 1987; Høeg 1995; Yamaguchi et al. 2008; Urano et al. 2009). There are no thecostracan species that consist exclusively of parthenogenetic females. In the present coding scheme, dioecy (separate sexes) is therefore equivalent with the presence of pure females (Fig. 1-7), since these always occur together with a separate male sex (Fig. 1-6). Our analysis indicates that dioecy (=Fig. 1-7: females) is the ancestral condition for the Thecostraca, and this sexual system has been retained in the Acrothoracica, Rhizocephala, and in all ascothoracidan species analyzed here. Hermaphroditism seems to have evolved only twice within the Thecostraca. It appears (Fig. 1-7) in the stem line of the Thoracica and is present in most species of that taxon. Hermaphroditism also occurs in the ascothoracidan family Petrarcidae. No petrarcid species were available for our analysis, but according to Grygier (1987b, 1996), their hermaphroditic condition also evolved secondarily from dioecy. It illustrates the complexity of cirripede sexual biology that a separate male sex (Fig. 1-6) reappeared convergently several times in the Thoracica from hermaphroditic ancestors, either as dioecy (e.g., *Ornatoscalpellum, Trianguloscalpellum*) or as androdioecy (e.g., *Scalpellum, Paralepas, and Calantica*). The ecological background for this was recently analyzed by mathematical modeling (Yusa et al. 2011). Males have even re-evolved from hermaphroditism in a few balanomorphan species, although none is part of our analysis (Henry and McLaughlin 1965; Gomez 1975; Dayton et al. 1982; Foster 1988).

Compared to their female or hermaphroditic partners, thecostracan males are always very small (Darwin, 1851, 1854; Klepal 1987; Buhl-Mortensen and Høeg 2006), but this dwarf male condition did not have a common evolutionary origin. In the Rhizocephala, the highly specialized dwarf males live cryptically within the tissue of the female and represent an apomorphy for the taxon (Høeg 1995). In the Thoracica, the situation is much more complex (Yusa et al. 2011), as dwarf males seem to have evolved convergently several times (Fig. 1-8). Finally, we note that a study of the still unknown adults of the Facetotecta could modify our conclusions on thecostracan sexual evolution.

Morphology of adults (Characters 9–12)

*Plate number* (Fig. 1-9): The number of mineralized shell plates has always been the primary character set when analyzing evolution in the Cirripedia. There are no shell plates in the Acrothoracica and Rhizocephala, and our phylogeny illustrates this as an ancestral condition. For the Thoracica, the most popular hypothesis posits that the ancestral condition was four plates (as in *Ibla*) or five (as in...
Lepas), from whence their number increased gradually through evolution (Newman 1987, 1996; Glenner et al. 1995). Although character 9 is an extreme simplification, our analysis agrees with Pérez-Losada et al. (2008) in finding no support for any such progression in plate number. Instead we find that the ancestral state for the Thoracica is more than eight plates. Five-plated thoracicans such as Lepas are not basally situated, but seem to have reached this number through secondary loss. Similarly, eight-plated thoracicans (e.g., Neolepas) are not an evolutionary step following five plates, but again evolved from multiplated forms. The total absence of shell plates in Paralepas is also derived. The four-plated state in Ibla appears as an apomorphy for this genus. We surmise that shell plate characters need to be reanalyzed and critically recoded before their information content can be evaluated. This is not least true for the complex and critical transition between pedunculated and balanomorphan barnacles, since shell plate homologies are exceedingly difficult to establish between these two groups (Glenner et al. 1995) due to processes of profound morphological modification, fusion of plates and secondary loss.

Adductor muscle in adults (Fig. 1-10): A preoral position of the adductor muscle is an apomorphy for all Thoracica except Ibla, and joins several other characters supporting a basal position of this genus (Glenner and Høeg 1998; Høeg et al. 2009b). Recently it was even argued from embryological evidence that Ibla is the sister-group to the Rhizocephala (Scholtz et al. 2009), but our extensive molecular-based analyses found no support for this hypothesis. There is no obvious correlation between the adductor muscle and shell plate character. The adductor is present and postoral in the primarily naked Ascothoracida and Acrothoracica. It also present in secondarily naked thoracicans such as Paralepas, but here in the apomorphic preoral position.

Peduncle (Fig. 1-11) and asymmetry (Fig. 1-12): Absence of a peduncle characterizes both the Balanomorpha and the asymmetric Verrucidae, both normally united as the Sessilia. The hydrothermal vent inhabiting genus Neoverruca has been much in focus when discussing the evolution of the Verrucidae, whose asymmetry is due to the opercular plates on one side having become part of the fixed wall plates. Our analyses and those of Pérez-Losada et al. (2008) show that absence of the peduncle and asymmetry in Neoverruca had no common origin with the Verrucidae but evolved independently from pedunculated and symmetric ancestors. It follows that Neoverruca should not be used as a model for understanding asymmetry in the Verrucidae. This is hardly surprising, since asymmetry in Neoverruca principally involves the lateral plates while, in verrucids, it is the operculum that is asymmetric (Newman and Hessler 1989; Glenner et al. 1995).

Conclusions

Within Thecostraca, the Thoracica and Rhizocephala have achieved major evolutionary success, with hundreds or thousands of species having immense diversity and ecological importance. One explanation could be the protective plate armament in the former and the advanced endoparasitism in the latter, both unique adaptations within the Crustacea. The enigmatic Facetotecta (y-larvae) may yet prove to be a similarly diverse taxon (Grygier 1991; Glenner et al. 2008), while the parasitic Ascothoracida and, within cirripedes, the burrowing Acrothoracica have had only limited success. Here, we show that thecostracan diversity evolved not only from unique innovations, such as the c-cyprid larva and some of its morphological features, but also from multiple events of convergence such as endoparasitism, plate number, and reproductive biology. Our conclusions provide an opportunity to re-evaluate previous classifications and theories relating to the origin and diversification of the Thecostraca.

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References


Darwin C. 1854. A monograph of the sub-class Cirripedia, with figures of all the species. The Balanidae (or sessile cirripedes); the Verrucidae, etc., etc., etc. London: Ray Society.


