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Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): Phylogenetic approach and test of the early-radiation event

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ABSTRACT

Clades that have undergone episodes of rapid cladogenesis are challenging from a phylogenetic point of view. They are generally characterised by short or missing internal branches in phylogenetic trees and by conflicting topologies among individual gene trees. This may be the case of the subfamily Trematominae, a group of marine teleosts of coastal Antarctic waters, which is considered to have passed through a period of rapid diversification. Despite much phylogenetic attention, the relationships among Trematominae species remain unclear. In contrast to previous studies that were mostly based on concatenated datasets of mitochondrial and/or single nuclear loci, we applied various single-locus and multilocus phylogenetic approaches to sequences from 11 loci (eight nuclear) and we also used several methods to assess the hypothesis of a radiation event in Trematominae evolution. Diversification rate analyses support the hypothesis of a period of rapid diversification during Trematominae history and only a few nodes in the hypothetical species tree were consistently resolved with various phylogenetic methods. We detected significant discrepancies among trees from individual genes of these species, most probably resulting from incomplete lineage sorting, suggesting that concatenation of loci is not the most appropriate way to investigate Trematominae species interrelationships. These data also provide information about the possible effects of historic climate changes on the diversification rate of this group of fish.

1. Introduction

Evolutionary radiations, lineages that have undergone a phase of rapid cladogeneses, are often characterised in phylogenetic reconstruction by "soft polytomies" (Maddison, 1989): putative speciation events that occur very rapidly in the past genealogical tree and lead either to short or absent internal branches in the reconstructed phylogenetic trees (i.e. unresolved phylogenetic relationships) or to conflicting topologies among gene trees when using multiple loci. Such topological conflicts among gene trees are generally not supported statistically because the time elapsed since these cladogeneses is longer than the time between any two cladogenetic events and the time during which derived states can be erased by multiple substitutions is greater than the time during which these derived states accumulated. For clusters of recent cladogenetic events the lack of congruence among gene trees can also be due to the failure of individual gene genealogies to coalesce along internal branches leading to incomplete lineage sorting. Phylogenetic studies often attribute a finding of conflicting genealogies and/or a lack of phylogenetic resolution coupled with short internal branches to rapid diversification in the taxon of interest and a high rate of incomplete lineage sorting (e.g. Lopez-Fernandez et al., 2005; Belfiore et al., 2008). However, exact tests for periods of increased rates of diversification are usually absent from such studies.

The Trematominae, a tribe of Notothenioid teleosts from Antarctica widely used as a model of cold adaptation, fall into this category as it has a problematic phylogeny. The species flock is strikingly diverse in morphology and has evolved a variety of ecological forms including species of benthic, pelagic or cryopelagic habits. Ruber and Zardoya (2005) applied a γ statistic (Pybus and Harvey, 2000) to published phylogenetic data and tested for evidences of periods with increased diversification rates in several groups of marine fish. They showed that Trematominae

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phylogenies deviate significantly from rate constancy and found negative γ values that support a scenario of early radiation in Trematominae history.

The Trematominae contains 15 described species in three genera; *Trematomus*, *Pagothenia* and *Cryothenia.* The latter two genera are embedded within the genus *Trematomus* (Sanchez et al., 2007; Kuhn and Near, 2009). Several studies have focused on the phylogeny of Trematominae using morphological (Balushkin, 2000; Klingenberg and Ekau, 1996; Ekau, 1991) and molecular approaches (Ritchie et al., 1996; Near, 2004; Sanchez et al., 2007; Near and Cheng, 2008; Kuhn and Near, 2009). Whereas initial molecular studies focused on one or a few mitochondrial genes that led to many unresolved relationships, the latter three studies increased the resolution power by concatenating the data from several mtDNA loci and one nuclear locus resulting in an almost fully resolved phylogenetic tree (especially Kuhn and Near (2009), which used data derived from 13 of 15 known species). Both morphological and molecular work suggested that pelagic species evolved from a benthic ancestor on at least two occasions. This suggests a high evolutionary versatility in the Trematominae. From morphological data, Balushkin (2000) postulated the existence of a cryopelagic clade (including two species foraging under or within the platelet ice; *T. newnesi* and *P. borchgrevinki*), which was recently corroborated by Kuhn and Near (2009). In contrast, Ritchie et al. (1996) and Sanchez et al. (2007) inferred that *T. scotti* and *T. newnesi* are basal species and concluded that the Trematominae crown-group diversified after their split from the common ancestor. Using standard mtDNA molecular clocks, Ritchie et al. (1996) suggested that average percentage sequence divergence among the crown-group species corresponds to 3.4 Mya, and proposed that the main Trematominae radiation coincided with deglaciation of coastal waters during the Pliocene warming. However, Near (2004) used fossil-based calibration and penalised likelihood to create an ultrametric tree and showed that the Trematominae as a whole are much older (mean estimate = 9.4 Mya), and that the most recent common ancestor of the crown-group (which should include *T. newnesi*; see Kuhn and Near, 2009) is as old as 7.4 Mya.

Despite the apparent resolution of Trematominae relationships in Kuhn and Near (2009), the signal of rapid diversification early in the history of Trematominae suggests that reservations about concatenating the data from unlinked loci apply to this group and one must be aware of possible conflicts among gene trees. Although increasing the number of loci and characters that are put into a single concatenated data set can improve the phylogenetic resolution, it may also lead to false estimates of species trees (Edwards et al., 2007), especially when the lengths of the internal branches are short and ancestral population sizes large. This is because concatenation assumes the same phylogenetic history for all sites in the dataset (Belfiore et al., 2008). Such an assumption can easily be violated especially in the case of incomplete lineage sorting (Takahata, 1989; Felsenstein, 2004; Knowles and Chan, 2008).

The primary aim of this study is to investigate potential phylogenetic conflicts among unlinked loci as well as to reconstruct a Trematominae phylogeny explicitly taking into account the possible effects of stochastic lineage sorting. We achieve this by inspecting conflicts among individual gene trees as well as by applying Bayesian estimation of species trees (BEST; Liu, 2008) that allows for stochastic differences of topology of individual gene trees resulting from lack of gene lineage coalescence between speciation events. BEST is particularly suitable for data where incongruence in the phylogenetic signal of individual genes is expected. It is claimed to perform better and produce fewer artefactual topologies than traditional concatenation (Edwards et al., 2007).

The second aim of the study is to investigate the character of the putative signal of changes in diversification rate in all loci. This is important since the γ statistic applied previously by Ruber and Zardoya, although correctly depicting the general signal of a decreasing diversification rate, does not assess whether this reduction in rate fits to a gradual or an abrupt decrease of speciation tempo. Furthermore, similar γ values may be generated by a simple decrease speciation rate or by a combination of decreasing speciation rate and increasing extinction rates (Rabosky and Lovette, 2008; Quental and Marshall, 2009). We used several methods including non-parametric approaches that allow testing of complex scenarios.

Previous works proposed particular extrinsic factors that could explain the origin of Antarctic *Notothenioidei* in general (rev. in Eastman and Clarke, 1998) and Trematominae in particular (see Ritchie et al. (1996) for hypothesised link with Pliocene warming). Pleistocene climatic oscillations might have also strongly affected the rate of diversification either acting as a diversity pump (Avise, 2000), or inducing mass extinctions, particularly of benthic species (Thatje et al., 2005; Janko et al., 2007). Therefore, our final aim is to compare observed patterns of diversification with the timing of several known extrinsic factors or events to better understand Trematominae radiation.

2. Material and methods

2.1. Taxon sampling

The Trematominae contains two genera with 15 described species; 11 species of the genus *Trematomus* (one missing in our study – *T. tokarevi*), two species of the genus *Pagothenia* (one missing – *P. brachysoma*) and two of *Cryothenia* (one missing – *C. peninsulae*). The 12 species used in this study are listed in Table 1. We used several outgroup species; namely *Chionodraco hamatus*, *Notothenia rossii*, *Patagonotothen tessellata* and *Lepidonotothen squamifrons*.

Table 1
Specimens of Trematominae used for sequencing in this study.

Species	Specimen number	Geographical location
T. scottii	1371 867	Weddell Sea Weddell Sea
T. bernacchii	TNB1-5 CH 629	Terra Nova Bay Cape Hallett
P. borchgrevinki	Psp. B 5 CA 113b	Terre Adelie Cape Armitage
T. nicolai	CR 296 1369 CA436	Cape Roberts Weddell Sea Cape Armitage
T. newnesi	Tn399TA6 Tn401TA2 CA450	Terre Adelie Terre Adelie Cape Armitage
T. hansoni	1216 CH1026	South Georgia Cape Hallett
T. lepidorhinus	1368 565	Weddell Sea Terra Nova Bay
T. eulepidotus	759 1370	Terra Nova Bay Weddell Sea
T. loennbergii	139 426	Terre Adelie Terre Adelie
T. vicarius	1214 256	South Georgia South Georgia
T. pennellii	CH610 CR321	Cape Hallett Cape Roberts
C. amphitreta	USNM 385901	McMurdo