Molecular Phylogenetics and Evolution 68 (2013) 176–184

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

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

Phylogenetic relationship among genera of Polymorphidae (Acanthocephala), inferred from nuclear and mitochondrial gene sequences

Martín García-Varela [a,*], Gerardo Pérez-Ponce de León [a], Francisco J. Aznar [b], Steven A. Nadler [c]

[a] Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Avenida Universidad 3000, Ciudad Universitaria, CP 04510, Distrito Federal, Mexico
[b] Departamento de Biología Animal, Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universitat de Valencia, Burjasot, Valencia, Spain
[c] Department of Entomology and Nematology, University of California, Davis, CA 95616-8668, United States

ARTICLE INFO

Article history:
Received 3 August 2012
Revised 22 March 2013
Accepted 27 March 2013
Available online 6 April 2013

Keywords:
Acanthocephala
Polymorphidae
Ribosomal DNA
Cytochrome *c* oxidase subunit I
Phylogeny

ABSTRACT

Acanthocephalans of the family Polymorphidae Meyer, 1931 are obligate endoparasites with complex life cycles. These worms use vertebrates (marine mammals, fish-eating birds and waterfowl) as definitive hosts and invertebrates (amphipods, decapods and euphausiids) as intermediate hosts to complete their life cycle. Polymorphidae has a wordwide distribution, containing 12 genera, with approximately 127 species. The family is diagnosed by having a spinose trunk, bulbose proboscis, double-walled proboscis receptacle, and usually four to eight tubular cement glands. To conduct a phylogenetic analysis, in the current study sequences of the small (18S) and large-subunit (28S) ribosomal RNA, and cytochrome *c* oxidase subunit 1 (*cox* 1) were generated for 27 taxa representing 10 of 12 genera of Polymorphidae, plus three additional species of acanthocephalans that were used as outgroups. Maximum likelihood (ML), maximum parsimony (MP), and Bayesian analyses were conducted on a combined nuclear rRNA (18S + 28S) data set and on a concatenated dataset of nuclear plus one mitochondrial gene (18S + 28S + *cox* 1). Phylogenetic analyses inferred with the concatenated dataset of three genes support the monophyly of nine genera (*Andracantha*, *Corynosoma*, *Bolbosoma*, *Profilicollis*, *Pseudocorynosoma*, *Southwellina*, *Arhythmorhynchus*, *Hexaglandula* and *Ibirhynchus*). However, the four sampled species of *Polymorphus* were nested within several clades, indicating that these species do not share a common ancestor, requiring further taxonomic revision using phylogenetic systematics, and reexamination of morphological and ecological data. By mapping definitive and intermediate host association onto the resulting cladogram, we observe that aquatic birds were the ancestral definitive hosts for the family with a secondary colonization and diversification to marine mammals. Whereas amphipods were ancestral intermediate hosts and that the association with decapods represent episodes of secondary colonization that arose several times during the evolutionary history of the family. Our results are useful to start testing hypothesis about the evolutionary history of this highly diverse family of acanthocephalans.

1. Introduction

Acanthocephalans of the family Polymorphidae Meyer, 1931 are obligate endoparasites with complex life cycles, that use vertebrates (marine mammals, fish-eating birds, and waterfowl) as definitive hosts and invertebrates (amphipod, decapod and euphausiids) as intermediate hosts to complete their life cycle. The involvement of paratenic hosts (mostly teleost fish) is often necessary to facilitate transmission to appropriate definitive hosts (Schmidt, 1985; Hoberg, 1986; Pichelin et al., 1998; Nickol et al., 1999, 2002; Kennedy, 2006). In some cases, species of Polymorphidae alter the behavior or coloration of their intermediate host (Kennedy, 2006). Currently the family includes 12 genera with approximately 127 species (Schmidt, 1973; Dimitrova and Georgiev, 1994; Nickol et al., 1999, 2002; Aznar et al., 2006; García-Varela et al., 2011). Polymorphidae is diagnosed by having a spinose trunk, bulbose proboscis, double-walled proboscis receptacle, and usually four to eight tubular cement glands (Nickol et al., 1999, 2002; Aznar et al., 2006; García-Varela et al., 2011). Recent molecular studies suggested that Polymorphidae represents one of the most derived clades within the class Palaeacanthocephala (García-Varela and Nadler, 2005, 2006; Verweyen et al., 2011).

Taxonomic study of this group of acanthocephalans based solely on morphological grounds resulted in an unstable classification scheme. For instance, Meyer (1931) described Polymorphidae containing four genera. Later, Petrochenko (1956) subdivided the family into three subfamilies, with 10 genera. The same classification scheme was followed by Yamaguti (1963).

* Corresponding author. Fax: +52 5 5550 0164.
E-mail address: garciav@unam.mx (M. García-Varela).

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2013.03.029

Schmidt (1973) reviewed the family and indicated that only eight genera were valid. Later, Schmidt (1975) erected the genus *Andracantha*, including three species classified previously as members of *Corynosoma* Lühe, 1904. Amin (1992) proposed the designation of *Hexaglandula*Petrochenko, 1950 and *Profilicollis*Meyer, 1931 as junior synonyms of *Polymorphus* Luhë, 1911. Dimitrova and Georgiev (1994) erected the genus *Ardeirhynchus*, which contains a single species from herons in Bulgaria. Nickol et al. (1999) considered ecological and morphological characters to reinstate *Profilicollis* and *Hexaglandula* as valid genera. Aznar et al. (2006) erected *Pseudocorynosoma* Aznar, Pérez-Ponce de León and Raga, 2006, for several species previously included in *Corynosoma*. In 2008, García-Varela and Pérez-Ponce de León confirmed the validity of the genera *Profilicollis* and *Hexaglandula* by using molecular data. Finally, García-Varela et al. (2011) erected the genus *Ibirhynchus* to contain a single species previously assigned to *Southwellina* Witenberg, 1932.

Clearly, the combination of morphological, molecular, and ecological studies has been very useful to resolve taxonomic controversies within the family (see Nickol et al., 2002; Aznar et al., 2006; García-Varela et al., 2011). Unfortunately, published phylogenetic studies have not included sampling representative of the diversity of Polymorphidae. Broader taxonomic sampling is essential to our understanding of the phylogenetic relationships among genera and to properly describe the ecological association of these parasites with their intermediate and definitive hosts. The main objective of the present research was to reconstruct the phylogenetic relationships among the genera of Polymorphidae based on the most comprehensive sampling for the family to date, with 10 of the 12 valid genera, and by using sequences of the near-complete 18S and 28S nuclear rRNA genes, and the mitochondrial gene cytochrome oxidase subunit 1 (*cox* 1). We then use these phylogenetic trees as a framework to discuss the host parasite association in terms of the definitive hosts that are parasitized by polymorphids and to improve our understanding about the evolutionary history of this group of acanthocephalans.

2. Material and methods

2.1. Specimens and DNA isolation

Acanthocephalans used for this study were collected from their naturally infected vertebrate or invertebrate hosts (Table 1). Worms were washed three times in normal saline solution, preserved in absolute ethanol, and stored at 4 °C. Representative acanthocephalan specimens were stained with Mayer's paracarmine, and mounted in Canada balsam for identification. Parasites were identified by conventional morphological criteria following keys by Petrochenko (1958), Yamaguti (1963) and Schmidt (1973) and the original and revised descriptions of the species. Avian definitive hosts were identified using the field guide by Howell and Webb (1995). Voucher specimens were deposited at the Colección Nacional de Helmintos, Instituto de Biología, UNAM, Mexico City, Mexico (Table 1). Birds were deposited in the Colección de Aves Museo de Zoología Alfonso L. Herrera, Facultad de Ciencias, UNAM, Mexico City and Colección Nacional de Aves, Instituto de Biología, UNAM, Mexico City.

A single specimen of each species of parasite was digested overnight at 56 °C in a solution containing 10 mM Tris–HCl (pH 7.6), 20 mM NaCl, 100 mM Na_2 EDTA (pH 8.0), 1% Sarkosyl, and 0.1 mg/ml proteinase K. Following digestion, DNA was extracted from the supernatant using the DNAzol reagent (Molecular Research Center, Cincinnati, Ohio) according to the manufacturer's instructions.

2.2. Amplification and sequencing of DNA

Two regions of nuclear ribosomal RNA (rRNA) were amplified using the polymerase chain reaction (PCR). The near-complete 18S rRNA (~1800 bp) was amplified in 1 fragment using the forward primer 5′-AGATTAAGCCATGCATGCGT and reverse primer 5′-GCAGGTTCACCTACGGAAA. The near-complete 28S rRNA (~2900 bp) was amplified using 2 overlapping PCR fragments of 1400–1500 bp. Primers for LSU amplicon 1 were forward 5′-CAAGTACCGTGAGGGAAAGTTGC and reverse 5′-CTTCTCCAAC(T/G)TCAGTCTTCAA; amplicon 2, forward 5′-CTAAGGAGTGTGTAACAACTCACC and reverse 5′-CTTCGCAATGATAGGAAGAGCC (García-Varela and Nadler, 2005). A partial (661 bp) sequence of mitochondrial cytochrome c oxidase subunit 1 (*cox* 1) was amplified using the forward primer 5′-AGTTCTAATCATAA(R)GATAT(Y)GG and reverse 5′-TAAACTTCAGGGTGACCAAAAAATCA (Folmer et al., 1994).

PCR reactions (25 μl) consisted of 10 μM of each primer, 2.5 μl of 10X buffer, 2 mM $MgCl_2$, and 1 U of Taq DNA polymerase (Platinum Taq, Invitrogen Corporation, São Paulo, Brazil). PCR cycling parameters for rDNA amplifications included denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50–58 °C (optimized for each rRNA amplification) for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 7 min. PCR cycling conditions for the *cox* 1 amplifications included denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, annealing at 40 °C for 1 min, and extension at 72 °C for 1 min, followed by a post-amplification incubation at 72 °C for 5 min.

Each PCR product was purified using Millipore columns (Amicon, Billerica, Massachusetts) and cloned by ligation into pGEM-T vector (Promega, Madison, Wisconsin) and used to transform competent *Escherichia coli* (JM109). Positive clones were identified by blue/white selection, and clone (insert) size was confirmed by PCR of DNA extracts prepared from bacterial colonies. Liquid cultures for minipreps were grown in Luria broth containing 50 μg/ml of ampicillin. Plasmids for DNA sequencing were prepared using commercial miniprep kits (Qiaprep, Qiagen, Valencia, California). At least two plasmids obtained from each species were sequenced for both DNA strands using universal (vector) and internal primers. Sequencing reactions were performed using ABI Big Dye (Applied Biosystems, Boston, Massachusetts) terminator sequencing chemistry, and reaction products were separated and detected using an ABI 3730 capillary DNA sequencer. Contigs were assembled and base-calling differences resolved using Codoncode Aligner version 3.5.4 (Codoncode Corporation, Dedham, Massachusetts).

2.3. Alignments and phylogenetic analyses

The rRNA 18S and 28S data sets were aligned separately using ProAlign version 0.5 (Loytynoja and Milinkovitch, 2003). For each alignment, a ProAlign guide tree was constructed using corrected (for multiple hits) pairwise distances; this guide tree was used to estimate the hidden Markov model parameters (δ and ε) for progressive multiple alignment. Program (Java) memory and bandwidth were increased as required to complete the alignment. The minimum posterior probability of sites was used as the criterion for detecting and removing unreliably aligned sequence. To reduce the likelihood of excluding correctly aligned sites, the filter threshold was set to 60% minimum posterior probability, a value intermediate between the threshold of posterior probabilities for correctly versus incorrectly aligned sites in simulation results (Loytynoja and Milinkovitch, 2003). For the 18S sequences, using ProAlign to detect and remove unreliably aligned sites by their posterior probabilities excluded 227 of 1762 sites. For the 28S dataset, 791 of 3055 sites were excluded based on posterior probability filtering. Thus, these combined rRNA datasets included 3799