ACADEMIC PRESS

Molecular Phylogenetics and Evolution 23 (2002) 288–292

MOLECULAR PHYLOGENETICS AND EVOLUTION

www.academicpress.com

Short Communication

Phylogenetic analysis based on 18S ribosomal RNA gene sequences supports the existence of class polyacanthocephala (acanthocephala)

Martín García-Varela,[a] Michael P. Cummings,[b] Gerardo Pérez-Ponce de León,[c] Scott L. Gardner,[d] and Juan P. Laclette[a,*]

[a] *Department of Immunology, Instituto de Investigaciones Biomédicas México, D.F., México*
[b] *Department of Zoology, Instituto de Biología, UNAM, 04510 México, D.F., México*
[c] *The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543-1015, USA*
[d] *Harold W. Manter Laboratory of Parasitology, W-529 Nebraska Hall, University of Nebraska-Lincoln, Lincoln, NE 68588-0514, USA*

Received 19 June 2001; received in revised form 5 December 2001

Abstract

Members of phylum Acanthocephala are parasites of vertebrates and arthropods and are distributed worldwide. The phylum has traditionally been divided into three classes, Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala; a fourth class, Polyacanthocephala, has been recently proposed. However, erection of this new class, based on morphological characters, has been controversial. We sequenced the near complete 18S rRNA gene of *Polyacanthorhynchus caballeroi* (Polyacanthocephala) and *Rhadinorhynchus* sp. (Palaeacanthocephala); these sequences were aligned with another 21 sequences of acanthocephalans representing the three widely recognized classes of the phylum and with 16 sequences from outgroup taxa. Phylogenetic relationships inferred by maximum-likelihood and maximum-parsimony analyses showed Archiacanthocephala as the most basal group within the phylum, whereas classes Polyacanthocephala + Eoacanthocephala formed a monophyletic clade, with Palaeacanthocephala as its sister group. These results are consistent with the view of Polyacanthocephala representing an independent class within Acanthocephala.

Keywords: Acanthocephala; 18S rRNA; Polyacanthocephala; *Polyacanthorhynchus caballeroi*

1. Introduction

The phylum Acanthocephala consists of endoparasites of arthropods and vertebrates, commonly referred to as thorny-headed worms, included among the most basal tripoblasts (Brusca and Brusca, 1990; Clark, 1979; Hyman, 1951; Marcus, 1958; Wallace et al., 1996; Winnepenninckx et al., 1995). The phylum has been traditionally divided into three classes, Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala (Amin, 1985; Bullock, 1969), although a new class, Polyacanthocephala, with one order, one family, one genus (*Polyacanthorhynchus*), and four species has been recently proposed (Amin, 1987). Three of these species, *P. macrorhynchus*, *P. caballeroi*, and *P. rhopalorhynchus*, inhabit the digestive tract of south American caimans. The fourth species, *P. kenyensis,* is only known in the larval stage, infecting freshwater fish in Kenya (Amin and Dezfuli, 1995). However, erection of this new class has been controversial because polyacanthocephalans were originally included within family Rhadinorhynchidae, belonging to Palaeacanthocephala.

Recent studies based on sequences of 18S rRNA suggested that the phylum Acanthocephala is a monophyletic group with Archiacanthocephala situated as the most basal class of the phylum and, therefore, Eoacanthocephala + Palaeacanthocephala form a derived clade (García-Varela et al., 2000; Near et al., 1998). However, these analyses did not include sequences from Polyacanthocephala species.

* Corresponding author. Fax: +525-622-3892.
E-mail address: laclette@servidor.unam.mx (J.P. Laclette).

1055-7903/02/$ - see front matter
PII: S1055-7903(02)00020-9

The initial proposal of Polyacanthocephala as a separate class (Bullock, 1969; Schmidt and Canaris, 1967) was not incorporated into the major taxonomic reviews of Acanthocephala (Amin, 1985; Bullock, 1969). More recently, characters such as trunk spination, lacunar canal location, number and size of proboscis hooks, female ligament sacs, and male cement gland nuclei have been used to support the Polyacanthocephala as a new class (Amin, 1987). In this study we sequenced the nearly complete 18S ribosomal RNA gene of *P. caballeroi* and *Rhadinorhynchus* sp., which were aligned with 21 sequences of acanthocephalans representing the classes Archiacanthocephala, Palaeacanthocephala, and Eoacanthocephala.

2. Materials and methods

2.1. Specimen collection

Specimens of *P. caballeroi* were collected from the intestine of a caiman (*Caiman yacare*) from Bolivia, whereas the specimens of *Rhadinorhynchus* sp. were collected from the intestines of fish belonging to the family Scianidae. The worms were washed three times in saline and preserved in liquid nitrogen until DNA extraction. The parasites were identified using conventional morphological criteria. The voucher specimens were deposited at the Colección Nacional de Helmintos, Inst. de Biología, UNAM (CNHE No: 4437-4438).

2.2. Characterization of 18S rDNA Gene of P. caballeroi and Rhadinorhynchus sp.

Genomic DNA from *P. caballeroi* and *Rhadinorhynchus* sp. were extracted and the near complete 18S rDNAs were amplified by PCR using primers Forward 5′-AGATTAAGCCATGCATGCGT-3′ and Reverse 5′-GCAGGTTCACCTACGGAAA-3′ as described elsewhere (García-Varela et al., 2000). PCR products were separated and evaluated by electrophoresis through 1% agarose gels. The band containing the amplified DNA was excised from the gel and PCR products were cleaned using the Wizard PCR purification system (Promega). The amplified products were ligated and cloned using plasmid vector pMOSBlue (Amersham) and *Escherichia coli* TG1 cells. After purification of the recombinant plasmid with the purification system (Promega), both strands of 18S rDNA gene were sequenced with an Applied Biosystems 310 automatic sequencer using ABI Prism dye terminator sequencing kits using Ml3 universal primers or primers annealing to conserved internal sequences. DNA sequences were inspected individually and assembled with the program DNAMAN (Lynnon Biosoff, 1994–1997). The near complete 18S rRNA gene sequences for *P. caballeroi* and *Rhadinorhynchus* sp. have been deposited in the GenBank/EMBL data sets with the Accession Nos. AF388660 and AY062433, respectively.

2.3. Taxa used and sequence alignment

The sequences obtained from *P. caballeroi* and *Rhadinorhychus* sp. were aligned within an expanded database of 18S rRNA genes, consisting of 37 taxa and 2031 aligned nucleotide positions (García-Varela et al., 2000), using the programs Clustal W (Thompson et al., 1994) and DNAMAN (Lynnon Biosaf, 1994–1997) and were then adjusted by eye. The complete alignment is available from the corresponding author upon request.

2.4. Phylogenetic analysis

The phylogenetic analysis was carried out with PAUP* 4.0b7a (Swofford, 2000). To determine which model of sequence evolution best fit our data set, a nested likelihood ratio test was performed using Modeltest program version 3.0 (Posada and Crandall, 1988). Phylogenetic relationships were inferred using maximum-likelihood (Felsenstein, 1981). Five random taxon addition heuristic searches with Tree Bisection–Reconnection (TBR) branch swapping were conducted to find an initial maximum-likelihood tree. In these searches, gamma shape parameter, proportion of invariable sites, and nucleotide frequencies were reestimated and the new parameters were used in another series of maximum-likelihood heuristic searches, carried out as above. To compare topologies representing specific phylogenetic hypotheses, constraints were defined, and searches for the maximum-likelihood tree were conducted using the same model and the same heuristic search strategy. Differences between maximum-likelihood values for trees representing alternative hypotheses were evaluated using the test of Kishino and Hasegawa (1989) implemented in PAUP*. The resulting tree was drawn using RETREE and DRAWGRAM from PHYLIP (Felsenstein, 1999). Parsimony analysis was also performed using a test version of PAUP 4.0b7a (Swofford, 2000). In all analyses the gaps were treated as missing data and 10 random-addition heuristic searches with TBR branch swapping were conducted to find the smaller tree. To support the inferred tree, bootstrap analyses were carried out with 1000 replications.

3. Results and discussion

Alignment of the near complete 18S rRNA gene sequences of 23 acanthocephalan species representing classes Archiacanthocephala (with three of four orders: Moniliformida, Gigantorhynchida, and Oligacanthorhynchida), Eoacanthocephala (with one of two or-

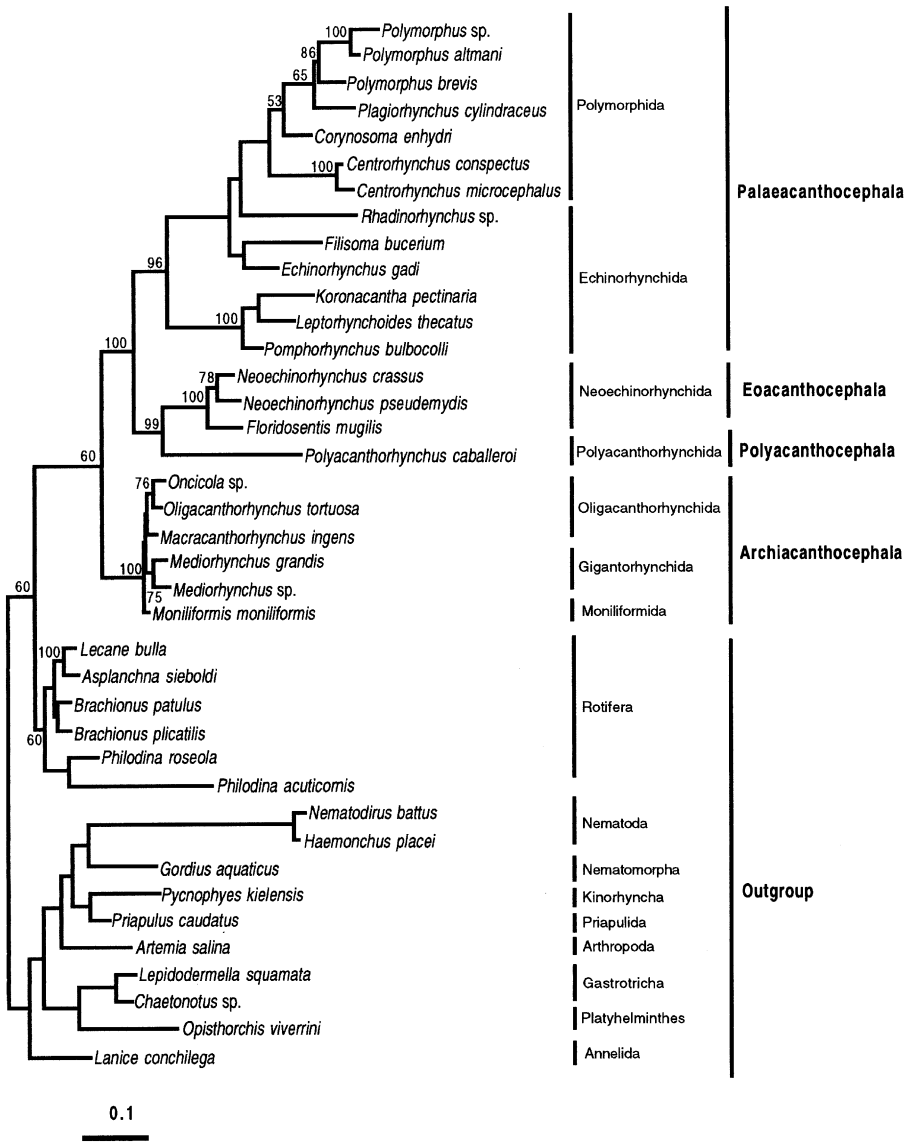

Fig. 1. Single best tree resulting from a maximum-likelihood analysis using our expanded 18S gene sequence data (García-Varela et al., 2000), supplemented with sequences of *Polyacanthorhynchus caballeroi* and *Rhadinorhynchus* sp. The −ln likelihood is 29512.680. Branch lengths are proportional to the inferred amount of nucleotide substitution. Numbers adjacent to branches show the bootstrap values (higher than 50%) from a parallel parsimony analysis. Taxa examined: *Centrorhynchus conspectus* (U41399); *Centrorhynchus microcephalus* (AF064813); *Corynosoma enhydri* (AF001837); *Echinorhynchus gadi* (U88335); *Filisoma bucerium* (AF064814); *Floridosenti mugilis* (AF064811); *Leptorhynchoide thecatus* (AF001840); *Koronacantha pectinaria* (AF092433); *Macracanthorhynchus ingens* (AF001844); *Mediorhynchus* sp. (AF064816); *Mediorhynchus grandis* (AF001843); *Moniliformis moniliformis* (Z19562); *Neoechinorhynchus pseudemydis* (U41400); *Neoechinorhynchus crassus* (AF001842); *Oligacanthorhynchus tortuosa* (AF064817); *Oncicola* sp., (AF064818); *Plagiorhynchus cylindraceus* (AF001839); *Polymorphus* sp. (AF064815); *Polymorphus brevis* (AF064812); *Polymorphus altmani* (AF001838); *Polyacanthorhynchus caballeroi* (AF388660); *Pomphorhynchus bulbocolli* (AF001841); *Rhadinorhynchus* sp. (AY062433); *Asplanchna sieboldi* (AF092434); *Brachionus plicatilis* (U29235); *Brachionus patulus* (AF154568); *Lecane bulla* (AF154566); *Philodina acuticornis* (U41281); *Philodina roseola* (AF154567); *Lepidodermella squamata* (U29198); *Chaetonotus* sp. (AJ001735); *Opisthorchis viverrini* (X55357); *Lanice conchilega* (X79873); *Haemonchusplacei* (L04154); *Nematodirus battus* (U01230); *Gordius aquaticus* (X87985); *Priapulus caudatus* (X87984); *Pycnophyes kielensis* (U67997); *Artemia salina* (X01723).

ders: Neoechinorhynchida), and Palaeacanthocephala (with two of two orders: Echinorhynchida and Polymorphida) plus 16 other outgroup taxa comprised a data set of 39 taxa and 2308 sites. The likelihood ratio test indicated that the best model to our data set was the general time reversible model (Rodríguez et al., 1990), with invariable sites (+I) and rate heterogeneity (+G; Yang, 1994). The proportion of invariable sites = 0.096 and the gamma shape parameter = 0.554. The maximum-likelihood analysis using this model yielded a single best tree with a likelihood score of 29512.680, and all branches were of significantly positive length. The topology of this tree was identical to that obtained in a previous analysis, except for the new branches for

P. caballeroi and *Rhadinorhynchus* sp. (García-Varela et al., 2000). The phylum Acanthocephala was monophyletic with Archiacanthocephala as the most basal class. Polyacanthocephala appeared to form a sister group with Eoacanthocephala, separated from Palaeacanthocephala (Fig. 1).

To test the support for this hypothesis, new maximum-likelihood analyses were carried out introducing the alternative topologies [((*Rhadinorhynchus* sp., Polyacanthocephala) Eoacanthocephala) (Archiacanthocephala)] or [((*Leptorhynchoides thecatus*, Polyacanthocephala) Eoacanthocephala) (Archiacanthocephala)] as constraints. *Rhadinorhynchus* sp. and *L. thecatus* are members of the Rhadinorhynchidae family to which Polyacanthocephala was previously assigned (Golvan, 1962). In both cases, all searches resulted in the same maximum-likelihood trees (not shown). The –ln likelihood score for the first alternative topology was 29804.552, whereas the score for the second was 29828.225. Based on the results of the Kishino–Hasegawa test, both alternative topologies are significantly less likely than that shown in Fig. 1. The difference in the –ln likelihood between trees for [((*Rhadinorhynchus* sp., Polyacanthocephala) Eoacanthocephala) (Archiacanthocephala)] is 291.872 (SD = of 34.624, $t = 8.429$, $P < 0.05$). The difference between trees for [((*L. thecatus*, Polyacanthocephala) Eoacanthocephala) (Archiacanthocephala)] is 315.544 (SD = of 36.009, $t = 8.762$, $P < 0.05$). Therefore, the hypothesis [((Eoacanthocephala, Polyacanthocephala) Palaeacanthocephala) (Archiacanthocephala)] is correct. This topology was also supported through a parsimony analysis, which yielded a single tree of 5629 steps long, with a consistency index of 0.441. Bootstrap values (higher than 50%) resulting from this analysis are presented on equivalent branches of the tree in Fig. 1. Relationships among classes of Acanthocephala were supported by high bootstrap values. Also, the position of *Rhadinorhynchus* sp. and *L. thecatus* within Palaeacanthocephala or the position of *P. caballeroi* as the sister group of Eoacanthocephala were also well supported.

Based on morphological characters, the four species of Polyacanthocephala were formerly included in the subfamily Rhadinorhynchidae, within Palaeacanthocephala (Golvan, 1962; Petrotschenko, 1956). However, our results based on sequence data showing Polyacanthocephala as the sister group of Eoacanthocephala are consistent with the concept that Polyacanthocephala represents a different class within the phylum Acanthocephala. Nevertheless, because only one of the two orders of Eoacanthocephala was represented in our study, the possibility that Polyacanthocephala constitutes a new order within Eoacanthocephala cannot be excluded. Additional sequences are required in the analysis to further detail the position of polyacanthocephalans within the phylum.

Acknowledgments

This work was supported in part by grants from CONACYT, L0042–M9607 (J.P.L.), DGAPA-UNAM IN-207195 (J.P.L.), PADEP 102324 (M.G.V.), and Fundación Miguel Alemán, A.C (J.P.L.). M.G.V. is being supported by scholarships from CONACYT and DGAPA-UNAM. M.P.C. is funded by grants from the National Aeronautics and Space Administration, the National Science Foundation, and the Alfred P. Sloan Foundation. S.I.G. was supported by grant BSR-9024816, United States National Science Foundation. We thank Patricia de la Torre for technical assistance during DNA sequencing.

References

Amin, O.M., 1985. Classification. In: Crompton, D.W.T., Nickol, B.B. (Eds.), Biology of the Acanthocephala. Cambridge University Press, Cambridge, UK, pp. 27–72.

Amin, O.M., 1987. Key to the families and subfamilies of Acanthocephala with the erection of a new class (Polyacanthocephala) and a new order (Polyacanthorhynchida). J. Parasitol. 73, 1216–1219.

Amin, O.M., Dezfuli, S.B., 1995. Taxonomic notes on *Polyacanthocephala kenyensis* (Acanthocephala: Polyacanthorhynchidae) from lake Naivasha, Kenya. J. Parasitol. 81, 76–79.

Brusca, R.C., Brusca, G.J., 1990. Invertebrates. Sinauer, Sunderland, MA.

Bullock, W.L., 1969. Morphological features as tool and pitfall in acanthocephalan systematics. In: Schmidt, D.G. (Ed.), Problems in Systematics of Parasites. University Park Press, Baltimore, pp. 9–45.

Clark, R.B., 1979. Radiation of the Metazoa. In: Houses, M.R. (Ed.), The Origins of Major Invertebrate Groups. Academic Press, New York, pp. 55–101.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1999. PHYLIP (Phylogeny Inference Package), ver. 3.572c. University of Washington, Seattle.

García-Varela, M., Pérez-Ponce de León, G., De la Torre, P., Cummings, M.P., Sarma, S.S.S., Laclette, J.P., 2000. Phylogenetic relationships of acanthocephala based on analysis of 18S ribosomal RNA gene sequences. J. Mol. Evol. 50, 532–540.

Golvan, Y.J., 1962. Le phylum des Acanthocephala. (Quatrieme note). La classe des Archiacanthocephala (A. Meyer 1931). Ann. Parasitol. Hum. Comp. 37, 1–72.

Hyman, L.B., 1951. The Invertebrates. Vol III: Pseudocoelomates Groups. McGraw-Hill, New York.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from sequence data and branching order in Hominoidea. J. Mol. Evol. 229, 170–179.

Marcus, E., 1958. On the evolution of the animal phyla. Q. Rev. Biol. 33, 24–58.

Near, J.T., Garey, J.R., Nadler, S.A., 1998. Phylogenetic relationships of the acanthocephala inferred from 18S ribosomal DNA sequences. Mol. Phylogenet. Evol. 10, 287–298.

Petrotschenko, V.I., 1956. Acanthocephala of wild and domestic animals. Akad. Nauk SSSR 1.

Posada, D., Crandall, K.A., 1988. Modeltest: testing the model of DNA substitution. Bioinformatics 9, 817–818.

Rodríguez, F., Oliver, J.F., Marin, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Schmidt, G.D., Canaris, A.G., 1967. Acanthocephala from Kenya with descriptions of two new species. J. Parasitol. 53, 634–637.

Swofford, D., 2000. PAUP 4.0b7a, Phylogenetic Analysis Using Parsimony (and Other Methods). Sinauer, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Wallace, R.L., Ricci, C., Melone, G., 1996. A cladistic analysis of pseudocoelomates (aschelminth) morphology. Invertebr. Biol. 115, 104–112.

Winnepenninckx, B., Backeljau, T., Mackey, L.Y., Brooks, J.M., Wachter, D.R., Kumar, S., Garey, J.R., 1995. 18S rRNA data indicate that the Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. Mol. Biol. Evol. 12, 1132–1137.

Yang, Z., 1994. Estimating the patterns of nucleotides substitution. J. Mol. Evol. 39, 105–111.