

Short communication

# The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences

Holger Herlyn,<sup>a,\*</sup> Oliver Piskurek,<sup>a</sup> Jürgen Schmitz,<sup>a</sup> Ulrich Ehlers,<sup>b</sup> and Hans Zischler<sup>a</sup>

<sup>a</sup> Primate Genetics, German Primate Center, Kellnerweg 4, Göttingen 37077, Germany

<sup>b</sup> Institut für Zoologie and Anthropologie, Abtlg. Morphologie and Systematik, Berliner Straße 28, Göttingen 37073, Germany

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## Abstract

The phylogeny of the Syndermata (Rotifera: Monogononta, Bdelloidea, Seisonidea; Acanthocephala: Palaeacanthocephala, Eoacanthocephala, Archiacanthocephala) is key to understanding the evolution of acanthocephalan endoparasitism from free-living ancestors. In the present study, maximum likelihood, distance/neighbor-joining, and maximum parsimony analyses have been carried out based on 18S rDNA data of 22 species (four new sequences). The results suggest a monophyletic origin of the Eurotatoria (Monogononta + Bdelloidea). *Seison* appears as the acanthocephalan sistergroup. Palaeacanthocephala split into an “*Echinorhynchus*”—and a “*Leptorhynchoidea*”—group, the latter sharing a monophyletic origin with the Eoacanthocephala and Archiacanthocephala. As inferred from the phylogeny obtained acanthocephalan endoparasitism evolved from a common ancestor of *Seison* and Acanthocephala that lived epizoically on an early mandibulate. Probably, an acanthocephalan stem species invaded the mandibulate host, thus establishing an endoparasitic lifestyle. Subsequently, vertebrates (or gnathostomes) became part of the parasite’s life cycle. In the stem line of the Archiacanthocephala, a terrestrial life cycle has evolved, with an ancestor of the Tracheata (Insecta, Myriapoda) acting as intermediate host.

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## 1. Introduction

The taxon Syndermata comprises the two pseudo-coelomate taxa Rotifera and Acanthocephala, both characterized by special ultrastructural features (Ahlrichs, 1997, 1998). Today, most authors distinguish three subclades within each of the taxa: the Monogononta, Bdelloidea, and Seisonidea within the Rotifera, and the Palaeacanthocephala, Eoacanthocephala, and Archiacanthocephala within the Acanthocephala (e.g., Nielsen, 1995). Since the Seisonidea only comprise two species (*Seison nebaliae* and *Seison annulatus*), the present study uses the term *Seison* instead of Seisonidea.

While the aquatic Rotifera are commonly free-living, the Acanthocephala are exceptionally obligate endoparasites with a fascinating life cycle. Adult acanthocephalans live in the intestine of their vertebrate, mostly gnathostomatan, definitive host. Inseminated females

excrete thousands of shelled and fully embryonated eggs that are voided with the faeces of the host. The mandibulate (Insecta, Myriapoda, and Crustacea) intermediate host becomes infected by swallowing the eggs. After ingestion the hatched larva (“acanthor”) pierces the gut wall, enters the body cavity and develops to a young adult (“acanthella”). The parasite’s life cycle is completed when a potential definitive host feeds on an infected intermediate host.

While the monophyly of the Acanthocephala as a whole has never been disputed, the monophyly of the Palaeacanthocephala is still matter of debate (Figs. 1A and B). Consequently, the assumed phylogeny within the Acanthocephala varies depending on the study, placing either archiacanthocephalans (García-Varela et al., 2000; Near et al., 1998) or palaeacanthocephalans at the base of the taxon (Herlyn et al., 2001).

Apart from the phylogeny within the Acanthocephala, the acanthocephalan sistergroup still remains unresolved. On the basis of morphological characters three competing possibilities have been proposed in the past decades, and recently, a fourth option has been intro-

\* Corresponding author. Fax: +49-551-3851-228.

E-mail address: [hherlyn@gwdg.de](mailto:hherlyn@gwdg.de) (H. Herlyn).

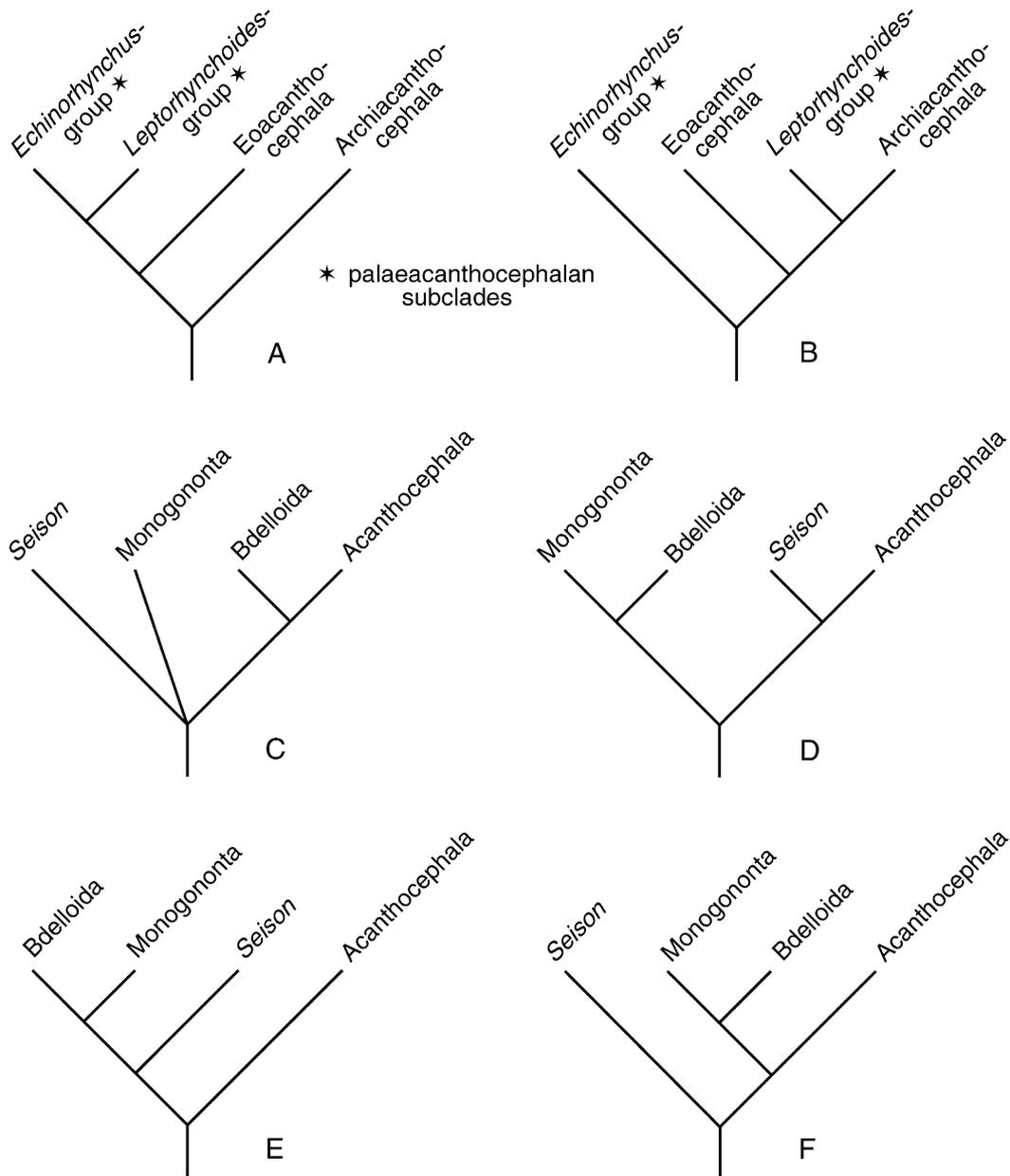


Fig. 1. (A) and (B) Possible phylogenetic relationships within the Acanthocephala. (A) Basal position of Archiacanthocephala and monophyly of Palaeacanthocephala (García-Varela et al., 2000; Near et al., 1998). (B) Paraphyly of “Palaeacanthocephala,” basal position of *Echinorhynchus*-group (present study; see also Herlyn et al., 2001). (C)–(F) Alternative phylogenetic hypotheses within the Syndermata: (C) Lemniscia-hypothesis (Lorenzen, 1985); (D) Pararotatoria-hypothesis (Ahlrichs, 1997, 1998); (E) classic theory of a monophyletic origin of the Rotifera (Nielsen, 1995); (F) Eurotatoria as the acanthocephalan sistergroup (Welch, 2000).

duced inferred from *hsp82* data (Figs. 1C–F; see also Garey et al., 1998). Phylogenetic analyses of rDNA (16S, 18S) data carried out so far corroborated a Bdelloidea–Acanthocephala sistergroup relationship (see e.g., Garey et al., 1996, 1998; García-Varela et al., 2000; Giribet et al., 2000; Near et al., 1998). However, *Seison* data have not been included in these approaches, even though the phylogenetic position of the taxon *Seison* is of special interest. Both representatives live epizoically—possibly even ectoparasitically (Segers and Melone, 1998)—on crustaceans of the taxon *Nebalia* (Leptost-

raca), in what can be described as an extant intermediate state between the free life style of the Monogononta and Bdelloidea and the endoparasitism of the Acanthocephala.

In the present study, we analyse the phylogeny of the Syndermata to elucidate the evolution of acanthocephalan endoparasitism from a free-living ancestor and its development within the Acanthocephala after becoming established. For this purpose we sequenced orthologous 18S rDNA-fragments of the palaeacanthocephalan species *Echinorhynchus truttiae* and *Acanthocephalus an-*

*guillae*, of the eoacanthocephalan species *Paratenuisentis ambiguus* and of *S. nebalia*. Apart from these four new sequences the present analyses include 18 additional sequences from GenBank (4 rotifers, 12 acanthocephalans, 1 gnathostomulid, and *Symbion pandora*). To assess the effect of possible homoplasies three datasets were generated with increasing stringency against variable sites. Based on these datasets, maximum likelihood (ML), distance/neighbor-joining (DNJ), and maximum parsimony (MP) analyses were carried out. Finally, alternative topologies were tested with respect to the significance of the relevant branching patterns.

## 2. Materials and methods

### 2.1. Collection of specimens

Adults of *E. truttae*, *P. ambiguus*, and *A. anguillae* (Acanthocephala) were collected in summer 2000 from the intestine of *Thymallus thymallus* (from river Leine at Göttingen, Germany), *Anguilla anguilla*, and *Barbus barbus* (both from river Weser, near Göttingen), and stored at  $-80^{\circ}\text{C}$ . Specimens of *S. nebaliae* were detached from *Nebalia bipes* (Crustacea) from eulittoral rockpools near Roscoff, France. To purge the intestinal tract of foreign DNA the *S. nebaliae*-specimens were starved for 1.5 days at  $4^{\circ}\text{C}$  before freezing ( $-80^{\circ}\text{C}$ ).

### 2.2. Nucleic acid isolation, polymerase chain reaction, and sequencing

Total genomic DNA was extracted from individual specimens using a commercial extraction kit (Qiagen, Germany). A 18S rDNA-fragment of  $\sim 1000$  bp was amplified [30–35 cycles:  $94^{\circ}\text{C}$  (40 s),  $61^{\circ}\text{C}$  (60 s), and  $72^{\circ}\text{C}$  (60 s)] using primers corresponding to conserved regions (5'-GTTG(CT)TGT(AG)GTTAAAA(AC)GC TCGT-3' and 5'-TAGCGACGGGCGGTGTGTAC-3'). Each fragment was electrophoretically separated on agarose gel, ligated into pGem T-vector (Promega, Madison, WI), transformed into *Escherichia coli* by electroporation, and sequenced in both directions using a commercial cycle-sequencing kit (Amersham, Cleveland, OH). Consensus-sequences were assessed on the basis of five clones in *S. nebaliae* and three clones in *P. ambiguus*, *E. truttae*, and *A. anguillae*, respectively.

### 2.3. Alignment and phylogenetic analyses of sequence data

The obtained sequences (AF469411–4) were combined with homologues of 18 additional species from GenBank (for GenBank accession numbers see Appendix A). Based on these 22 sequences, three datasets with increasing stringency against variable sites were generated. Dataset 1 was a gap-cleaned CLUSTAL W-alignment

(default parameters; Thompson et al., 1994) of 911 characters, 312 of which were parsimony-informative. To generate dataset two the non-conserved blocks were excised from the CLUSTAL W-alignment with GBLOCKS (default parameters; Castresana, 2000) using only one representative per cluster (*Haplognathia spec.*, *Symbion pandora*, *Asplanchna sieboldi*, *S. nebaliae*, *E. truttae*, *Neoechinorhynchus crassus*, *Macracanthorhynchus ingens*, and *Koronacantha pectinaria*). The remaining 867 sites comprised 264 parsimony-informative characters. For dataset 3 the sequences were aligned according to a secondary structure model of the 18S rRNA of the platyhelminth *Calicophorum calicophorum* ([http://rrna.uia.ac.be/secmodel/Cca1\\_Ssu.html](http://rrna.uia.ac.be/secmodel/Cca1_Ssu.html)). After blocking dataset 3 included 842 sites 249 of which were parsimony-informative.

Based on dataset 1–3 ML, DNJ, and MP analyses were carried out using PUZZLE 4.0.2 (Strimmer and von Haeseler, 1996) and the 4.0b8 beta version of PAUP\* (Swofford, 1999) assuming the HKY85 model of sequence evolution. For PAUP\* *H. spec.* and *S. pandora* were defined as paraphyletic outgroup. According to PUZZLE, only *H. spec.* served as outgroup in the rooted ML trees. Robustness of clades was assessed by examining 1000 bootstrap replicates and puzzling steps, respectively.

To assess the phylogenetic affiliation of *S. nebaliae* as well as the species' influence on the general tree topology within the Acanthocephala, additional PAUP\*-analyses (basal polytomies permitted) of dataset 1 were carried out with varying outgroup samplings. Additionally, the likelihood mapping analysis in PUZZLE was performed assuming the  $\chi^2$  model, and alternative topologies were compared using the 5% significance KH-test on the basis of likelihood criteria. Finally, unrooted trees were calculated using Splits-Tree-2.4 (Huson, 1998) to assess the phylogeny within the Acanthocephala irrespective of the taxonomic outgroup sampling.

## 3. Results

Maximum likelihood analysis of dataset 1 was based on 7315 quartets, 0.7% of which remained unresolved. The majority-rule consensus tree ( $\log L -6974.96$ ) was completely resolved (Fig. 2). Apart from weak support for the phylogenetic position of *E. truttae* (61%) none of the quartet puzzling support values (QPSV) amounted to less than 73%. Eurotatoria (Monogononta + Bdelloidea) were monophyletic in 100% of the replicates. With 97% support, *S. nebaliae* appeared as the acanthocephalan sistertaxon. Eoacanthocephala and Archiacanthocephala constituted monophyla in 100% and 99% of the replicates, respectively. In contrast, Palaeacanthocephala split into two separate clades, that

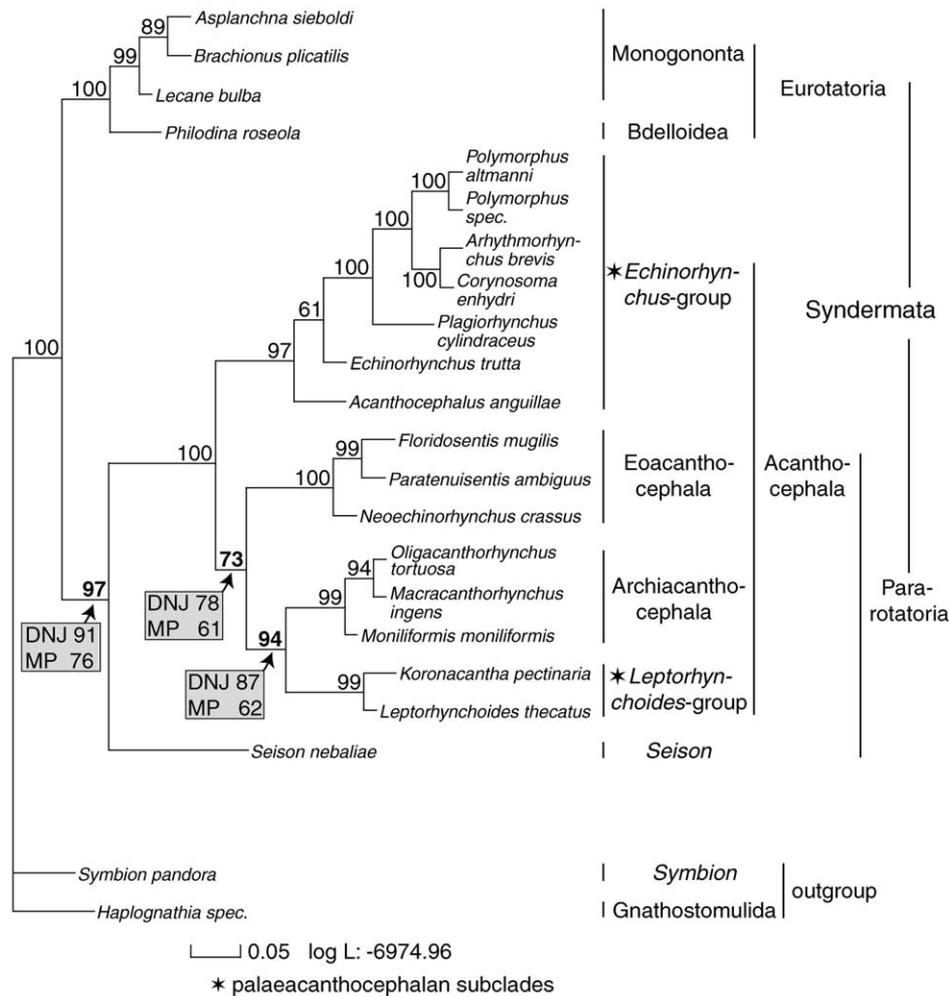


Fig. 2. ML tree based on dataset 1. Numbers indicate QPSV (%) of branches (1000 puzzling steps). The QPSV of the crucial branches are bold faces. Numbers in rectangles refer to bootstrap percentages (1000 replicates) of DNJ and MP analyses based on dataset 1.

are both supported: one comprising *K. pectinaria* and *Leptorhynchoides thecatus* (99% support, termed “*Leptorhynchoides*-group” in the following), and one comprising the other palaeacanthocephalan species that were included in the present approach (97% support, termed “*Echinorhynchus*-group” in the following). In 73% of the replicates Eoacanthocephala, Archiacanthocephala, and *Leptorhynchoides*-group had a common monophyletic origin. Within this monophylum Archiacanthocephala and the *Leptorhynchoides*-group appeared as sistergroups in 94% of the replicates. The average distance over all possible pairs of sequences was 0.212. Apart from differences on species level within *Echinorhynchus*-group and Eurotatoria DNJ and MP (1181 steps, consistency index = 0.612) analyses of dataset 1 yielded the same topology as the ML analysis (Fig. 2; Appendix B). Even if *H. spec.* and *S. pandora* were excluded from dataset 1 and the monogont and bdelloidean species as well as *S. nebaliae* were defined as outgroup, in none of the ML, DNJ, and MP analyses *S. nebaliae* fell to the Eurotatoria. Moreover, the general

tree topology within the Acanthocephala did not change under these conditions.

The ML mapping analysis of dataset 1 again mirrored the general tree topology described above. (1) When Monogononta, Bdelloidea, *Seison*, and Acanthocephala were defined as separate clusters *S. nebaliae* was grouped with the Acanthocephala in 100% of the quartets. (2) When the the four acanthocephalan sub-clades were defined as clusters the *Leptorhynchoides*-group fell to the Archiacanthocephala, again in 100% of the quartet samplings.

Regarding the described general tree topology, analyses of dataset 2 and 3 yielded the same results as did analysis of dataset 1, partly with even higher supports for the crucial branches (see Appendix B): in most trees *Seison* appeared as the acanthocephalan sistergroup, and even in the only exception (MP tree of dataset 2) *Seison* did not fall to the Eurotatoria. Moreover, in none of the trees, the Palaeacanthocephala were monophyletic, and in most of trees the *Leptorhynchoides*-group fell to Eoacanthocephala and Archiacanthocephala.

#### 4. Discussion

The described general congruence in tree topology regardless of dataset and tree building method (see Appendix B) confirms the negligible influence of possible homoplasies. The waning resolution from analysis of dataset 1 and 2 to dataset 3 probably was caused by the decreasing fragment lengths from dataset 1 (911 bp), 2 (867 bp), and 3 (842 bp) and the concomitant loss of information.

##### 4.1. The phylogeny within the Acanthocephala (see Figs. 1A and B and 2)

Most previous molecular approaches (*hsp82*, 16S rDNA, and 18S rDNA) on the syndermatan phylogeny included too few acanthocephalan sequences to draw valid conclusions on the phyletic status of the acanthocephalan subclades (see e.g., Garey et al., 1998; Giribet et al., 2000; Welch, 2000). Where the analyses were based on broader samplings including representatives of Archiacanthocephala, Eoacanthocephala, and *Echinorhynchus*- and *Leptorhynchoides*-group the authors (García-Varela et al., 2000; Near et al., 1998) reasoned a monophyletic origin of the Palaeacanthocephala and a basal position of the Archiacanthocephala.

To assess a possible influence of taxonomic sampling outside the Acanthocephala on the tree topology we compared an unrooted DNJ tree based on the acanthocephalan sequences of the original dataset of García-Varela et al. (2000) to an unrooted tree based on the acanthocephalan sequences in dataset 1 (see Appendix C). In both trees the Palaeacanthocephala split into an *Echinorhynchus*- and a *Leptorhynchoides*-group, the latter of which clustered with the Eoacanthocephala and Archiacanthocephala. This broad congruence of the unrooted trees suggests that the acanthocephalan phylogeny in previous 18S rDNA analyses was biased by sampling outside the Acanthocephala.

Finally, the assumption of a palaeacanthocephalan paraphyly and a monophyletic origin of *Leptorhynchoides*-group, Eoacanthocephala and Archiacanthocephala fits well with morphological data on the presence/absence of sensory organs and correlated structures at the base (lateral sense organs) and the tip (apical sense organs) of the acanthocephalan proboscis (Herlyn et al., 2001). However, we won't conceal that a recent morphological attempt came to different conclusions, even though on the basis of partly subjective character states that often lack homologues in the outgroup (see Monks, 2001).

##### 4.2. The acanthocephalan sistergroup (see Figs. 1C–F and 2)

The present results placing *Seison* as the acanthocephalan sistergroup differ from those of most previous

rDNA (16S and 18S) approaches which in principle endorsed Lorenzen's (1985) Lemniscea-hypothesis of a Bdelloidea–Acanthocephala sistergroup relationship (e.g., Garey et al., 1996, 1998; Near et al., 1998). These approaches, however, did not consider *Seison* data at all. We statistically compared the true ML tree based on dataset 1 with a user-defined one that reflected the Lemniscea-hypothesis and found that the true ML tree was significantly better with a log likelihood of  $-6974.96$  vs.  $-7028.54$  (S.E. 14.78). Moreover, the morphological data assumed as synapomorphies of Bdelloidea and Acanthocephala (see e.g., Lorenzen, 1985) can be doubted (see also Herlyn and Ehlers, 2001; Ricci, 1998). On the other hand, the monophyly of the Eurotatoria (Monogononta + Bdelloidea) seems well confirmed by morphological characters (see Ahlrichs, 1995) as well as the 18S rDNA approaches carried out to date including more than two eurotarian representatives (García-Varela et al., 2000, present study).

Welch (2000) was first to include data of the Monogononta, Bdelloidea, and of *S. nebaliae* in a molecular analysis based on sequence data of the heat shock protein 82 (*hsp82*) using a homologue of *Caenorhabditis elegans* as outgroup sequence. Inferred from this sampling the Eurotatoria (Monogononta + Bdelloidea) appeared as the acanthocephalan sistergroup whereas *S. nebaliae* represented the earliest emerging syndermatan taxon. We re-examined Welch's original dataset and found that *C. elegans* is problematic as outgroup: its HKY85 distances reach up to 55%, values that clearly exceed the desirable maximum of 30% recommended for outgroup sequences (see Hillis and Dixon, 1991). From this it follows that Welch's results possibly were biased by the usage of an inappropriate outgroup.

So far, none of the molecular analyses supported a monophyletic origin of Monogononta, Bdelloidea, and *Seison*. Also the morphological features discussed as apomorphies of a monophylum Rotifera in its classic sense (e.g., mastax, see Nielsen, 1995) are ambiguous, more likely representing plesiomorphies retained from the base patterns of higher taxa such as Syndermata, Gnathifera, and Eurotatoria (see e.g., Herlyn and Ehlers, 1997; Kristensen and Funch, 2000). Therefore, it seems at least improbable that Monogononta, Bdelloidea, and *Seison* as a whole constitute the acanthocephalan sistergroup.

In contrast, a grouping of *Seison* and Acanthocephala as it appears in almost all ML, DNJ, and MP trees of the 18S rDNA based phylogenetic reconstructions presented (see Appendix B) seems well founded due to the presence of special epidermal filaments and so-called densed bodies in the sperm flagellum (Ahlrichs, 1997, 1998). A *Seison*–Acanthocephala sistergroup relationship makes sense also in light of the different sexual biology within the Syndermata: both *Seison* and the Acanthocephala always reproduce bisexually. In con-

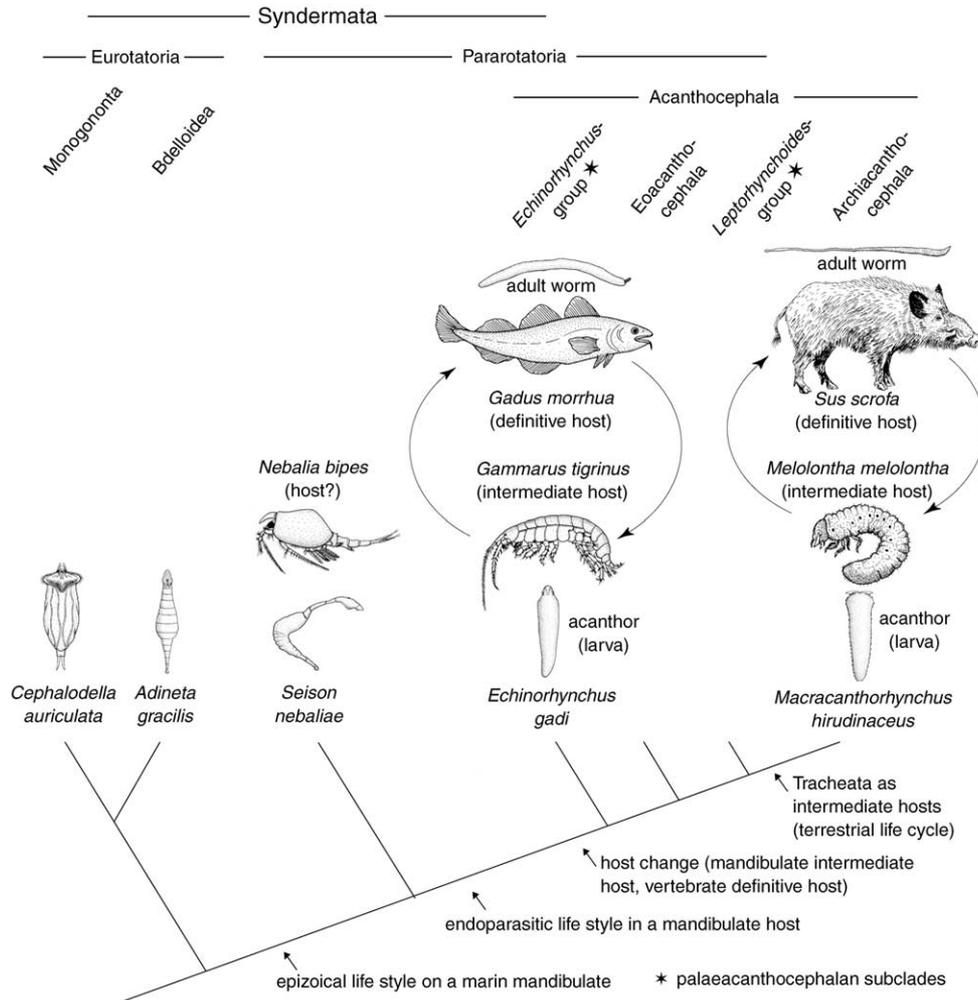


Fig. 3. Evolution of the acanthocephalan endoparasitism as inferred from the present 18S rDNA based phylogenetic analyses. The depicted species represent the superspecific taxa at the top of the figure. *Not shown*. Eoacanthocephala and *Leptorhynchoides*-group retained an aquatic life cycle and a mandibulate intermediate host from the last acanthocephalan stem species in their base patterns.

trast, in Eurotatoria parthenogenesis prevails accompanied by a reduction of the males with facultative dwarf males in monogononts and the absence of males in bdelloids (see e.g., Nielsen, 1995).

#### 4.3. Phylogenetic conclusion

(1) The taxon “Palaeacanthocephala” represents a paraphyletic assemblage of two clades, termed *Echinorhynchus*- and *Leptorhynchoides*-group in the present study the latter of which has a monophyletic origin with the Eoacanthocephala and Archiacanthocephala. (2) The two species comprising taxon *Seison* represents the sistergroup of the Acanthocephala.

#### 4.4. The evolution of acanthocephalan endoparasitism

The syndermatan phylogeny as obtained from 18S rDNA allows some fascinating insights into the evolu-

tion of acanthocephalan endoparasitism (Fig. 3; see also Near et al., 1998). Most likely, the common ancestor of *Seison* and Acanthocephala developed an epizoical life style on a marine mandibulate ancestor as can be seen today in *S. nebaliae* and *S. annulatus*. Possibly, the *Seison*–Acanthocephala stem species even established an ectoparasitic life style, as is currently discussed for at least *S. annulatus* (see e.g., Segers and Melone, 1998). The stem species of the Acanthocephala invaded the body cavity of the mandibulate host, thus establishing an endoparasitic life cycle. Vertebrates (especially gnathostomates) subsequently became part of the life cycle by feeding on infected mandibulates. The integration of a second host entailed a bipartite ontogenesis as observed in extant acanthocephalans: (1) the acanthocephalan larva (acanthor) hatches in the intestinal tract of the mandibulate intermediate host, penetrates the gut wall and develops in the body cavity to a resting stage that in principle represents a young adult (acanthella)

and (2) the acanthella matures to a fertile adult and reproduces in the intestine of the vertebrate definitive host. This host change and the correlated bipartite ontogenesis doubtlessly represent apomorphies of the Acanthocephala. The archiacanthocephalan stem species parasitized an ancestor of the Tracheata as intermediate host and evolved a terrestrial life cycle alongside its tracheatan host. Similarly, a terrestrial life cycle has been established where representatives of the crustaceans, namely isopods, secondarily became terrestrial.

### Acknowledgments

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### Appendix A

#### Taxa examined in the present study

Species	Code	Classification	Acc. No.	Reference
<i>Haplognathia spec.</i>	Hsp	Gnathostomulida, Filospermoidea	AF119084	Giribet et al. (2000)
<i>Symbion pandora</i>	Spa	Cycliophora	Y14811	Winnepenninckx et al. (1998)
<i>Asplanchna sieboldi</i>	Asi	Rotifera, Monogononta	AF092434	García-Varela et al. (2000)
<i>Brachionus plicatilis</i>	Bpl	Rotifera, Monogononta	BPU29235	Winnepenninckx et al. (1995)
<i>Lecane bulba</i>	Lbu	Rotifera, Monogononta	AF154566	García-Varela et al. (2000)
<i>Philodina roseola</i>	Pro	Rotifera, Bdelloidea	AF154567	García-Varela et al. (2000)
<i>Seison nebaliae</i>	Sne	Rotifera, Seisonidea	AF469411	This study
<i>Acanthocephalus anguillae</i>	Aan	Acanthocephala, Palaeacanthocephala	AF469413	This study
<i>Arhythmorhynchus brevis</i>	Abr	Acanthocephala, Palaeacanthocephala	AF064812	García-Varela et al. (2000)
<i>Corynosoma enhydri</i>	Cen	Acanthocephala, Palaeacanthocephala	AF001837	Near et al. (1998)
<i>Echinorhynchus truttae</i>	Etr	Acanthocephala, Palaeacanthocephala	AF469412	This study
<i>Koronacantha pectinaria</i>	Kpe	Acanthocephala, Palaeacanthocephala	AF092433	García-Varela et al. (2000)
<i>Leptorhynchoides thecatus</i>	Lth	Acanthocephala, Palaeacanthocephala	AF001840	Near et al. (1998)
<i>Plagiorhynchus cylindraceus</i>	Pcy	Acanthocephala, Palaeacanthocephala	AF001839	Near et al. (1998)
<i>Polymorphus altmanni</i>	Pal	Acanthocephala, Palaeacanthocephala	AF001838	Near et al. (1998)
<i>Polymorphus spec.</i>	Psp	Acanthocephala, Palaeacanthocephala	AF064815	García-Varela et al. (2000)
<i>Floridosentis mugilis</i>	Fmu	Acanthocephala, Eoacanthocephala	AF064811	García-Varela et al. (2000)
<i>Neoechinorhynchus crassus</i>	Ncr	Acanthocephala, Eoacanthocephala	AF001842	Near et al. (1998)
<i>Paratenuisentis ambiguus</i>	Pam	Acanthocephala, Eoacanthocephala	AF469414	This study
<i>Macracanthorhynchus ingens</i>	Min	Acanthocephala, Archiacanthocephala	AF001844	Near et al. (1998)
<i>Moniliformis moniliformis</i>	Mmo	Acanthocephala, Archiacanthocephala	Z19562	Telford and Holland, 1993

**Appendix A** (continued)

Species	Code	Classification	Acc. No.	Reference
<i>Oligacanthorhynchus tortuosa</i>	Oto	Acanthocephala, Archiacanthocephala	AF064817	García-Varela et al. (2000)
Additional taxa incorporated in the original dataset of García-Varela et al. (2000)				
<i>Centrorhynchus conspectus</i>	Cco	Acanthocephala, Palaeacanthocephala	U41399	Garey et al. (1996)
<i>Centrorhynchus microcephalus</i>	Cmi	Acanthocephala, Palaeacanthocephala	AF064813	García-Varela et al. (2000)
<i>Echinorhynchus gadi</i>	Ega	Acanthocephala, Palaeacanthocephala	U88335	Aleshin et al. (1998)
<i>Filisoma bucerium</i>	Fbu	Acanthocephala, Palaeacanthocephala	AF064814	García-Varela et al. (2000)
<i>Pomphorhynchus bulbocolli</i>	Pbu	Acanthocephala, Palaeacanthocephala	AF001841	Near et al. (1998)
<i>Neoechinorhynchus pseudemydis</i>	Nps	Acanthocephala, Eoacanthocephala	U41400	Garey et al. (1996)
<i>Mediorhynchus spec.</i>	Msp	Acanthocephala, Archiacanthocephala	AF064816	García-Varela et al. (2000)
<i>Mediorhynchus grandis</i>	Mgr	Acanthocephala, Archiacanthocephala	AF001843	Near et al. (1998)
<i>Oncicola spec.</i>	Osp	Acanthocephala, Archiacanthocephala	AF064818	García-Varela et al. (2000)

**Appendix B**

Bootstrap and quartet puzzling support values (%) of crucial branches (%)

Dataset	Tree building method	<i>Seison</i> + Acanthocephala	Eoacanthocephala + Archiacanthocephala + <i>Leptorhynchooides</i> -group	Archiacanthocephala + <i>Leptorhynchooides</i> -group
1	ML	97	73	94
	DNJ	91	78	87
	MP	76	61	62
2	ML	71	80	94
	DNJ	75	89	89
	MP	— <sup>a</sup>	62	68
3	ML	96	54	— <sup>b</sup>
	DNJ	92	— <sup>c</sup>	— <sup>c</sup>
	MP	57	— <sup>c</sup>	— <sup>c</sup>

<sup>a</sup> Polytomy of *Eurotatoria*, *Seison* and Acanthocephala.

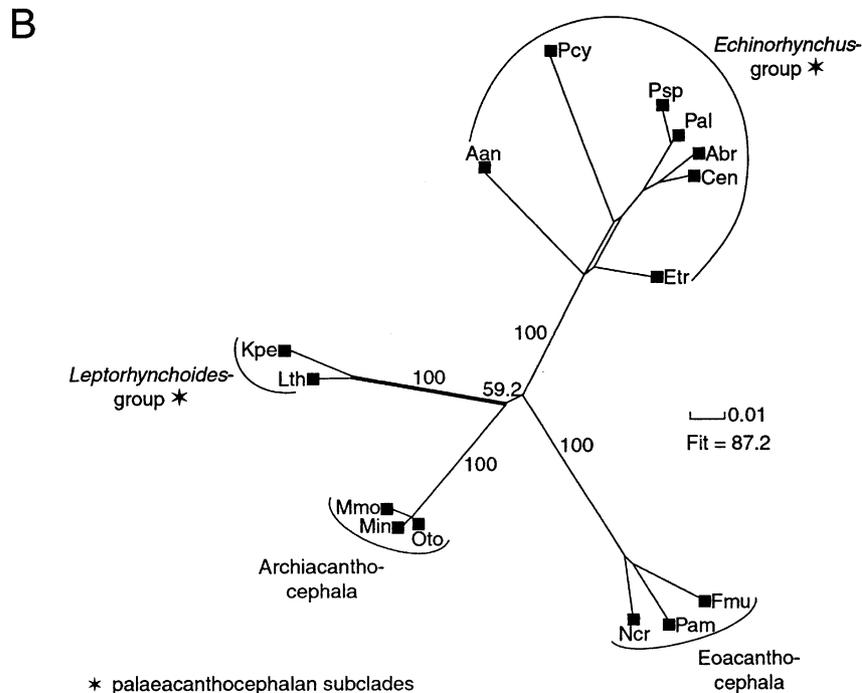
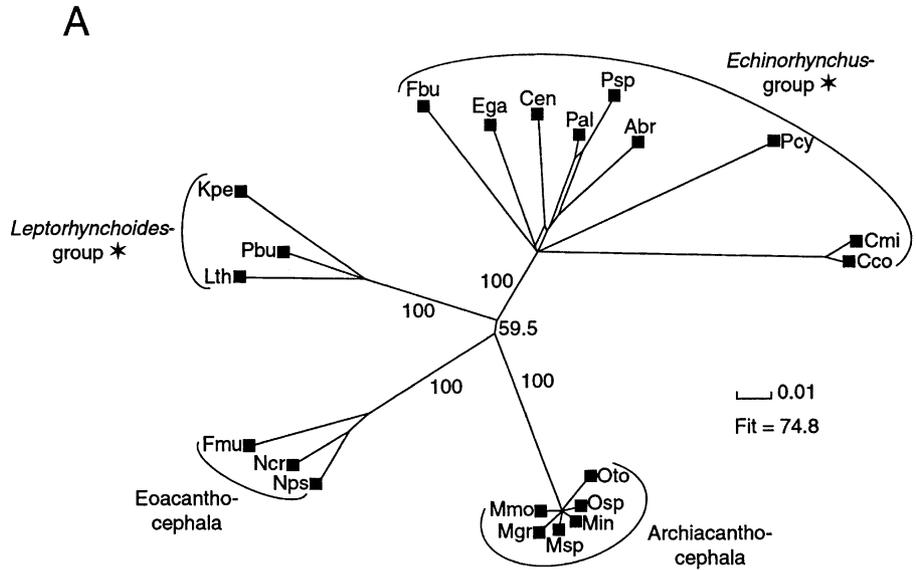
<sup>b</sup> Polytomy of Eoacanthocephala, Archiacanthocephala, and *Leptorhynchooides*-group.

<sup>c</sup> Polytomy of *Acanthocephalus*-group, Eoacanthocephala, Archiacanthocephala and *Leptorhynchooides*-group.

**Appendix C**

Unrooted DNJ trees of the acanthocephalan sequences included in the original dataset of García-Varela et al. (2000) (A) and dataset 1 (B). The Hamming

model was used for distance correction. Numbers indicate bootstrap percentages (1000 replicates) of the branches in focus. Regarding the three-letter code see Appendix A.



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