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Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea)

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Abstract

Phylogenetic interrelationships of 32 species belonging to 18 genera and four families of the superfamily Microphalloidea were studied using partial sequences of nuclear lsrDNA analysed by Bayesian inference and maximum parsimony. The resulting trees were well resolved at most nodes and demonstrated that the Microphalloidea, as represented by the present data-set, consists of three main clades corresponding to the families Lecithodendriidae, Microphallidae and Pleurogenidae + Prosthogonimidae. Interrelationships of taxa within each clade are considered; as a result of analysis of molecular and morphological data, *Floridatrema* Kinsella & Deblock, 1994 is synonymised with *Maritrema* Nicoll, 1907, *Candidotrema* Dollfus, 1951 with *Pleurogenes* Looss, 1896, and *Schistogonimus* Lühe, 1909 with *Prosthogonimus* Lühe, 1899. The taxonomic value of some morphological features, used traditionally for the differentiation of genera within the Lecithodendriidae and Prosthogonimidae, is reconsidered. Previous systematic schemes are discussed from the viewpoint of present results, and perspectives of future studies are outlined.

Introduction

The superfamily Microphalloidea Ward, 1901 is among the most derived taxa of digenean trematodes (Brooks et al., 1985; Tkach et al., 2001; Cribb et al., 2001; Olson et al., 2003). Since this superfamily was established by Morozov (1955), there has been considerable disagreement among authors concerning the taxa circumscribed by, and phylogenetic relationships within the superfamily, and thus the taxonomy of the group has been labile and confusing. Morozov (1955) included in the Microphalloidea only the Microphallidae Ward, 1901 and Gymnophallidae Odhner, 1905. Subsequently, Odening (1964a,b) included in this superfamily Microphallidae, Pleurogenidae Looss, 1899, Stomylotrematidae Poche, 1926, Lecithodendriidae Lühe, 1901, Allassogonoporidae Skarbilovich, 1948, Eumegacetidae Travassos, 1922, Anenterotrematidae Yamaguti, 1958 and Cortrematidae Yamaguti, 1958. However, in his later work, Odening (1971) changed his opinion, leaving only the Microphallidae within the Microphalloidea, and recognised the superfamily Lecithodendrioidea Lühe, 1901, comprising 10 families. Yamaguti (1958) included the Gymnophallinae Odhner, 1905 in the Microphallidae, but this systematic arrangement was not supported by subsequent workers (Yamaguti, 1971; Deblock, 1971; Richard & Prévot, 1974; Brooks et al., 1985, 1989; Bayssade-Dufour et al., 1993).

The taxonomic history of many taxa belonging to the Microphalloidea is complex and opinions on their

positions vary greatly. For instance, the subfamily Pleurogeninae established by Looss (1899) was first included in the Brachycoeliidae Johnston, 1912, but this arrangement was not supported by other authors and Odhner (1910) considered it a subfamily within the Lecithodendriidae. This viewpoint was accepted by a majority of authors (see Skarbilovich, 1948) until Odening (1959) raised the Pleurogeninae to full family level. Yamaguti (1971) and Prudhoe & Bray (1982) did not accept the familial status of the Pleurogenidae and again placed it in the Lecithodendriidae. Cladistic analysis of the Digenea by Brooks et al. (1985) considered the Microphalloidea and Lecithodendrioidea as separate superfamilies, but ignored the position of the Pleurogenidae entirely. More recently, Sharpilo & Iskova (1989) and other authors again recognised the Pleurogenidae as a separate family. This brief historical account of just one microphalloidean family clearly indicates that morphological characters alone cannot resolve the existing systematic confusion.

In their phylogenetic studies of the suborder Plagiorchiata La Rue, 1957 based on the partial sequences of nuclear large subunit ribosomal DNA (lsrDNA), Tkach et al. (2000, 2001) demonstrated that a group of taxa belonging to the families Lecithodendriidae, Pleurogenidae, Allassogonoporidae, Microphallidae and Prosthogonimidae Lühe, 1909 form a strongly supported clade that is the sister-group to another strongly supported clade comprising members of the superfamily Plagiorchioidea Lühe, 1901. Although these works have been devoted mainly to the study of the relationships of higher taxa (families, superfamilies) within the Plagiorchiata and analysis of its position within the Digenea, it was shown that the Microphalloidea comprises three sub-clades, including representatives of the Lecithodendriidae, Microphallidae and Pleurogenidae + Allassogonoporidae + Prosthogonimidae.

In the most recent work devoted to the phylogeny of this group, Tkach et al. (2002) analysed in detail the phylogenetic affinities of two genera possessing a seminal vesicle lying freely in parenchyma, *Ophiosacculus* Macy, 1935 and *Allassogonoporus* Oliver, 1938, that were placed by Yamaguti (1971) in different subfamilies of the Lecithodendriidae and by Sharpilo & Iskova (1989) in the family Allassogonoporidae. Molecular data have shown that *Ophiosacculus* and *Allassogonoporus* belong to different evolutionary lineages of the Microphallidea and were allocated within the Lecithodendriidae and Pleurogenidae, correspondingly. In the present paper, we explore relationships among a larger number of microphalloidid genera and species in order to test existing phylogenetic hypotheses and systematic schemes and to establish a basis for further molecular phylogenetic studies of this taxon-rich digenean superfamily.

Materials and methods

Specimen collection

Adult specimens of 13 species, belonging to different families of the Microphalloidea, were collected from amphibians, birds and small mammals in Europe, primarily in the Ukraine, and North America (Table 1). A specimen of *Loxogenes macrocirra* (Caballero & Bravo, 1949) collected in Guatemala was kindly provided by Dr Valerie McKenzie. Larval stages of five species of the Microphallidae collected from marine molluscs in Northern Ireland were kindly provided by Dr Sam Irwin. For species determination, whole-mounted voucher specimens of all sequenced species were made. When possible, e.g. in the case of the prosthogonimids, only a part of the digenean body was used for DNA extraction, while the rest of the same specimens were mounted on slides. Sequences of 14 other species belonging to the Microphalloidea as well as of seven outgroup species, published by Tkach et al. (2000, 2001, 2002), have been retrieved from GenBank. Outgroup taxa have been selected from the families Plagiorchiidae, Telorchiidae Looss, 1899, Haematoloechidae Freitas & Lent, 1939 and Brachycoeliidae, all belonging to the superfamily Plagiorchioidea, which has been demonstrated to be a sister-group of Microphalloidea according to the previous molecular phylogenetic studies (Tkach et al., 2000, 2001). Taxonomic names, definitive hosts, collecting localities and GenBank accession numbers are provided in Table 1.

Living worms recovered from the host were rinsed thoroughly in saline, fixed in 70% or 95% ethanol for molecular analyses and additionally in AFA for morphological study. In some cases living specimens were placed directly in guanidine thiocyanate lysis buffer, which facilitated further DNA extraction. Most specimens were identified while living before further processing.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from single specimens of adult or larval stages of worms following the

Table 1. Digenean species used in this study, their hosts, geographical origin of material and GenBank accession numbers for corresponding sequences. Specimens were collected in Ukraine unless otherwise is stated.

Digenean taxa	Host species	Geographical origin	GenBank N	Reference
Brachycoeliidae				
Brachycoelium salamandrae (Froelich, 1789)	*Salamandra salamandra* (L.) - Fire salamander	Rakhiv, Zakarpatska region	AF151935	Tkach et al., 2000
Haematoloechidae				
Haematoloechus longiplexus Stafford, 1902	*Rana catesbeiana* (Shaw) - North American bullfrog	Cage Co., Nebraska, USA	AF387801	Snyder & Tkach, 2001
Haematoloechus varioplexus Stafford, 1902	*Rana clamitans* (Latreille) - Green frog	Winnebago Co., Wisconsin, USA	AF387798	Snyder & Tkach, 2001
Lecithodendriidae				
Lecithodendrium linstowi Dollfus, 1931	*Nyctalus noctula* (Schreber) - Noctule bat	Kirikovka, Sumy region	AF151919	Tkach et al., 2000
Ophiosacculus mehelyi (Mödlinger, 1930)	*Eptesicus serotinus* (Schreber) - Serotine bat	near Odessa, Odessa region	AF480167	Tkach et al., 2002
Prosthodendrium chilostomum Mehlis, 1931	*Nyctalus noctula* (Schreber) - Noctule bat	Kirikovka, Sumy region	AF151920	Tkach et al., 2000
Prosthodendrium hurkovaae Dubois, 1960	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AF151922	Tkach et al., 2000
Prosthodendrium longiforme (Bhalerao, 1926)	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AF151921	Tkach et al., 2000
Prosthodendrium parvouterus (Bhalerao, 1926)	*Miniopterus schreibersi* (Kuhl) - Bent-winged Bat	Rubielos de Mora, Spain	AY220617	This study
Pycnoporus heteroporus (Dujardin, 1845)	*Pipistrellus kuhli* (Kuhl) - Kuhl's bat	Golopristansky district, Kherson region	AF151918	Tkach et al., 2000
Pycnoporus megacotyle (Ogata, 1939)	*Pipistrellus kuhli* (Kuhl) - Kuhl's bat	Golopristansky district, Kherson region	AF151917	Tkach et al., 2000
Microphallidae				
Floridatrema heardi Kinsella & Deblock, 1994	*Oryzomys palustris* (Harlan) - Marsh rice rat	Florida, USA	AY220632	This study
Maritrema arenaria Hadley & Castle, 1940	barnacle	Belfast Lough, Northern Ireland	AY220629	This study
Maritrema oocysta Lebour, 1907	*Hydrobia ulvae* (Pennant) - Laver spire shell	Belfast Lough, Northern Ireland	AY220630	This study
Maritrema subdolum Jägerskiöld, 1909	*Tringa erythropus* (Pallas) - Spotted redshank	Golopristansky district, Kherson region	AF151926	Tkach et al., 2000
Maritrema neomi Tkach, 1998	*Neomys anomalus* Cabrera - Miller's water shrew	Rakhiv, Zakarpatska region	AF151927	Tkach et al., 2000
Maritrema prosthometra Deblock & Heard, 1969	*Oryzomys palustris* (Harlan) - Marsh rice rat	Cedar Key, Florida, USA	AY220631	This study
Microphallus abortivus Deblock, 1974	*Hydrobia ulvae* (Pennant) - Laver spire shell	Belfast Lough, Northern Ireland	AY220626	This study
Microphallus basodactylophallus (Bridgman, 1969)	*Oryzomys palustris* (Harlan) - Marsh rice rat	Cedar Key, Florida, USA	AY220628	This study
Microphallus primas Jägerskiöld, 1908	*Hydrobia ulvae* (Pennant) - Laver spire shell	Belfast Lough, Northern Ireland	AY220627	This study
Microphallus similis Jägerskiöld, 1900	*Carcinus maenas* (L.) - Shore crab	Belfast Lough, Northern Ireland	AY220625	This study
Microphallidae gen. sp.	*Hydrobia ulvae* (Pennant) – Laver spire shell	Belfast Lough, Northern Ireland	AY220633	This study

Table 1 continued.

Digenean taxa	Host species	Geographical origin	GenBank N	Reference
Plagiorchiidae				
Haplometra cylindracea (Zeder, 1800)	*Rana arvalis* Nillsson - Moor frog	Kostylivka, Rakhiv district, Zakarpatska region	AF151933	Tkach et al., 2000
Lecithopyge rastellus Perkins, 1928	*Bombina variegata* (L.) - Yellow-bellied toad	Yaremcha, Nadvirna district, Ivano-Frankivsk region	AF151932	Tkach et al., 2000
Plagiorchis vespertilionis (Müller, 1780)	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AF151931	Tkach et al., 2000
Pleurogenidae				
Allassogonoporus amphoraeformis (Mödlinger, 1930)	*Pipistrellus kuhli* (Kuhl) - Kuhl's bat	Golopristansky district, Kherson region	AF151924	Tkach et al., 2000
Allassogonoporus amphoraeformis (Mödlinger, 1930)	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AY220620	This study
Brandesia turgida (Brandes, 1888)	*Rana lessonae* Camerano - Pool frog	near Lesniki, Kiev-Svyatoshin district, Kiev region	AY220622	This study
Candidotrema loossi (Africa, 1930)	*Rana ridibunda* Pallas - Lake frog	Vilkovo, Kiliya district, Odessa region	AY220621	This study
Loxogenes macrocirra (Caballero & Bravo-Hollis, 1949)	*Rana berlandieri* (Baird) - Rio Grande leopard frog	Guatemala	AY220624	This study
Parabascus joannae (Zdzitowiecki, 1967)	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AY220619	This study
Parabascus duboisi Hurková, 1961	*Myotis daubentoni* (Kuhl) - Daubenton's bat	Kiev	AY220618	This study
Parabascus semisquamosus (Braun, 1900)	*Pipistrellus kuhli* (Kuhl) - Kuhl's bat	Golopristansky district, Kherson region	AF151923	Tkach et al., 2000
Pleurogenes claviger (Rudolphi, 1819)	*Rana temporaria* L. - Common frog	Kiev	AF151925	Tkach et al., 2000
Pleurogenoides medians (Olsson, 1876)	*Rana lessonae* Camerano - Pool frog	near Lesniki, Kiev-Svyatoshin district, Kiev region	AF433670	Tkach et al., 2002
Prosotocus confusus (Looss, 1894)	*Rana lessonae* Camerano - Pool frog	Kiev region	AY220623	This study
Prosthogonimidae				
Prosthogonimus cuneatus (Rudolphi, 1809)	*Sturnus vulgaris* (L.) - European starling	Nezhin, Chernigiv region	AY220634	This study
Prosthogonimus ovatus (Rudolphi, 1803)	*Pica pica* (L.) – Magpie	Nezhin district, Chernigiv region	AF151928	Tkach et al., 2000
Schistogonimus rarus (Braun, 1901)	*Anas querquedula* L. – Garganey	Golopristansky district, Kherson region	AY116869	This study
Telorchiidae				
Telorchis assula (Dujardin, 1845)	*Natrix natrix* (L.) - Grass snake	near Lesniki, Kiev-Svyatoshin district, Kiev region	AF151915	Tkach et al., 1999

protocol of Tkach & Pawlowski (1999) or using a Qiagen® DNeasy™ tissue kit according to the manufacturer's instructions. In the latter case, the ethanol in the tissue samples was replaced with 1 M Tris-EDTA (pH 8) buffer via repeated washings, and specimens were lysed overnight in a rotating incubator.

The 5′ end of the lsrDNA gene spanning the D1-D3 variable domains was amplified using forward primers dig12 (5′-AAG CAT ATC ACT AAG CGG- 3′) or LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) with the reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′).

PCR reactions were performed in a total volume of 51 μl containing 42 μl water, 5 μl Taq buffer, 1 μl dNTP at a concentration of 10 pM/μl, 1 μl of each primer at concentration 10 pM/μl, 1 μl of Biotools' Taq polymerase at a concentration of 2 units/μl or 0.25 μl of Taq polymerase from Roche, and 1–1.5 μl of template gDNA extract. Alternatively, 25 μl PCR amplifications were performed using Ready-To-Go™ (Amersham Pharmacia Biotech) PCR beads (each containing ~1.5 units Taq DNA polymerase, 10 mM Tris-HCl at pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP and stabilisers, including BSA), 1 μl of gDNA extract and 10 mM of each PCR primer. The thermocycling profile was as follows: 3 min denaturation hold at 94 °C; 40 cycles of 30 sec at 94 °C, 30 sec at 52–56 °C, 2 min at 72 °C; and 7 min extension hold at 72 °C.

PCR products were purified using Qiagen Qiaquick™ columns and sequenced directly on an ABI Prism 377™ automated sequencer using ABI BigDye™ chemistry according to manufacturer's protocols. DNA products were sequenced in both directions using the two PCR primers and internal primers 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′) and ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′), as well as primers 400R (5′-GCA GCT TGA CTA CAC CCG-3′) and 900F (5′-CCG TCT TGA AAC ACG GAC CAA G-3′) in some cases. Contiguous sequences were assembled and edited using Sequencher™ ver. 3.1.1 (GeneCodes Corp.) and submitted to GenBank (accession numbers in Table 1).

Alignment and phylogenetic analyses

The new sequences have been aligned with sequences previously published by Tkach et al. (2000, 2001). Sequences were aligned initially with the aid of ClustalX using default parameters (Jeanmougin et al., 1998), and alignments then refined by eye using MacClade ver. 4.03 (Maddison & Maddison, 2000). The full alignment has been deposited with EBI and is available by anonymous FTP from FTP.EBI.AC.UK in directory /pub/databases/embl/align and via the EMBLALIGN database via SRS at http://srs.ebi.ac.uk, under the following accession ALIGN_000523. Exclusion sets are added as notes and the alignment may be adapted as a NEXUS file.

Regions that could not be aligned unambiguously were excluded from the analyses. Maximum parsimony (MP) was performed using PAUP* (Swofford, 2002, ver. 4.0b10) and Bayesian inference (BI) using MrBayes software (Huelsenbeck & Ronquist, 2001, ver. 2.01). The resulting networks were rooted with the outgroup taxa. Analyses by MP were performed using a heuristic search strategy (100 search replicates), random-addition of sequences and tree-bisection-reconnection (TBR) branch-swapping options. All characters were run unordered and equally weighted. Gaps were treated as missing data. Bayesian inference (BI) was employed using the following nucleotide substitution parameters: lset nst=6, rates=invgamma, ncat=4, shape=estimate, inferrates=yes and basefreq=empirical, that correspond to a general time reversible (GTR) model including estimates of the proportion of invariant sites (I) and gamma (G) distributed among-site rate variation. This model showed the best fit to the data using Modeltest (Posada & Crandall, 1998, ver. 3.06). Posterior probabilities were approximated over 300,000 generations, log-likelihood scores plotted and only the final 85% of trees where the log-likelihood had reached a plateau were used to produce the consensus tree. Nodal support was assessed by bootstrap resampling in MP (1,000 replicates); nodal support from majority-rule consensus trees found with BI were also utilised.

Results

In the analyses, members of six genera belonging to four families of the Plagiorchioidea, were used as outgroups (Figures 1, 2; Table 1). Another major superfamily-level lineage within the Plagiorchiata La Rue, 1957, namely the Renicoloidea Dollfus, 1939 (comprising the Renicolidae Dollfus, 1939 and the Eucotylidae Skrjabin, 1924), according to Tkach et al. (2001) and our new unpublished results, includes long-branching taxa which therefore were not included in the outgroup.

A total of 1,284 sites were available for alignment, of which 1,231 could be aligned unambiguously. Of the aligned positions, 729 were constant and 391 parsimony informative. There were few problematical regions within the alignment. The Lecithodendridae, with the exception of *Ophiosacculus*, were characterised by large deletions at positions 429–446, 449–459 and 463–472. With the possibility to compare lecithodendriid sequences with a large and diverse database of digeneans and other parasitic flatworms, we may say confidently that these fragments represent deletions, especially taking into account the highly derived nature of this family and the Plagiorchiata as a whole

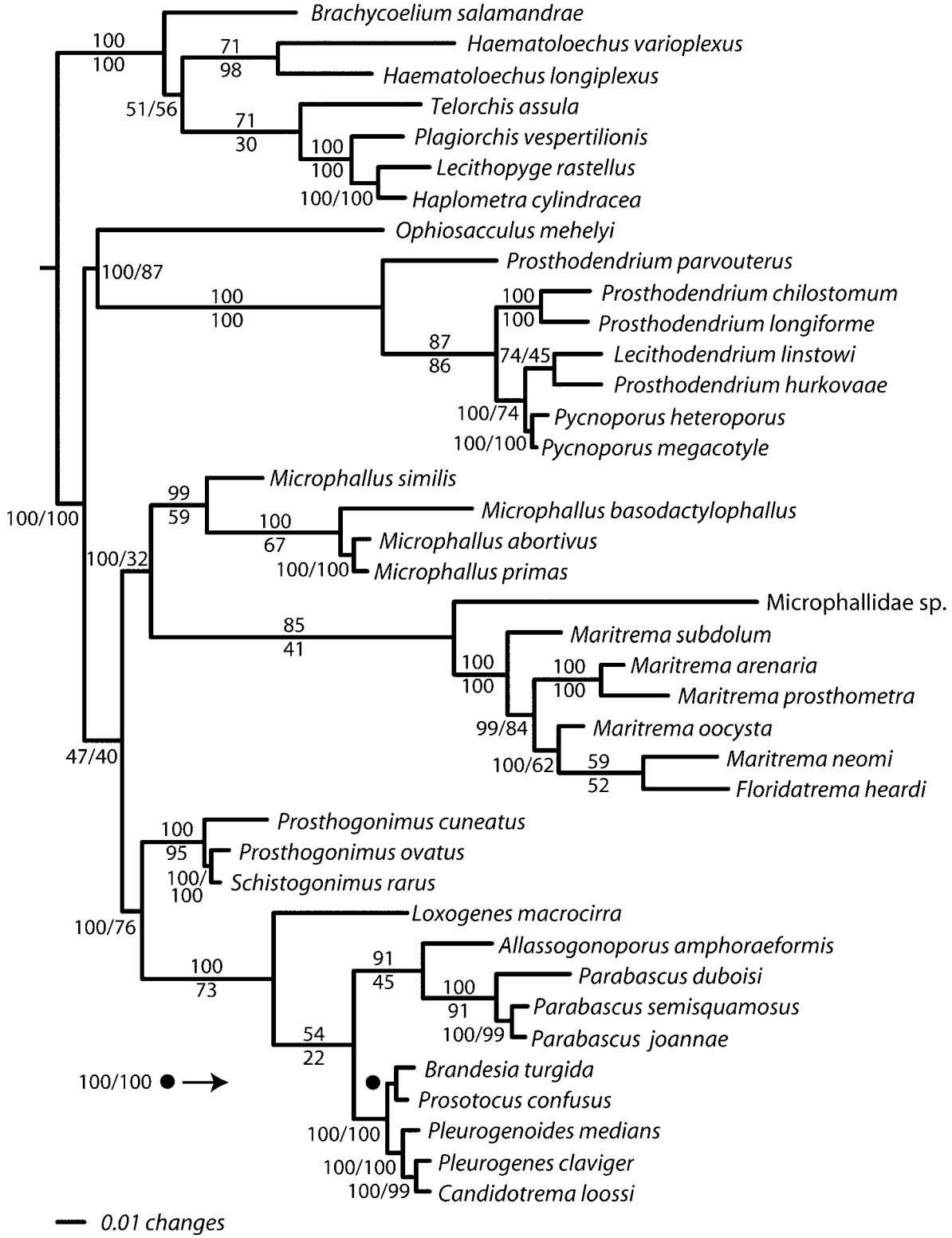

Figure 1. Bayesian inference phylogram depicting the interrelationships of the Microphalloidea. The tree represents the majority-rule consensus of 2,260 trees using the option contype=allcompat with the sumt command in MrBayes (Huelsenbeck, 2000) in order to provide mean branch lengths. Nodal support is indicated as Bayesian posterior probabilities above and maximum parsimony bootstrap percentages (n=1,000) below the branches. Taxonomic names are those given in Table 1.

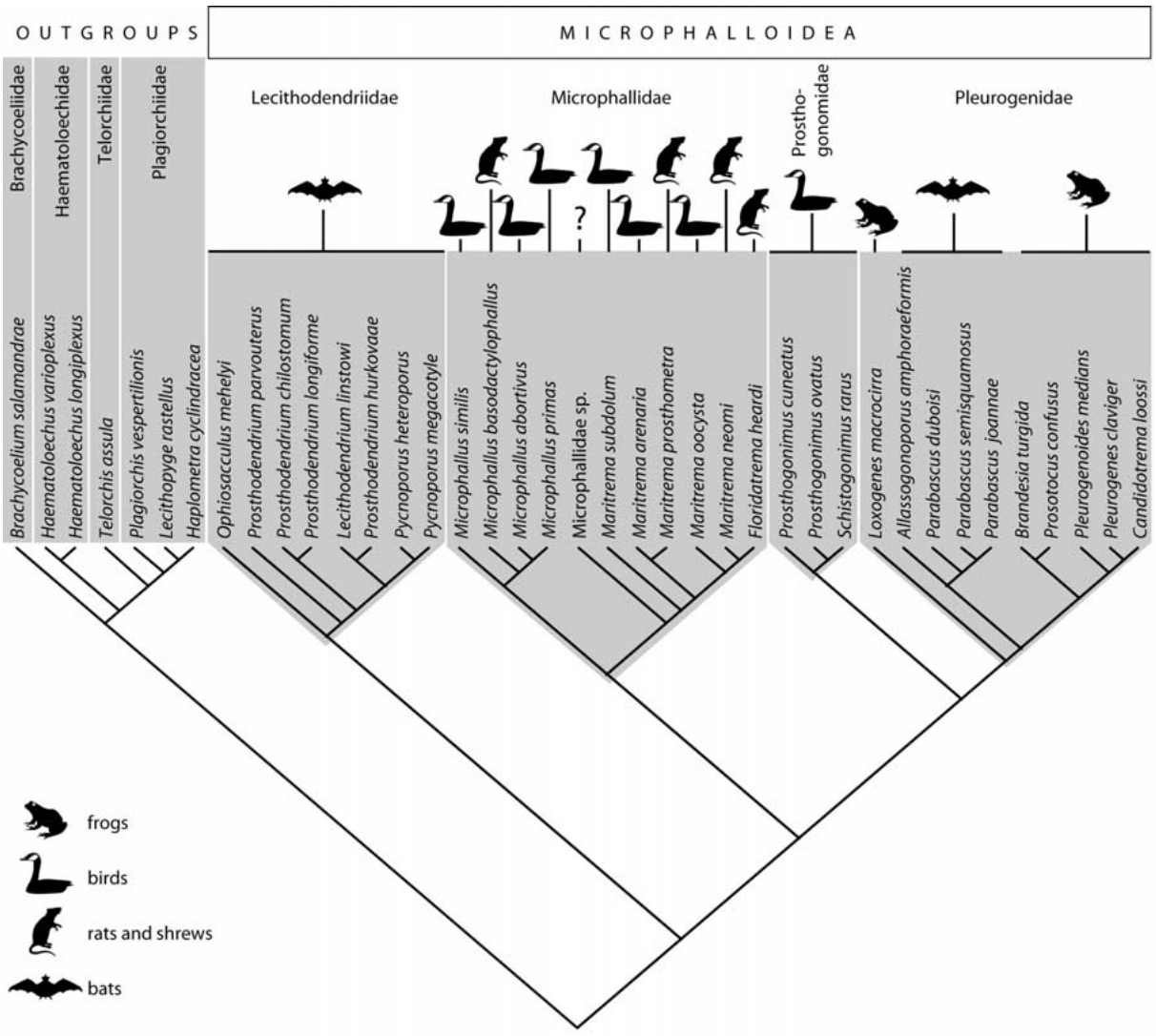

Figure 2. Cladogram indicating major taxonomic groups and host affinities.

(Brooks et al., 1985; Tkach et al., 2001; Cribb et al., 2001). Analyses with or without these regions had no effect on the final topology of the trees; here we present solutions including these regions, as they increase resolution among the remaining taxa. Among the ingroup taxa, lecithodendriids were characterised by the shortest sequences, with the shortest in *Lecithodendrium linstowi* Dollfus, 1931 (1,190 bp). The longest sequences were found in the microphallid genus *Maritrema* Nicoll, 1907 (1,258 bp in *M. subdolum* Jägerskiöld, 1908 and 1,270 bp in *M. arenaria* Hadley & Castle, 1940).

MP analysis yielded six equally parsimonious trees. The strict consensus was entirely compatible with the BI solution and we present the BI topology, with MP bootstrap and Bayesian nodal support, in Figure 1. Of the 300,000 generations computed, the first 74,000 were ignored when estimating the BI solution; the 'burn-in' was therefore 740 trees. In the tree, each of the families is very well supported, as is the clade uniting the Pleurogenidae with the Prosthogonimidae. The node placing *Loxogenes* Stafford, 1905 as the sister group to all other members of the Pleurogenidae is poorly resolved by both MP and BI analyses. The clade uniting the Microphallidae and Pleurogenidae + Prosthogonimidae is also poorly (47/40%) supported by both BI and MP analyses and therefore, we did not consider this topology meaningful. Within the

Lecithodendriidae (Figures 1, 2), *Ophiosacculus* occupies a basal position and is characterised by a long branch. Unexpectedly, *Prosthodendrium parvouterus* Bhalerao, 1926 is situated basally to the cluster uniting *Lecithodendrium* Looss, 1896, *Pycnoporus* Looss, 1899 and three other members of the genus *Prosthodendrium* Dollfus, 1931, one of which, *P. hurkovaae* Dubois, 1960, grouped together with *L. linstowi* in a weakly supported clade.

The second clade representing the Microphallidae contains two sub-clades, one of them containing species of *Microphallus* Ward, 1901 and the other members of *Maritrema* and *Floridatrema* Kinsella & Deblock, 1994, as well as an unidentified microphallid (DNA extraction obtained from a sporocyst) which may represent either another species of *Maritrema* or a member of one of the phylogenetically close genera.

The third major clade of the ingroup is the Pleurogenidae + Prosthogonimidae. In this generally well-resolved clade, weak (54/22%) support of the single node separating *Loxogenes* from other pleurogenids may suggest that this genus represents a separate lineage within the Pleurogenidae. *Allassogonoporus* forms a sub-clade with three species of *Parabascus* Looss, 1907, while five genera that include parasites of anuran amphibians in Europe (*Pleurogenes* Looss, 1896, *Pleurogenoides* Travassos, 1921, *Prosotocus* Looss, 1899, *Candidotrema* Dollfus, 1951 and *Brandesia* Stossich, 1899) form a well-resolved subclade with all nodes strongly supported (Figures 1, 2).

Discussion

The topology of the Microphalloidea obtained in the present study (Figures 1, 2) generally corresponds to the results obtained by Tkach et al. (2001, 2002) based on fewer taxa. The present phylogenetic estimate of the Microphalloidea corresponds best to that of Odening (1964a), with the exception of the Prosthogonimidae which he considered as a separate superfamily-level lineage of the Plagiorchiata. The Prosthogonimidae here belong to the Microphalloidea as a sister-taxon of the Pleurogenidae (see also Tkach et al., 2001). Representatives of some families included by Odening (1964a) in the Microphalloidea, namely the Eumegacetidae, Stomylotrematidae, Anenterotrematidae and Cortrematidae, were absent from our analyses. Brooks et al. (1989) included in it the families Microphallidae, Prosthogonimidae and Lecithodendriidae, which is in agreement with our results. It is unclear, however, where Brooks et al. (1985, 1989) would place the Pleurogenidae, as this family was not considered. In an earlier paper of Brooks et al. (1985), the Lecithodendrioidea and Microphalloidea were considered independent superfamilies.

Weak support for the Microphallidae + (Pleurogenidae + Prosthogonimidae) supports the results of Tkach et al. (2001) in which the Microphallidae appeared as an independent clade in some analyses or clustered together with other families with weak nodal support. In particular, it confirmed the presence of three main clades within the Microphalloidea, a strong affinity between the Pleurogenidae and Prosthogonimidae and lack of a close affinity between the Lecithodendriidae and Pleurogenidae; this is in contrast with the opinion of many authors, beginning with Odhner (1910) who was first to unite Lecithodendriinae and Pleurogeninae within the Lecithodendriidae (see 'Introduction'). Besides revealing the inter-familial relationships, the inclusion of many new taxa in our study allowed a greater resolution among species and genera within each of the major lineages of the Microphalloidea.

Among the Lecithodendriidae, the current analysis confirmed the position of *Ophiosacculus* within the family. Unlike other lecithodendriids, *O. mehelyi* (Mödlinger, 1930) possesses a seminal vesicle lying freely in the parenchyma, which, combined with the results of the molecular study, allowed Tkach et al. (2002) to establish a separate subfamily, the Ophiosacculinae, for this genus. The position of *Prosthodendrium parvouterus* in the phylogenetic tree is of particular interest, because it suggests that this species does not actually belong to *Prosthodendrium*. In our analysis, *Prosthodendrium* is represented also by *P. chilostomum* (Mehlis, 1831) and *P. longiforme* (Bhalerao, 1926), which are typical representatives of the genus in terms of morphology. The latter two species form a well-supported group in a clade derived in relation to *P. parvouterus* and containing members of *Lecithodendrium* and *Pycnoporus*. The separation of these three genera was traditionally based primarily on the position of vitelline glands and the position of reproductive system organs (Figure 3). *Prosthodendrium parvouterus* generally corresponds to the current diagnosis of *Prosthodendrium*, but its body is much wider than average across the genus. The vast majority of representatives of this genus possess a more or less elongate, elliptical body shape, while *P. parvouterus* has a relatively wider body, which is

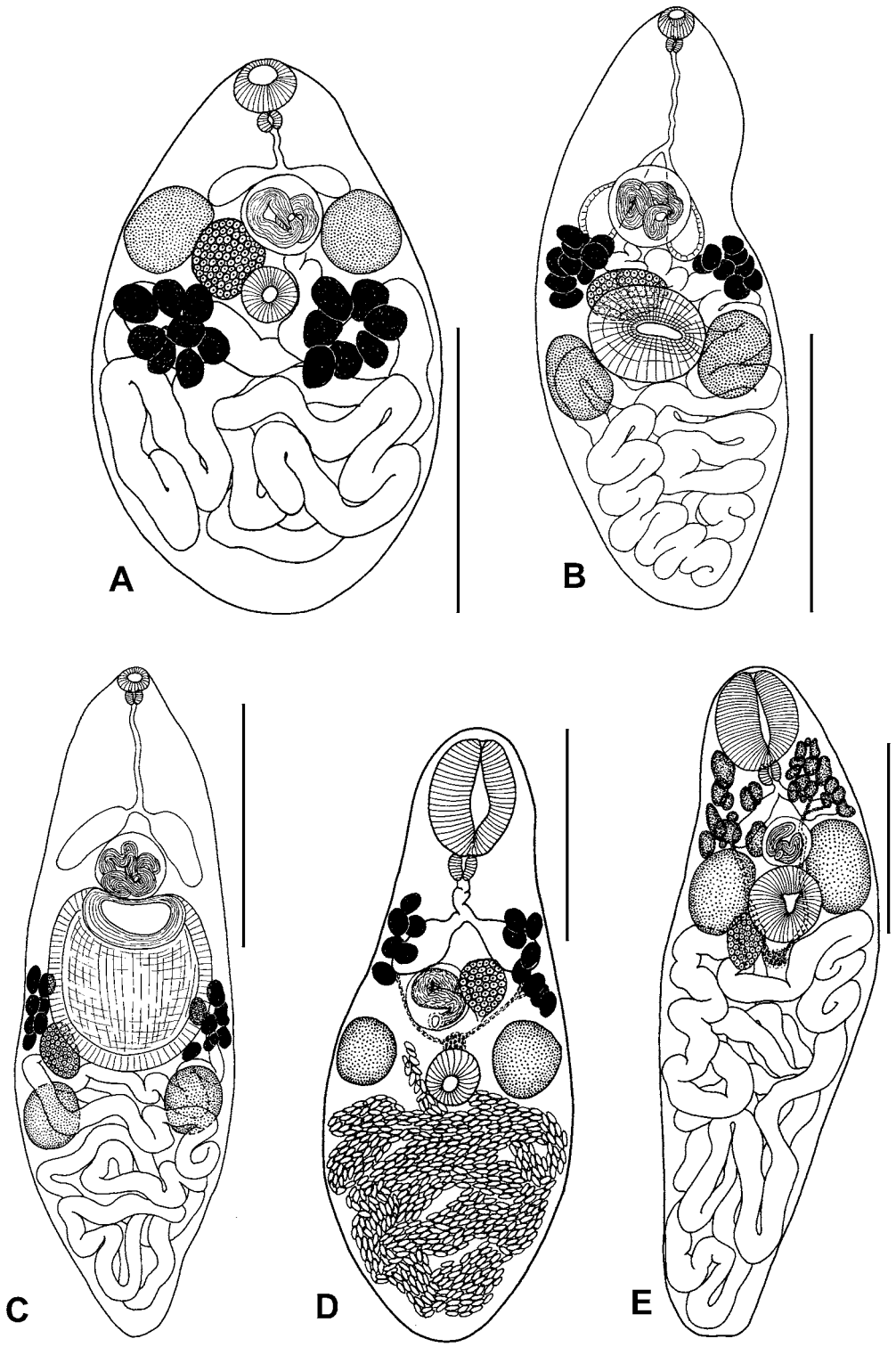

Figure 3. General morphology of members of lecithodendriid genera represented in our study. Note the differences in the position of vitelline follicles and testes. A. *Lecithodendrium linstowi*, B. *Pycnoporus megacotyle*, C. *Pycnoporus heteroporus*, D. *Prosthodendrium chilostomum*, *E. Prosthodendrium longiforme*. *Scale-bars*: 0.5 mm.

at least as wide as it is long, but can be wider than long (Figures 3, 4). In some respects, the body shape and arrangement of internal organs of *P. parvouterus* is more similar to that of *Paralecithodendrium* Odhner, 1911. The latter genus differs from *Prosthodendrium* only by the lobed ovary and therefore some (Dubois, 1960, 1962; Yamaguti, 1958, 1971; Odening, 1964b) have considered *Paralecithodendrium* as a synonym or subgenus of *Prosthodendrium*, while others (Skarbilovich, 1948; Salem, 1971) preferred to designate it as a separate genus. To test the utility of such morphological characters as the lobed ovary for generic differentiation in the Lecithodendriidae, and to verify the systematic position of *P. parvouterus*, some representatives of *Paralecithodendrium* must be included in further molecular phylogenetic studies. The situation may be more complicated, as there are several other lecithodendriid genera, which are morphologically similar to *Prosthodendrium* but differ from it by rather minor morphological characters. For instance, spination of the genital atrium area in *Acanthatrium* Faust, 1919 and presence of a lip-like structure around the genital atrium in *Ochoterenatrema* Caballero, 1943 render these taxa as candidates for further study. It was not possible for us to obtain specimens of representatives of these genera for analysis, but their examination is highly desirable to better elucidate interrelationships within the Lecithodendriidae. The present study also highlights the curious position of *Prosthodendrium hurkovaae* in the cluster including members of *Lecithodendrium* and *Pycnoporus*, while the typical *Prosthodendrium* (*P. chilostomum* and *P. longiforme*) form a separate clade. Moreover, *P. hurkovaae* seems to be closer to *Lecithodendrium* than to *Pycnoporus*. Although the majority of authors (Hurková, 1959, 1963; Dubois, 1960; Odening, 1964b; Sharpilo & Iskova, 1989) placed this species in *Prosthodendrium*, others (Caballero, 1961; Yamaguti, 1971) have allocated it to *Pycnoporus*. *Pycnoporus* appears at present to be a complex, most probably polyphyletic, group uniting species with very different body shapes, positions of different internal organs and sucker structure (Dubois, 1960; Yamaguti, 1958, 1971; Salem, 1971). It appears to us that a most prominent feature, characteristic for *Pycnoporus* spp. used in the present study [including the type-species, *P. heteroporus* (Dujardin, 1845)] is the unusual structure of the ventral sucker, which can be sac-like or at least deeply embedded under the tegumental surface (Figure 3B,C). The main morphological character differentiating *Prosthodendrium* and *Lecithodendrium* is the position of vitelline follicles, which are pre-testicular and pre-acetabular in *Prosthodendrium* but post-testicular and post-acetabular in *Lecithodendrium* (Figure 3). Vitelline follicles in typical members of *Pycnoporus* are pre-testicular (Figure 3). Curiously, in *P. hurkovaae*, the rosettes of vitelline follicles nearly overlap the testes with slight variations in the relative positions of these organs (Figure 5). According to the results of our molecular study, *P. hurkovaae* does not belong either to *Prosthodendrium* or to *Pycnoporus*. Taking into account the weak support for its position with *Lecithodendrium linstowi*, the most adequate solution of this problem might be establishing a separate genus for this species, but this is beyond the scope of the present study.

Separation of the Microphallidae into two subclades (Figures 1, 2) was expected, although some authors considered the Maritrematidae Nicoll, 1907 as a separate family (Baer, 1943; Bayssade-Dufour et al., 1993). Many more microphallid genera should be included in future studies in order to resolve the internal phylogeny of the Microphallidae and to test previously proposed hypotheses on the relationships and taxonomic status of its genera and subfamilies.

A point of particular interest is the position of *Floridatrema heardi* Kinsella & Deblock, 1994 nested in the tree among species of *Maritrema* Nicoll, 1907. The only morphological feature that allowed Kinsella & Deblock (1994) to establish a separate genus *Floridatrema* for this species, was the possession of anterior pre-caecal extensions of uterine loops reaching, on both sides, the level of the intestinal bifurcation or even the pharynx. Otherwise, Kinsella & Deblock (1994) considered *Floridatrema* to be the closest genus to *Maritrema*. However, molecular data convincingly demonstrate that this morphological feature is insufficient to constitute a basis for generic differentiation in the Microphallidae. Therefore, we transfer *F. heardi* to *Maritrema,* which thus acquires the name *Maritrema heardi* (Kinsella & Deblock, 1994) n. comb. and consequently, the monotypic *Floridatrema* becomes a junior synonym of *Maritrema*.

It is interesting that *Maritrema heardi* appears in a derived position within the Maritrematinae sub-clade, together with *M. neomi* Tkach, 1998, the only other species of microphallid from mammals present in our study. The two species are morphologically similar. Moreover, the uterus in *M. neomi* also extends pre-caecally on one side of the body (Tkach, 1998). Nodal support for this assemblage is, however, quite weak. *M. heardi* was described from a marsh rice rat

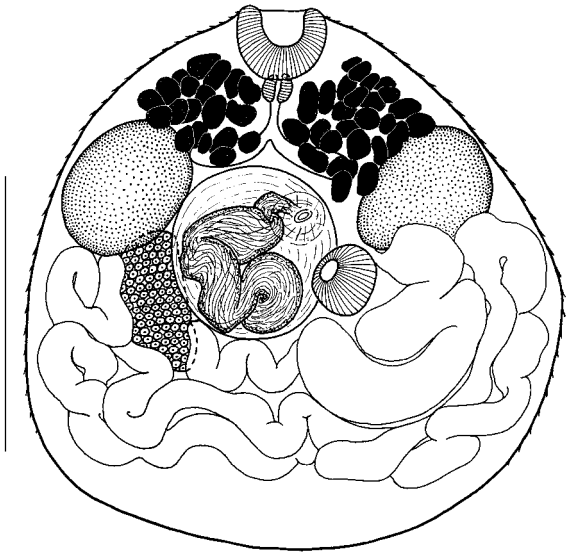

Figure 4. Morphology of *Prosthodendrium parvouterus*. *Scale-bar*: 0.5 mm.

Oryzomys palustris in Florida whereas *M. neomi* is a specific parasite of the water shrews *Neomys anomalus* and *N. fodiens* found in the Carpathian Mountains of Europe. The position of the two species in the phylogenetic tree suggests that these small mammals likely acquired microphallids from birds secondarily. Rice rats in salt marshes along the Gulf of Mexico coast have so far been found to host ten species of microphallids, eight of them also known from birds in the same area or elsewhere (Kinsella, 1988). Thus, it is probable that *M. heardi* is normally a bird parasite and only secondarily found in rice rats. It is interesting that marsh rice rats examined in the inland areas did not have microphallids. In contrast, *M. neomi* occurs in water shrews in the mountainous streams of the Carpathian Mountains, similar to other microphallids known from insectivores in Europe, namely *M. feliui* Gracenea, Montoliu & Deblock, 1993, *M. pyrenaica* Deblock & Combes, 1965 and *M. carpathica* Matskasi, 1984. No microphallids are known in birds from the Carpathian mountains which are situated very far from the closest sea coast. Therefore, in the case of microphallids parasitic in insectivores in Europe, the evolutionary 'capture' from birds seems to be a more complicated and longer evolutionary process than the acquisition of some species from birds inhabiting the same biotopes.

The third major clade within the ingroup includes two sub-clades corresponding to the Prosthogonimidae and Pleurogenidae. The taxa considered as pleurogenids in the present paper (Figure 2) have been allocated to different subfamilies of the Lecithodendriidae in several major monographic reviews of this group (Skarbilovich, 1948; Yamaguti, 1958, 1971; Sharpilo & Iskova, 1989).

Within the Prosthogonimidae, *Schistogonimus rarus* (Braun, 1901) surprisingly nests between two members of *Prosthogonimus*. As a result, *P. ovatus* (Rudolphi, 1803) appears to be much closer to *Schistogonimus rarus*, than to its congener *P. cuneatus* (Rudolphi, 1809). Pairwise comparison has shown that in the studied lsrDNA fragment, *P. ovatus* and *S. rarus* have only seven nucleotide differences, while *P. ovatus* and *P. cuneatus* differ in 47 sites, and *S. rarus* and *P. cuneatus* differ in 49 sites. These results suggest some morphological characters traditionally used in the generic differentiation of prosthogonimids require re-assessment. The only morphological character separating *Prosthogonimus* and *Schistogonimus* is the relative position of the male and female genital pores which are distinctly separate in *Schistogonimus rarus* (the type and only known member of the genus), while in all *Prosthogonimus* they open very close to each other. Apparently, this feature does not reflect a deep phylogenetic divergence between these taxa. In contrast, the presence of numerous coils of the uterus anterior to the ventral sucker unites *S. rarus* and *P. ovatus* (as well as some other *Prosthogonimus* species) and separates them from *P. cuneatus* (Figure 6). Until now, this feature was used for differentiation among different *Prosthogonimus* species, but may be indicative for some larger evolutionary lineages. However, we consider it premature to draw new generic boundaries and make any substantial systematic reshuffling in the Prosthogonimidae based on the limited data available. At the same time, our results convincingly demonstrate that *Schistogonimus* is not a valid genus and *S. rarus* should belong to *Prosthogonimus*. Moreover, since Braun (1901) originally placed *Schistogonimus rarus* in *Prosthogonimus*, we return to this species the name *Prosthogonimus rarus* Braun, 1901, while the name *Schistogonimus* Lühe, 1909 becomes a junior synonym of *Prosthogonimus* Lühe, 1899.

The well-supported sub-clade of the Pleurogenidae (Figures 1, 2) includes representatives of six genera containing parasites of amphibians and two genera parasitic in frogs. Somewhat surprisingly, a parasite of Central American anuran amphibians, *Loxogenes macrocirra* (Caballero & Bravo-Hollis, 1949), forms a branch basal to a well-supported, and internally well-resolved, clade comprising two subclades. One

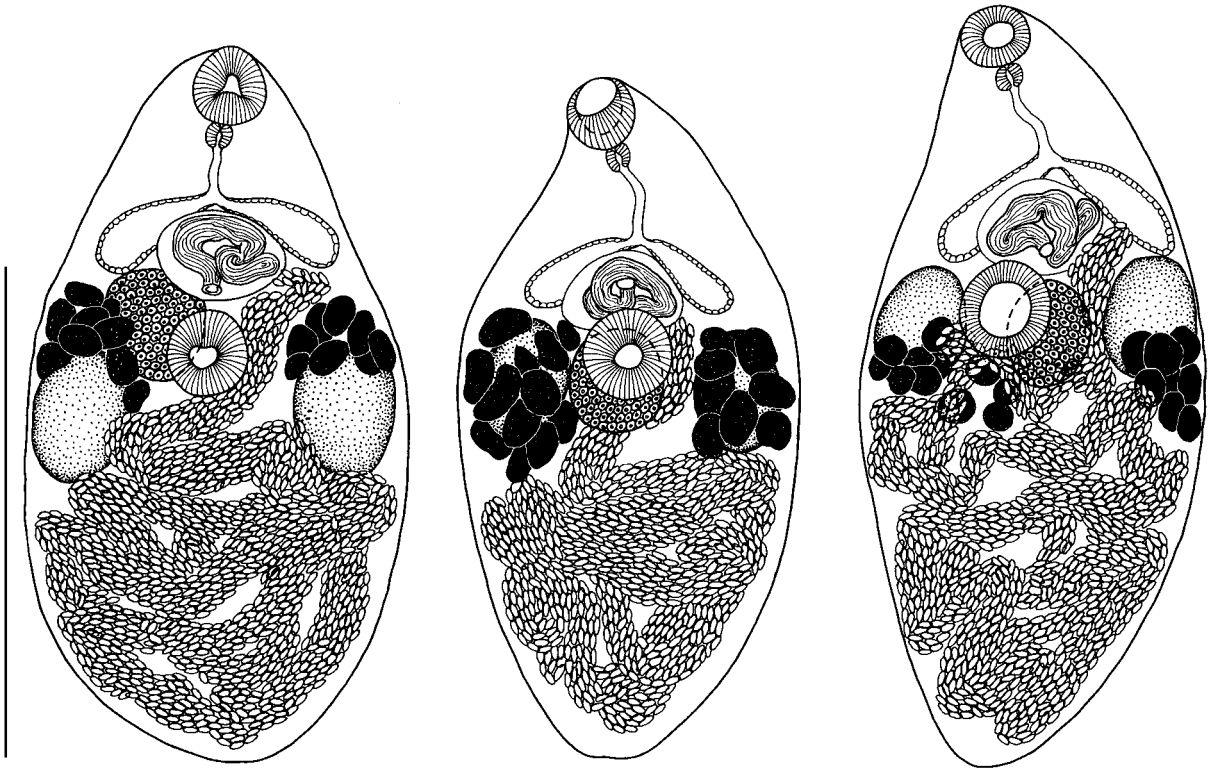

Figure 5. Variations in the relative position of the vitelline follicles and testes in *Prosthodendrium hurkovaae*. *Scale-bar*: 0.5 mm.

of these consists of five typical members of the Pleurogeninae, parasites of anuran amphibians in Europe (Yamaguti, 1971; Prudhoe & Bray, 1982; Sharpilo & Iskova, 1989); the second includes *Parabascus* + *Allassogonoporus*, both of which are represented in our study by parasites of bats. Unlike the five genera from amphibians represented in the phylogenetic tree (*Pleurogenes*, *Pleurogenoides*, *Candidotrema*, *Prosotocus* and *Brandesia*) that possess lateral or slightly sublateral genital atria, the genital pore in *Loxogenes* is situated relatively far from the lateral body margin. We did not find in the literature any information/suggestions regarding the phylogenetic relationships between these genera. Our results clearly indicate close affinities between *Pleurogenes* + *Candidotrema*, with *Pleurogenoides* being basal to them, on one side, and *Brandesia* + *Prosotocus*, on the other. Morphological differences between *Candidotrema* and *Pleurogenes* are very slight. They are somewhat more pronounced comparing *Candidotrema loossi* (Africa, 1930) and *Pleurogenes claviger* (Rudolphi, 1819) due to the differences in the position of the testes (in about the middle of the body in *C. loossi* and in the last third of the body, at the end of the caeca, in *P. claviger*). Differences between *C. loossi* and some other species of *Pleurogenes*, for instance, *P. intermedius* Issaichikov, 1926, are much less pronounced and are only found in the body shape and relative sucker size, which, in our opinion, can be considered only as interspecific differences. In fact, there is no morphological feature in *Candidotrema* that could reliably differentiate it from *Pleurogenes*. Obvious difficulties in such differentiation are seen in the keys presented by Yamaguti (1971) and Sharpilo & Iskova (1989). In both cases attempts to differentiate the two genera based on the position of the testes (for instance, intercaecal in *Candidotrema* against near posterior extremity in *Pleurogenes*, according to Yamaguti, 1971) omit the fact that some members of *Pleurogenes* also possess testes that are situated in the middle third of the body intercaecally or overlapping the caeca. Based on the high morphological similarity and the results of the molecular study, we return *C. loossi* to *Pleurogenes*, where it was originally described as *Pleurogenes loossi* Africa, 1930 (Africa, 1930; Srivastava, 1934; Khotenovsky, 1970). The monotypic *Candidotrema* Dollfus, 1951 is thus

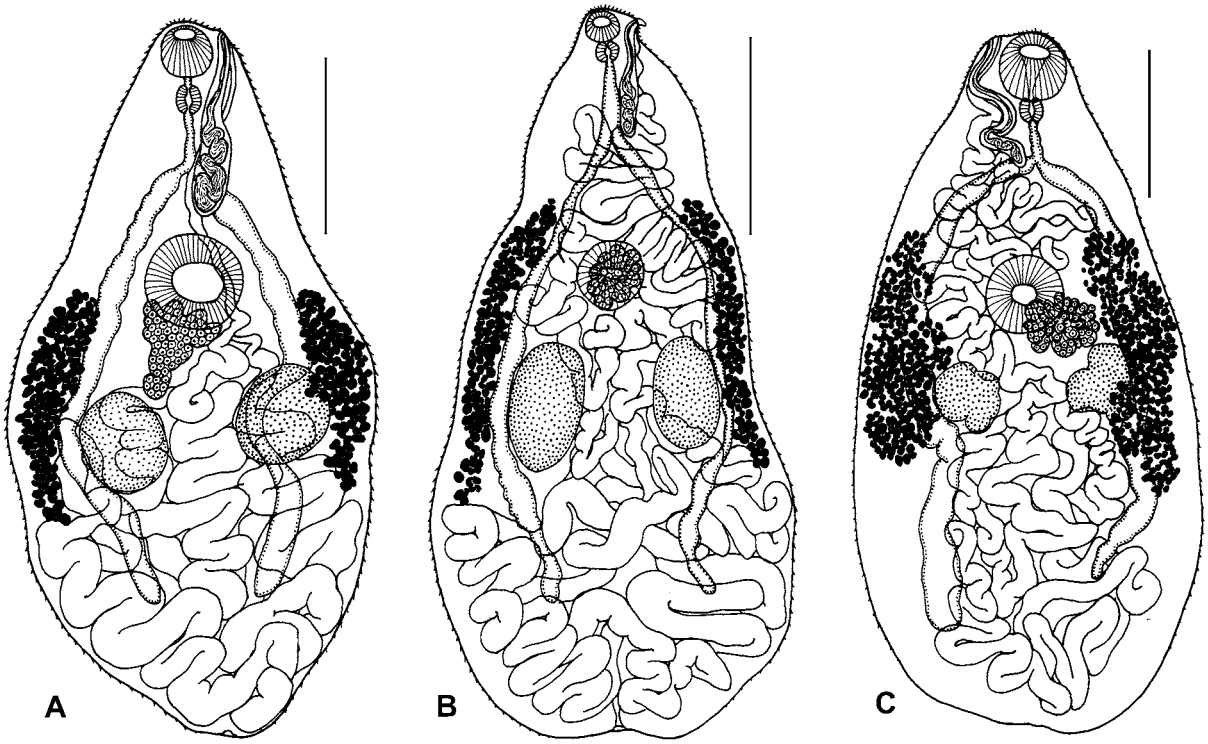

Figure 6. Morphology of representatives of Prosthogonimidae used in the present study. A. *Prosthogonimus cuneatus*, B. *P. ovatus*, C. *Schistogonimus rarus.* Note the differences in the relative position of male and female genital openings and presence/absence of the pre-acetabular uterine loops. *Scale-bars*: 1 mm.

considered here as a junior synonym of *Pleurogenes* Looss, 1896.

A close relationship between *Brandesia* and *Prosotocus* is supported by many morphological features, such as short caeca which end anteriorly to the ventral sucker, large vitelline follicles situated in clusters on both sides of the oral sucker, pre-acetabular testes and ovary, and the presence of uterine loops both posterior and anterior to the ventral sucker. *Brandesia* is, nevertheless, very different from *Prosotocus* due to its practically non-flattened body (resulting from the localisation in capsules in the intestinal wall), highly reduced hindbody and the position of the genital pore in relation to the ventral sucker.

Parabascus joannae Zdzitowiecki, 1967 has been described by Zdzitowiecki (1967) as *Czosnowia joannae*, transferred *Parabascus* by Khotenovsky (1970) and synonymised with *Parabascus duboisi* Hurková, 1961 by Skvortsov (1970). Yamaguti (1971) again considered *C. joannae* as the only species in *Czosnovia* Zdzitowiecki, 1967, but it is likely that, at the time of submission of Yamaguti's (1971) book for printing, he had not yet seen the papers of Skvortsov (1970) and Khotenovsky (1970). The species was considered valid and has subsequently been placed in *Parabascus* by Khotenovsky (1985), a viewpoint accepted by Sharpilo & Iskova (1989). Molecular data once again support the status of *P. joannae* (Zdzitowiecki, 1967) as an independent species and its position within *Parabascus*.

We would like to use this opportunity to correct the long-persisting problem of the authorship of the family Lecithodendriidae, which is most frequently attributed in the literature to Odhner (1910). On the other hand, some authors mention Looss (1902) as the author of the subfamily Lecithodendriinae. In fact, the author of both Lecithodendriinae and Lecithodendriidae should be Lühe (1901), who was the first to erect a member of the family-group, the Lecithodendriinae (see Lühe, 1901, p. 173).

In summary, the systematic implications of this study are:

1. With the present set of examined taxa, the Microphalloidea comprises three main lineages represented by the Lecithodendriidae, Microphallidae and Pleurogenidae + Prosthogonimidae. We anticipate, however,

that the addition of several important taxa (e.g. representatives of the Eumegacetidae and Cortrematidae) in future studies will increase the number of family-level clades.

2. At the level of families, it is confirmed that the Pleurogenidae and Lecithodendriidae are separate families and that *Allassogonoporus* (formerly the only genus of the Allassogonoporidae) should be included in the Pleurogenidae.

3. At the generic level, results of the present molecular study and analysis of morphological features suggests the synonymy of *Floridatrema* with *Maritrema*, *Candidotrema* with *Pleurogenes,* and *Schistogonimus* with *Prosthogonimus*. In the last case, a spatial separation between male and female genital pores in *Prosthogonimus rarus* n. comb. proved to be less important than was suggested by authors who considered *Schistogonimus* to represent a distinct genus. Our results have also demonstrated that *Prosthodendrium parvouterus* and *P. hurkovaae* do not belong to *Prosthodendrium,* which indicates that the utility of some morphological characters traditionally used in the generic differentiation of lecithodendriids from bats, in particular the position of the vitelline follicles, should be reconsidered. The exact systematic position of the above two species will likely be revealed upon inclusion of other lecithodendriids from bats in future studies.

We consider the present work a preliminary phylogenetic study of the Microphalloidea. In the future, such studies should include representatives of remaining genera and suprageneric taxa lacking in the present work due to the difficulties in obtaining specimens. We hope that this study will provoke further enquiries into the systematics and evolution of the Microphalloidea and thus enhance our knowledge of this diverse and highly derived group of digeneans.

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References

Africa, C.M. (1930) *Pleurogenes loossi* sp. nov. in the intestine of *Rana esculenta* Germani. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*. I. Abteilung **115**, 448–451.

Baer, J.G. (1943) Les trématodes parasites de la musraigne d'eau *Neomys fodiens* (Schreb.). *Bulletin de la Société Neuchâteloise Science Naturelles*, **68**, 33–84.

Bayssade-Dufour, C., Hugot, J.-P. & Albaret, J.-L. (1993) Analyse phénétique des Microphalloidea (Trematoda) d'après la chétotaxie des cercaires. *Systematic Parasitology*, **25**, 1–24.

Braun, M. (1901) Trematoden der Bursa Fabricii, des Eileiters und der Eier der Vogel. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, I. Abteilung, **29**, 12–19.

Brooks, D.R., O'Grady, R.T. & Glen, D.R. (1985) Phylogenetic analysis of the Digenea (Platyhelminthes: Cercomeria) with comments on their adaptive radiation. *Canadian Journal of Zoology*, **63**, 411–443.

Brooks, D.R., Bandoni, S.M., MacDonald, C.A. & O'Grady, R.T. (1989) Aspects of the phylogeny of the Trematoda Rudolphi, 1808 (Platyhelminthes: Cercomeria). *Canadian Journal of Zoology*, **67**, 2609–2624.

Caballero, E.C. (1961) Trematodos de los murcielagos de Mexico. VIII. Catalogo taxonomico de los trematodos que parasitan a los murcielagos (Mammalia, Chiroptera). *Anales del Instituto de Biología*, **31**, 215–287.

Cribb, T.H., Bray, R.A., Littlewood, D.T.J., Pichelin, S.P. & Herniou, E.A. (2001) The Digenea. *In*: Littlewood, D.T.J. & Bray, R.A. (Eds) *Interrelationships of the Platyhelminthes*. London: Taylor & Francis, pp. 168–185.

Deblock, S. (1971) Contribution à l'étude des Microphallidae Travassos, 1920 (Trematoda). XXIV. Tentative de phylogénie et de taxonomie. *Bulletin du Muséum National d'Histoire Naturelle, 3ème Série, 7, Zoologie*, **7**, 353–468.

Dubois, G. (1960) Contribution à l'étude des Trématodes de Chiroptères. Revision du sous-genre *Prosthodendrium* Dollfus, 1931 et des genres *Lecithodendrium* Looss, 1896 et *Pycnoporus* Looss, 1899. *Revue Suisse de Zoologie*, **67**, 1–80.

Dubois, G. (1962) Contribution à l'étude des Trématodes de Chiroptères. Revision du sous-genre *Paralecithodendrium* Odhner, 1911. *Revue Suisse de Zoologie*, **69**, 385–405.

Huelsenbeck, J.P. (2000) MrBayes: Bayesian inference of phylogeny. Rochester, NY: Department of Biology, University of Rochester.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Hurková, J. (1959) *Prosthodendrium* (*Prosthodendrium*) *carolinum* n. sp. and some less known bat trematodes in ČSR. *Vestnik Československé Zoologické Společnosti*, **23**, 23–33.

Hurková, J. (1963) Bat trematodes in Czechoslovakia. I. A systematical review of occurring species. *Vestnik Československé Zoologické Společnosti*, **27**, 250–276.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G. & Gibson, T.J. (1998) Multiple sequence alignment with Clustal X. *Trends in Biochemical Sciences*, **23**, 403–5.

Khotenovsky, I.A. (1970) [Family Pleurogenidae Looss, 1899]. *Osnovy Trematodologii*, **23**, 137–296. (In Russian).

Khotenovsky, I.A. (1985) Trematodes of the genus *Parabascus* (Trematoda, Pleurogenidae) from the bats of the Holarctic. *Parazitologicheskii Sbornik*, 1985, 125–133. (In Russian).

Kinsella, J.M. (1988) Comparison of helminths of rice rats, *Oryzomys palustris*, from freshwater and saltwater marshes in Florida. *Proceedings of the Helminthological Society of Washington*, **55**, 275–280.

Kinsella, J.M. & Deblock, S. (1994) Contribution à l'étude des Microphallidae Travassos, 1920 (Trematoda). XLVI. Description de *Floridatrema heardi* n. gen., n. sp., parasite d'*Oryzomys palustris* (Mammifere) des États-Unis. *Parasite*, **1**, 45–50.

Looss, A. (1899) Weitere Beitrage zur Kenntnis der Trematodenfauna Aegyptens, zugleich Verseuch einer natürlichen Gliederung des Genus *Distomum* Reitzius. *Zoologischer Jahrbücher*, *Abteilung für Systematik*, **12**, 521–784.

Looss, A. (1902) Über neue und bekannte Trematoden aus Seeschildkröten. Nebst Erörterungen zur Systematik und Nomenclatur. *Zoologischer Jahrbücher, Abteilung für Systematik*, **16**, 411–894.

Lühe, M.F.W. (1901) Zweie neue Distomen aus indischen Anuren. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, I. Abteilung, **30,** 166–177.

Maddison, W.P. & Maddison, D.R. (2000) MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.03. Sunderland, Massachusetts: Sinauer Associates.

Morozov, F.N. (1955) [Family Gymnophallidae Morozov, 1955]. *Osnovy Trematodologii*, **10**, 315–335. (In Russian).

Odening, K. (1959) Das Exkretionssystem von *Omphalometra* und *Brachycoelium* (Trematoda, Digenea) und die Taxonomie der Unterordnung Plagiorchiata. *Zeitschrift für Parasitenkunde,* **19**, 442–457.

Odening, K. (1964a) Zur Taxonomie der Trematodenunterordnung Plagiorchiata. *Monatsberichte der Deutschen Akademie der Wissenschaften zu Berlin*. **6**, 191–198.

Odening, K. (1964b) Exkretionssystem und systematische Stellung einiger Fledermaustrematoden aus Berlin und Umgebung nebst Bemerkungen zum lecithodendrioiden Komplex. *Zeitschrift für Parasitenkunde*, **24**, 453–483.

Odening, K. (1971) Möglichkeiten der Herstellung des bisher unbekannten Zusammenhangs von Cercarien und adulten Trematoden mit Hilfe detailierter Kenntnisse des Exkretionssystems nebst Ausführungen zum weiteren Ausbau des Systems der Plagiorchiata. *Perspektiven der Cercarienforschung. Parasitologische Schriftenreihe*, **21**, 57–72.

Odhner, T. (1910) Nordostafrikanische Trematoden, grösstenteils vom Weissen Nil (von der schweidischen zoologischen Expedition gesammelt). *Results of the Swedish Zoological Expedition to Egypt and White Nile (1901)* (Jägerskiöld) (23A), 170 pp.

Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A. & Littlewood, D.T.J. (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology*, **33**, 733-755.

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Prudhoe, S. & Bray, R.A. (1982) *Platyhelminth parasites of the Amphibia*. London: British Museum (Natural History) and Oxford University Press, 217 pp.

Richard, J. & Prévot, G. (1974) Chétotaxie des cercaires de *Gymnophallus nereicola* Rebecq et Prévot, 1962 et *Gymnophallus fossarum* Bartoli, 1965 (Trematoda, Gymnophallidae). *Bulletin du Muséum National d'Histoire Naturelle*, 3ème Série, 233, Zoologie, **157**, 845–853.

Salem J.B. (1971) Studies of the trematodes of bats found in Hederabad, Andhra Pradesh (India). Part: 1. *Rivista di Parassitologia*, **32**, 159–178.

Sharpilo, V.P. & Iskova, N.I. (1989) [The Fauna of the Ukraine: Trematoda: Plagiorchiata.] *Fauna Ukrainy*, **34**, 1–278. (In Russian).

Skarbilovich, T.S. (1948) [Family Lecithodendriidae Odhner, 1911]. *Osnovy Trematodologii*, **2**, 237–590. (In Russian).

Skvortsov, V.G. (1970) [Trematodes of the family Lecithodendriidae from bats in Moldavia]. *Parazity Zhivotnych i Restenij*. Kishinev: Kartya Moldovenyaske, **5**,17–36. (In Russian).

Srivastava, H.D. (1934) On new trematodes of frogs and fishes of the United Provinces, India. Part III. On a new genus *Mehraorchis* and two new species of *Pleurogenes* (Pleurogenetinae) with a systematic discussion and revision of the family Lecithodendriidae. *Bulletin of the Academy of Sciences*, Allahabad, **3**, 239–256.

Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony *and Other Methods. 4 ed., Sunderland, Massachusetts: Sinauer Associates.

Tkach, V.V. (1998) *Maritrema neomi* n. sp. (Digenea: Microphallidae) from water shrews (*Neomys*). *Journal of Parasitology*, **84**, 846–849.

Tkach, V., Grabda-Kazubska, B., Pawlowski, J. & Swiderski, Z. (1999) Molecular and morphological evidences for close phylogenetic affinities of the genera *Macrodera*, *Leptophallus*, *Metaleptophallus* and *Paralepoderma* (Digenea, Plagiorchioidea). *Acta Parasitologica*, **44**, 170–179.

Tkach, V.V., Lotz, J., Swiderski, Z. & Esteban, J.G. (2002) On the systematic position of *Ophiosacculus* Macy, 1935 (Digenea: Lecithodendriidae), with the erection of the Ophiosacculinae n. subfam. *Systematic Parasitology*, **53**, 159–167.

Tkach, V. & Pawlowski J. (1999) A new method of DNA extraction from the ethanol-fixed parasitic worms. *Acta Parasitologica*, **44**, 147–148.

Tkach, V., Pawlowski J. & Mariaux, J. (2000) Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. *International Journal for Parasitology*, **30**, 83–93.

Tkach, V., Pawlowski, J., Mariaux, J. & Swiderski, Z. (2001) Molecular phylogeny of the suborder Plagiorchiata and its position in the system of Digenea. *In*: Littlewood, D.T.J. & Bray, R.A. (Eds) *Interrelationships of the Platyhelminthes*. London: Taylor & Francis, pp. 186–193.

Yamaguti, S. (1958) *Systema helminthum. The digenetic trematodes of vertebrates*. Vols.1, 2. New York – London: Interscience, 1575 pp.

Yamaguti, S. (1971) *Synopsis of digenetic trematodes of vertebrates*. Tokyo: Keigaku Publishing Co., 1074 pp.

Zdzitowiecki, K. (1967) *Czosnowia joannae* n. g., n. sp. (Lecithodendriidae) a new trematode species from the bat, *Myotis daubentoni* (Kuhl, 1819). *Acta Parasitological Polonica*, **14**, 405–408.