**When proglottids and scoleces conflict: phylogenetic relationships and a family-level
classification of the Lecanicephalidea (Platyhelminthes: Cestoda)**

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24 Abstract

25 This study presents the first comprehensive phylogenetic analysis of the interrelationships of the
26 morphologically diverse elasmobranch-hosted tapeworm order Lecanicephalidea, based on
27 molecular sequence data. With almost half of current generic diversity having been erected or
28 resurrected within the last decade, an apparent conflict between scolex morphology and proglottid
29 anatomy has hampered the assignment of many of these genera to families. Maximum likelihood
30 and Bayesian analyses of two nuclear markers (D1–D3 of *lsrDNA* and complete *ssrDNA*) and two
31 mitochondrial markers (partial *rnrL* and partial *cox1*) for 61 lecanicephalidean species representing
32 22 of the 25 valid genera were conducted; new sequence data were generated for 43 species and 11
33 genera, including three undescribed genera. The monophyly of the order was confirmed in all but
34 the analyses based on *cox1* data alone. *Sesquipedalapex* placed among species of *Anteropora* and
35 was thus synonymized with the former genus. Based on analyses of the concatenated dataset, eight
36 major groups emerged which are herein formally recognized at the familial level. Existing family
37 names (i.e., Lecanicephalidae, Polypocephalidae, Tetragonocephalidae, and Cephalobothriidae) are
38 maintained for four of the eight clades, and new families are proposed for the remaining four groups
39 (Aberrapecidae n. fam., Eniochobothriidae n. fam., Paraberrapecidae n. fam., and Zanobatocestidae
40 n. fam.). The four new families and the Tetragonocephalidae are monogeneric, while the
41 Cephalobothriidae, Lecanicephalidae and Polypocephalidae comprise seven, eight and four genera,
42 respectively. As a result of their unusual morphologies, the three genera not included here (i.e.,
43 *Corrugatocephalum*, *Healyum* and *Quadcuspibothrium*) are considered incertae sedis within the
44 order until their familial affinities can be examined in more detail. All eight families are newly
45 circumscribed based on morphological features and a key to the families is provided. Aspects of
46 morphological evolution and host associations are discussed in a phylogenetic context for each
47 family and for the order as a whole. Lecanicephalidean genera lacking apical structures were
48 confirmed as the earliest diverging lineages. Proglottid anatomy was determined to be much more
49 conserved and indicative of phylogenetic affinities than scolex morphology. Collectively, the

50 Lecanicephalidea parasitize three of the four orders of Batoidea—their almost exclusive absence
51 from skates (Order Rajiformes) appears to be real; only a few records from sharks exist. At the
52 family level, the breadth of host associations is correlated with taxonomic diversity of the family.
53 The degree to which factors such as intermediate host use or host specificity at any stage in the life-
54 cycle shape these patterns is currently unknown.

55

56 *Keywords:* Elasmobranchs, Tapeworms, Evolution, Molecular phylogeny, Morphology, Host
57 associations, New families

58

1. Introduction

The Lecanicephalidea Yamaguti, 1959 is one of the more morphologically diverse (Fig. 1) and speciose of the nine orders of cestodes hosted by elasmobranchs. The order gained brief notoriety early in the 20th century when their larvae were implicated as pearl-inducing agents in the pearl oyster industry in Ceylon (Shipley and Hornell, 1904, 1906; Herdman and Hornell, 1906) and the South Pacific (Seurat, 1906a, b). For the most part, as adults, lecanicephalideans parasitize batoids (i.e., stingrays, guitarfish, etc.), although a few records from sharks also exist (e.g., Caira et al., 1997; Jensen, 2005). Historically, possession of the following combination of features has been used to typify its members: suckers or simple bothridia (i.e., muscular holdfast organs), an apical modification of the scolex proper and an apical organ (sensu Caira et al., 1999) essential in attachment, and a unique combination of features associated with the reproductive organs (i.e., a vagina that opens into the genital atrium posterior to the cirrus sac, and a sizeable vas deferens often expanded into a sacciform external seminal vesicle that extends from the level of the ovarian bridge to the cirrus sac; Jensen, 2005).

In her excellent treatment of its taxonomic history, Butler (1987) referred to the group as “a collection of little-known genera,” the taxonomy of which is “in a very confusing state” (Butler, 1987; p. 105). Indeed, throughout its history, many genera once assigned to the group have since been transferred to other orders. Even its ordinal status has been contested. It has been recognized as a family, most often within the order Tetraphyllidea, by many authors (e.g., Braun, 1894–1900; Meggitt, 1924; Southwell, 1925; Fuhrmann, 1931; Riser, 1955; Euzet, L., 1959. *Recherches sur les cestodes tétraphyllides des sélaciens des côtes de France*, Docteur és Sciences Naturelles. Université de Montpellier, Montpellier, p. 263; Joyeux and Baer, 1961), but others (e.g., Hyman, 1951; Wardle and McLeod, 1952; Yamaguti, 1959; Schmidt, 1970, 1986) have recognized it as an independent order—an opinion shared by both Euzet (1994) and Jensen (2005) who are responsible for the two most recent comprehensive revisions of the group. However, even these workers differed in their treatments of families and genera. Whereas Euzet (1994) recognized five valid

genera, and four families, all but one of which (i.e., Tetragonocephalidae) was monogeneric, Jensen (2005) recognized 12 genera, but refrained from assigning them to families until such time as their phylogenetic relationships could be formally assessed—ideally following collection of new material fixed for both morphological and molecular work. The substantial conflict between scolex morphology and proglottid anatomy that appears to exist across the order (Jensen, 2005) has impeded formalization of family-level classification in this order.

As a result of intensive global collecting efforts over the past two decades, focused on hosts not previously examined for tapeworms, since 2005, eight new lecanicephalidean genera have been erected (Jensen et al., 2011, 2014; Koch et al., 2012; Cielocha and Jensen, 2013; Cielocha et al., 2014; Jensen and Russell, 2014). In addition, five poorly known genera are now considered valid (Cielocha and Jensen 2011; Cielocha et al., 2013; Caira and Jensen, 2014). As a consequence, the order currently comprises 25 genera and approximately 88 valid species, and is in even more dire need of a family-level classification.

A key component of phylogenetic revision is resolution of the apparent conflict between scolex morphology and proglottid anatomy seen across the order (Jensen, 2005). A number of genera exhibit scoleces that are remarkably similar (e.g., Fig. 2) but have proglottids with widely different anatomies. Similarly, a number of genera with conspicuously divergent scolex morphologies (e.g., Fig. 1R, S, V–Z) exhibit proglottid anatomies that are almost identical (e.g., Fig. 3). It is unclear which, if either, of these suites of characters is phylogenetically more informative than the other.

The most extensive previous phylogenetic analyses, which included as many as 18 lecanicephalidean species from 12 genera, were based on morphological data alone (Caira et al., 1999, 2001; Jensen, 2005). Previous studies employing molecular sequence data have generally focused on the higher-level relationships among cestodes and thus have included only a handful of lecanicephalidean species (e.g., Olson and Caira, 1999; Olson et al., 1999, 2001; Healy et al., 2009; Waeschenbach et al., 2012). Most recently, Caira et al. (2014) included 18 species representing 11 lecanicephalidean genera in their investigation of the interrelationships and ordinal rearrangements

of elasmobranch-hosted cestodes based on complete *ssrDNA* (small subunit [18S] nuclear rDNA) and partial *lsrDNA* (D1–D3) (partial large subunit [28S] nuclear rDNA) sequence data. However, even those analyses were insufficient to allow lecanicephalidean interrelationships to be rigorously assessed given the diversity across the order.

The Lecanicephalidea is the only remaining major elasmobranch-hosted tapeworm order for which the interrelationships have not been rigorously explored. The goals of this study are: (i) to conduct the first comprehensive phylogenetic analyses of lecanicephalidean interrelationships based on molecular sequence data for the majority of known genera; (ii) to use the results of those analyses to inform the circumscription of families based on morphological features; (iii) to revise the family diagnoses presented by Euzet (1994), and resurrect or establish new families as needed to formulate a robust family-level classification for the order. With this phylogenetic framework in place, we discuss aspects of morphological evolution and host associations in this circumglobally distributed tapeworm order.

2. Materials and methods

2.1. Specimen preparation

All specimens for which sequence data were generated de novo as part of this study were collected between 1996 and 2013; all were fixed in 95% ethanol in the field. DNA was extracted from the middle or posterior portions of most specimens, and the remaining portion(s) (i.e., scolex and posterior proglottid[s]) were prepared as whole mounts to serve as hologenophores (Pleijel et al., 2008). In the cases of two species (*Elicilacunosus* sp. and *Anteropora comicus* [Jensen, Nikolov & Caira, 2011] n. comb.), DNA was extracted from a free proglottid or a strobila; photo vouchers were taken of the specimens prior to extraction. No whole-mounted vouchers or photo-vouchers are available for two species: the whole worm of New Genus 11 n. sp. 2, the smallest species included in the analysis, and the proglottid of *Paraberrapex manifestus* Jensen, 2001.

Specimens prepared as whole mounts, as histological sections or for scanning electron microscopy (SEM) were fixed in 10% formalin buffered with seawater, transferred to 70% ethanol for storage and prepared following the methods described by Jensen et al. (2014). Photomicrographs of whole mounts and histological sections were taken using a Leica FireCam DFC 320 or 480 (Leica Microsystems Inc., Buffalo Grove, IL, USA) mounted on a Zeiss Axioskop 2 plus (Zeiss, Thornwood, NY, USA), using differential interference contrast. Specimens prepared for SEM were examined with a FEI Versa 3D Dual Beam scanning electron microscope (FEI, Hillsboro, OR, USA) at the Microscopy and Analytical Imaging Laboratory at the University of Kansas, Lawrence, Kansas, USA.

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2.2. Taxon and gene selection

In total, 67 lecanicephalidean specimens representing 61 species were included in the analysis (Table 1). These consisted of representatives of three undescribed genera (Figs. 1G, V and W) and 22 of the 25 valid genera—not included were the ditypic *Healyum* Jensen, 2001, and the monotypic *Corrugatocephalum* Caira, Jensen & Yamane, 1997 and *Quadcuspibothrium* Jensen, 2001. The four *genera inquirendae* of Jensen (2005) remain poorly known and were also not included (i.e., *Kystocephalus* Shipley and Hornell, 1906; *Sephenicephalum* Shinde, Sarwade and Jadhav, 1980 or 1982; *Staurobothrium* Shipley and Hornell, 1905, and *Thysanobothrium* Shipley and Hornell, 1906). It is important to note that because much of the available material came from survey work involving novel geographic regions and/or host taxa, 38 of the 61 species have not yet been formally described. The advantages of the expanded taxon sampling provided by these specimens were considered to far outweigh issues that might arise from preliminary specific determinations. However, to verify identities and facilitate future identification of specimens sequenced, hologenophores and photo vouchers of specimens sequenced were deposited in the Lawrence R. Penner Parasitology Collection (LRP) at the University of Connecticut, USA (see Table 1). Host

161 and locality data, and GenBank and museum accession numbers for all ingroup specimens included
 162 in the analyses are provided in Table 1.

163 The four markers targeted in this study consisted of two nuclear markers (the variable domains
 164 D1–D3 of *lsrDNA* [~1,500 bp] and complete *ssrDNA* [~2,000 bp]) and two mitochondrial markers
 165 (partial large mitochondrial ribosomal RNA subunit [*rrnL*; ~900 bp] and partial cytochrome *c*
 166 oxidase subunit I [*cox1*; ~600 bp towards the 5' end]). Nuclear sequence data were included for all
 167 ingroup specimens. These data were generated de novo for 50 of the 67 lecanicephalidean
 168 specimens; data for the remaining 17 ingroup specimens and for the four outgroup species came
 169 from Caira et al. (2014). Mitochondrial DNA (mtDNA) sequence data for *rrnL* and *cox1* sequence
 170 data were included for 54 and 62 of the 67 lecanicephalidean specimens, respectively. These data
 171 were generated de novo for all ingroup specimens and for two of the four outgroup species; mtDNA
 172 sequence data for *Litobothrium nickoli* Olson & Caira, 2001 came from Waeschenbach et al. (2012)
 173 and those for *Triaenophorus stizostedionis* Miller, 1945 from Scholz et al. (2013).

174 The following four species were used as outgroups: (i) *Paragrillotia similis* (Linton, 1909)
 175 Dollfus, 1969 (Trypanorhyncha) (TE-51; LRP No. 4280 [hologenophore]) from *Ginglymostoma*
 176 *cirratum* (Bonnaterre) (GC-1), Florida Bay, Florida, USA (**KF685909** [*lsrDNA*]; **KF685779**
 177 [*ssrDNA*]; **KU248946** [*rrnL*]; **KU249102** [*cox1*]); (ii) *Rhabdotobothrium anterophallum* Campbell,
 178 1975 (Rhinebothriidea) (Rhab; BMNH2001.1.31.3–4 [paragenophore]) from *Mobula hypostoma*
 179 (Bancroft) (M-99-2442), Gulf of Mexico, USA (**AF286961** [*lsrDNA*]; **AF287000** [*ssrDNA*];
 180 **KU248947** [*rrnL*]; **KU249103** [*cox1*]); (iii) *Litobothrium nickoli* (Litobothriidea) (TE-113; LRP
 181 No. 8321 [hologenophore]) from *Alopias pelagicus* Nakamura (BJ-713), Santa Rosalia, Baja
 182 California Sur, Mexico (**KF685907** [*lsrDNA*]; **KF685844** [*ssrDNA*]) and *L. nickoli* from *A.*
 183 *pelagicus* (BJ-719), Santa Maria, Baja California, Mexico (**JQ268549** [*rrnL*, *cox1*]); and (iv)
 184 *Triaenophorus stizostedionis* (Bothriocephalidea) (PBI-41; IPCAS No. 657 [paragenophore]) from
 185 *Sander vitreus* (Mitchill), Lake Winnebago, Wisconsin, USA (**KR780900** [*lsrDNA*]; **KR780947**
 186 [*ssrDNA*]; **KR780861** [*rrnL*]; **KR780809** [*cox1*]).

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188 2.3. Generation of sequence data

189 Extractions of genomic DNA were done using a BioSprint 96 robotic workstation in
 190 combination with the BioSprint 96 DNA Blood Kit (QIAGEN, Valencia, CA, USA) following the
 191 manufacturer's instructions. Methodology for PCR protocols and PCR purification follows Scholz
 192 et al. (2013). For PCR, the following primers were used: ZX-1 (5'-
 193 ACCCGCTGAATTTAAGCATAT-3'; modified from van der Auwera et al., 1994) and 1500R (5'-
 194 GCTATCCTGAGGGAACTTCG-3'; Olson et al., 2003; Tkach et al., 2003) for amplification of
 195 partial *lsrDNA* (variable domains D1–D3); WormA (5'-GCGAATGGCTCATTAATCAG-3') and
 196 WormB (5'-CTTGTTACGACTTTTACTTCC-3') (Littlewood and Olson, 2001) for amplification
 197 of complete *ssrDNA*; Cest16SFgen (5'-TRCCTTTTGCATCATG-3') and Cest_16SRc (5'-
 198 AATAGATAAGAACCGACCTGGC-3') (; (Scholz et al., 2013) for amplification of partial *rrnL*;
 199 and PBI-cox1F_PCR (5'-CATTTTGCTGCCGGTC**ARCAYATGTTYTGRTTTTTTGG**-3') and
 200 PBI-cox1R_PCR (5'-CCTTTGTCGATACTGCCAA**RTAATGCATDGGRAA**-3') (Scholz et
 201 al., 2013) for amplification of partial *cox1*. Because of the high sequence variability in *cox1*,
 202 degenerate primers were designed to ensure amplification across a range of cestode species
 203 (emboldened regions in primers listed above are complementary to the target sequence). These
 204 primers were then extended at the 5' end to provide non-ambiguous sequences for sequencing
 205 (although for the 5' end a successful forward sequencing primer was subsequently designed in the
 206 emboldened region and was extended at the 3' end; see below). PCR products were sequenced
 207 using the following sequencing primers: the two PCR primers and internal primers 300F (5'-
 208 CAAGTACCGTGAGGGAAAGTTG-3'; Littlewood et al., 2000), 300R (5'-
 209 CAACTTTCCCTCACGGTACTTG-3'; Littlewood et al., 2000), ECD2 (5'-
 210 CTTGGTCCGTGTTTCAAGACGGG-3'; Littlewood et al., 2000), 400R (5'-
 211 GCAGCTTGACTACACCCG-3'; Reyda and Olson, 2003), 1090F (5'-
 212 TGAAACACGGACCAAGG-3'; Littlewood et al., 2008), and 1200F (5'-

213 CCCGAAAGATGGTGAAGTATGC-3'; Telford et al., 2003) for *lsrDNA*; the two PCR primers
 214 and internal primers 300F (5'-AGGGTTCGATTCCGGAG-3'; Elwood et al., 1985), 300R (5'-
 215 TCAGGCTCCCTCTCCGGA-3'; Littlewood and Olson, 2001), 600R (5'-
 216 ACCGCGGCKGCTGGCACC-3'; Littlewood and Olson, 2001), 1270F (5'-
 217 ACTTAAAGGAATTGACGG-3'; Littlewood et al., 2000), 1270R (5'-
 218 CCGTCAATTCCTTTAAGT-3'; Littlewood et al., 2000), 1200F (5'-CAGGTCTGTGATGCCC-
 219 3'; Littlewood and Olson, 2001), and 1200R (5'-GGGCATCACAGACCTG-3'; Littlewood and
 220 Olson, 2001) for *ssrDNA*; the two PCR primers were used to sequence *rrnL*; and PBI-cox1F_seq
 221 (5'-CATTTTGCTGCCGGTCA-3') and PBI-cox1R_seq (5'-TAATGCATDGGRAAAAAAC-3')
 222 (Scholz et al., 2013) for *cox1*; emboldened letters in PBI-cox1R_seq indicate the 3' end extension
 223 beyond the PCR primer annealing position.

224

225 2.4. Sequence alignment and phylogenetic analyses

226 Contigs were assembled using Sequencher 4.8 (GeneCodes Corporation, Ann Arbor, MI, USA).
 227 Primer sites were excluded from all sequences. Sequences were aligned using MAFFT version
 228 6.611b (Kato, 2008) with 1000 cycles of iterative refinement and the *genafpair* algorithm. An
 229 alignment mask, excluding sites of uncertain positional homology, was generated using ZORRO
 230 (Wu et al., 2012). ZORRO uses a pair Hidden Markov Model and a weighted sum of pairs scheme
 231 (guided by a guide tree) that sums up the probability that a given alignment column appears over
 232 the total alignment landscape, thus providing an objective estimate of whether positions consist of
 233 correctly aligned, homologous residues. Default settings were used, except for the invocation of the
 234 "-sample" option; positions with confidence scores <4 were excluded from subsequent analyses.

235 In total, five phylogenetic analyses were conducted using Maximum Likelihood (ML). Details
 236 of each analysis are as follows: (i) Analysis 1, ML of complete *ssrDNA* for all 71 specimens; (ii)
 237 Analysis 2, ML of partial *lsrDNA* for all 71 specimens; (iii) Analysis 3, ML of *rrnL* for 58
 238 specimens; (iv) Analysis 4, ML of *cox1* for 66 specimens; (v) Analysis 5, ML of all four genes

combined for all 71 specimens. JModeltest 2.1.7 (Guindon and Gascuel, 2003; Darriba et al., 2012) was used to estimate the best-fitting models of nucleotide substitution (GTR+I+ Γ for *lsrDNA*, *ssrDNA* and *cox1*, and TPM3uf+I+ Γ for *rrnL*). ML analyses of the datasets for each individual locus (Analysis 1–4) and the concatenated dataset were conducted using the desktop version of Garli 2.01 (Zwickl, D.J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Doctoral dissertation, University of Texas at Austin, TX, USA). For all five analyses, 20 independent ML runs were conducted using default settings with the following adjustments: ‘genthreshfortopoterm = 100000’ and ‘significanttopochange = 0.0001’. Analyses of the individual loci were run unpartitioned using the model specified in JModeltest 2.1.7 (see above). Partitioned models (GTR+I+ Γ for *lsrDNA*, *ssrDNA* and *cox1*, and TPM3uf+I+ Γ for *rrnL*) were used for the individual loci in the concatenated dataset. Model parameters were estimated separately for each partition. ML bootstrap support was estimated using Garli 2.01 implemented in the Extreme Science and Engineering Discovery Environment (XSEDE) and accessed via the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway (Miller et al., 2010). Three separate runs of 100 bootstrap replicates each were conducted using the same configuration, models, and partition strategies as were used for the five ML analyses with following changes from the defaults settings: ‘genthreshfortopoterm = 10000’, ‘significanttopochange = 0.01’, and ‘treerejectionthreshold = 20.0’. Bootstrap results were summarized on the ‘best’ tree resulting from the 20 independent ML runs using SumTrees 4.0.0 in DendroPy 4.0.3 (Sukumaran and Holder, 2010; available at <https://github.com/jeetsukumaran/DendroPy>.)

For Bayesian inference (BI) of the concatenated dataset, partitioned models as determined by PartitionFinder v1.1.1 (Lanfear et al., 2012) were used and run as unlinked across partitions. The partitioned models were as follows: GTR+I+ Γ for partitions *lsrDNA+ssrDNA*, *rrnL*, and *cox1*. A Bayesian analysis was performed using Mr. Bayes v3.2.6 (Ronquist et al., 2012) in two Markov chain Monte Carlo (MCMC) runs of four chains each, with a burn-in of 25% and default heating

parameters. Parameters and trees were sampled every 5000 generations. The program Tracer v1.6.0 (Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1.6, available from <http://beast.bio.ed.ac.uk/Tracer>) was used to obtain the effective sample size (ESS) of all parameters in both analyses and to assess parameter stationarity. Convergence of Bayesian analyses was further assessed using the Program ‘are we there yet?’ (AWTY; Nylander et al., 2008). After just over 45 million generations the BI analysis was terminated, and the parameters and resulting 9022 trees for each run (6767 trees post-burn-in) were evaluated. Following the exclusion of the initial 25% of sampled trees, the average S.D. of split frequencies was 0.0287, which indicates that the runs had converged with regards to the well-supported nodes on the tree. Comparison of posterior probability values for each node in the samples of trees obtained from the two separate analyses using AWTY suggested some degree of discordance between posterior probability values and topologies. However, overall, all parameters appeared to have converged on a stationary distribution and possessed ESSs of >2500, suggesting that the posterior distributions of each parameter had been satisfactorily sampled. In addition, the Potential Scale Reduction Factor (PSRF) for all parameter values was either 1.000 or 1.001.

Trees resulting from the ML analyses are shown with ML bootstrap values. In addition, posterior probability values, and summary matrices (four-squares) of individual locus support for lineages and major clades resulting from the separate analyses (i.e., Analysis 1–4) are provided on the tree resulting from analysis of the concatenated dataset of the four loci.

3. Results

3.1. *Lecanicephalidean interrelationships*

The aligned *ssrDNA* sequence data consisted of 2395 bp, 444 of which were excluded because of uncertain positional homology; 1473 of the remaining 1951 bp were invariable. The aligned *lsrDNA* sequence data consisted of 1668 bp, 200 of which were excluded; 786 of the remaining 1468 bp were invariable. The aligned *rrnL* sequence data consisted of 912 bp, 261 of which were

291 excluded; 285 of the remaining 651 bp were invariable. The aligned *cox1* sequence data consisted
 292 of 557 bp none of which were excluded; 261 were invariable.

293 ML analyses of the individual loci yielded tree topologies that differed substantially from one
 294 another and also from those resulting from analysis of the concatenated dataset. The BI tree of the
 295 concatenated dataset was essentially congruent with that resulting from the ML analysis of the same
 296 data (i.e., ML Analysis 5) with the exception of *Eniochobothrium* Shipley & Hornell, 2006
 297 grouping in a polytomy with Clade 4+Clade 5 and Clade 7+Clade 8, rather than as the sister clade
 298 to Clade 7+Clade 8, and the placement of *Polypocephalus helmuti* Jensen, 2001 as sister taxon to
 299 members of *Hornellobothrium* Shipley & Hornell, 2006, rather than as sister to *Polypocephalus* sp.
 300 3. The topology resulting from ML Analysis 5 has been chosen for presentation and discussion here
 301 (Fig. 4). The trees resulting from analyses of the four individual loci (Analyses 1–4) are provided in
 302 Supplementary Figs. S1–S4.

303 The monophyly of the Lecanicephalidea overall was highly supported in trees resulting from all
 304 analyses except Analysis 4 (ML of *cox1*) which yielded a topology in which some
 305 lecanicephalideans grouped among the outgroup taxa. The existence of a major clade to the
 306 exclusion of *Aberrapex* Jensen, 2001, *Paraberrapex* Jensen, 2001, and *Zanobatocestus* Jensen,
 307 Mojica & Caira, 2014 was also supported in trees resulting from all analyses except Analysis 4
 308 (*cox1* only).

309 A total of eight groups of lecanicephalideans emerged from the trees resulting from the analyses
 310 of the concatenated dataset (Fig. 4) and also from analysis of *lsrDNA* (Analysis 2). The eight clades
 311 are as follows: Clade 1, more appropriately referred to as a lineage because it is represented by only
 312 a single species, was *Paraberrapex*. Clade 2, also represented by only a single species, was
 313 *Aberrapex*. Clade 3 consisted of the two species of *Zanobatocestus*. Clade 4 consisted of four
 314 species of *Tetragonocephalum* Shipley & Hornell, 1905; this clade was highly supported in all
 315 analyses, except in those of *cox1* alone (Analysis 4). Clade 5 consisted of species of *Flapocephalus*
 316 Deshmukh, 1979; *Polypocephalus* Braun, 1878; *Hornellobothrium* Shipley & Hornell, 1906; New

317 Genus 11; *Seussapex* Jensen & Russell, 2014; New Genus 12; *Anthemobothrium* Shipley &
 318 Hornell, 1906; *Anteropora* Subhapradha, 1955; and *Sesquipedalapex* Jensen, Nikolov & Caira,
 319 2011. This group received strong support in Analysis 2 and 5, and the BI analysis, reduced support
 320 in Analysis 1, but was not recovered in the trees resulting from the remaining two analyses. Clade 6
 321 consisted of the four species of *Eniochobothrium* Shipley & Hornell, 1906 and was also highly
 322 supported in all analyses except Analysis 4 (i.e., of *cox1* alone). Clade 7 consisted of species of
 323 *Elicilacunosus* Koch, Jensen & Caira, 2012; *Rexapex* Koch, Jensen & Caira, 2012; *Collicocephalus*
 324 Koch, Jensen & Caira, 2012; *Stoibocephalum* Cielocha & Jensen, 2013; and two of the species
 325 originally assigned to *Tylocephalum* Linton, 1890 (i.e., *T. campanulatum* Butler, 1987 and *T.*
 326 *koenneckeorum* Jensen, 2005), but whose position on the tree suggests assignment to
 327 *Stoibocephalum*, *Hexacanalisis* Perrenoud, 1931; *Lecanicephalum* Linton, 1890; and
 328 *Floriparicapitus* Cielocha, Jensen & Caira, 2014. This clade was highly supported in Analysis 2
 329 and 5, and the BI analysis, weakly supported in Analysis 1 and 3, and was not recovered in the tree
 330 resulting from Analysis 4. Clade 8 consisted of *Tylocephalum*, New Genus 3, and all species
 331 identified as belonging to *Cephalobothrium* Shipley & Hornell, 1906 and *Adelobothrium* Shipley,
 332 1900. This clade was well supported in the tree resulting from Analysis 2, recovered with strong
 333 support in the tree resulting from Analysis 1 and the BI analysis (albeit excluding *Tylocephalum*),
 334 and was weakly supported in Analysis 5; no support for this clade was seen in Analysis 4; it was not
 335 recovered in Analysis 3 because *rrnL* sequence data for *Tylocephalum* could not be obtained.

336 The tree resulting from analysis of *cox1* alone (Analysis 4) diverged most from those resulting
 337 from the other analyses. With the exception of the monophyly of Clade 3, consisting solely of the
 338 two species of *Zanobatocestus*, the clades supported in the other analyses were not recovered in the
 339 *cox1* tree. This gene was considered to be least informative. In fact, the tree resulting from analysis
 340 of this gene alone also failed to support the congeneric affinities of species of *Eniochobothrium* and
 341 *Tetragonocephalum*, contrary to molecular support from all other analyses and unique
 342 morphological features.

In spite of various instabilities across some analyses, we believe our results provide sufficient evidence to justify the recognition of eight families within the Lecanicephalidea. Existing family names can be applied to four of the eight clades; new families are established for the remaining four clades.

4. Discussion

The eight families recognized here accommodate all but the three lecanicephalidean genera not treated herein. In each case, morphological features allowing for effective circumscription of each family are presented. This revised classification scheme requires reconfiguration of the four existing families beyond erection of the four new families. Diagnoses for all eight families are presented below, along with discussion of membership and host associations. The schematic in Fig. 5 illustrates generic membership and host associations for each family based on the topology in Fig. 4.

4.1. *Lecanicephalidean families*

Clade 1: Paraberrapecidae n. fam. (e.g., Fig. 1Q)

Diagnosis: Scolex with 4 acetabula; apical modification of scolex proper and apical organ absent. Acetabula in form of oval, uniloculate bothridia. Cephalic peduncle absent. Worms euapolytic. Proglottids craspedote, non-laciniate; immature proglottids of anterior strobila not laterally expanded; circumcortical longitudinal muscle bundles absent. Testes in 2 columns, 1 layer deep in cross-section, located in single field mainly anterior to genital pore. Vas deferens extending from level of ovarian bridge to cirrus sac, not expanded to form external seminal vesicle; internal seminal vesicle absent. Cirrus sac pyriform; cirrus unarmed, thin-walled. Genital pores lateral, alternating irregularly; genital atrium shallow. Ovary H-shaped in dorso-ventral view, bilobed in cross-section. Vagina medial, opening into genital atrium posterior to cirrus sac. Seminal receptacle absent. Uterus saccate, medial, extending from anterior of ovary to level of genital pore. Vitellarium

369 follicular; vitelline follicles in 2 lateral bands; each band consisting of 2 columns of follicles,
 370 extending entire length of proglottid, uninterrupted by ovary. Excretory vessels not observed. Eggs
 371 packaged in cocoons; cocoons oblong, containing more than 50 eggs. Parasites of *Squatina* Duméril
 372 (Squatinidae Bonaparte); eastern Pacific Ocean, western Atlantic Ocean.

373 Type and only known genus: *Paraberrapex* Jensen, 2001.

374 Remarks: The Paraberrapecidae n. fam. is proposed here to house *Paraberrapex*, which is
 375 currently ditypic (see Jensen, 2001; Mutti and Ivanov, 2016), and has not previously been assigned
 376 to a family. The genus is represented in our analyses by only one of its two known species. This
 377 appears to be the earliest, or among the earliest, diverging lineage of lecanicephalideans. In addition
 378 to representing a molecularly distinct lineage, this is one of only two families characterized by the
 379 absence of an apical structure (apical modification of the scolex proper or apical organ) in the adult
 380 form. It differs from the other family that lacks an apical structure (i.e., Aberrapecidae n. fam.) in
 381 that, for example, the testicular field begins approximately at the level of the cirrus sac, rather than
 382 at the anterior margin of the ovary, and in that the ovary is bilobed, rather than tetralobed, in cross-
 383 section.

384

385 Clade 2: Aberrapecidae n. fam. (e.g., Fig. 1A)

386 Diagnosis: Scolex with 4 acetabula; apical modification of scolex proper and apical organ
 387 absent. Acetabula in form of oval, uniloculate bothridia. Cephalic peduncle absent. Worms
 388 euapolytic. Proglottids craspedote, laciniate or non-laciniate; immature proglottids of anterior
 389 strobila not laterally expanded; circumcortical longitudinal muscle bundles absent. Testes in 1–2
 390 columns, 1 layer deep in cross-section, in single field anterior to ovary. Vas deferens extending
 391 from level of Mehlis' gland to cirrus sac, expanded to form external seminal vesicle in some;
 392 internal seminal vesicle present. Cirrus sac pyriform; cirrus unarmed, thin-walled. Genital pores
 393 lateral, alternating irregularly; genital atrium shallow. Ovary H-shaped in dorso-ventral view,
 394 tetralobed in cross-section. Vagina lateral, opening into genital atrium posterior to cirrus sac.

395 Seminal receptacle absent. Uterus saccate, medial, extending from ovarian bridge to posterior
 396 margin of cirrus sac or to near anterior margin of proglottid. Vitellarium follicular; vitelline follicles
 397 in 2 lateral bands; each band consisting of 2–3 columns of follicles, extending entire length of
 398 proglottid and interrupted by ovary, or extending only to anterior margin of ovary. Excretory
 399 vessels in single lateral pair. Eggs and cocoons unknown. Parasites of *Myliobatis* Cuvier and
 400 *Aetomylaeus* Garman (Myliobatidae Bonaparte), and *Taeniura* Müller & Henle (Dasyatidae
 401 Jordan); Celebes Sea, Gulf of California, Gulf of Carpentaria, and southwestern Atlantic Ocean.
 402 Type and only known genus: *Aberrapex* Jensen, 2001.

403 Remarks: The name Aberrapecidae n. fam. is erected to accommodate *Aberrapex*, which has not
 404 previously been assigned to a family (see Jensen, 2001). Although represented in our analyses by
 405 only one of its five known species, this lineage is sufficiently divergent from all other
 406 lecanicephalideans to justify its designation as a distinct family. Not only did it group as an
 407 independent lineage based on analysis of molecular sequence data, but its members differ from all
 408 but one other family in lacking an apical structure in the adult form. It differs from the
 409 Paraberrapecidae n. fam. in that, for example, the testicular field begins at the anterior margin of the
 410 ovary, rather than at the level of the cirrus sac, in that the ovary is tetralobed, rather than bilobed, in
 411 cross-section, and in that the distal and proximal bothridial surfaces are covered with conspicuous
 412 hastate (Fig. 1A) rather than inconspicuous gladiate (Fig. 1Q) spinitriches.

413

414 Clade 3: Zanobatocestidae n. fam. (e.g., Fig. 1Ba)

415 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 416 scolex proper and apical organ. Acetabula in form of biloculate bothridia. Apical modification of
 417 scolex proper narrower or wider than scolex proper, invaginable or not. Apical organ internal or
 418 external, retractable or not, non-invaginable, primarily glandular or muscular. Cephalic peduncle
 419 absent. Worms euapolytic. Proglottids craspedote, non-laciniate; immature proglottids of anterior
 420 strobila not laterally expanded; circumcortical longitudinal muscle bundles absent. Testes in 1

421 irregular column, 1 layer deep in cross-section, in single field anterior to ovary. Vas deferens
 422 extending from near posterior margin of ovary to level anterior to cirrus sac, not expanded to form
 423 external seminal vesicle; internal seminal vesicle absent. Cirrus sac pyriform; cirrus unarmed, thin-
 424 walled. Genital pores lateral, irregularly alternating; genital atrium shallow. Ovary H-shaped in
 425 dorso-ventral view, lobate, tetralobed in cross-section. Vagina sublateral, opening into genital
 426 atrium posterior to cirrus sac. Seminal receptacle absent. Uterus saccate, medial, extending from
 427 ovarian bridge to level of anterior-most vitelline follicles. Vitellarium follicular; vitelline follicles in
 428 2 lateral bands; each band consisting of 2–3 columns of follicles, extending from posterior margin
 429 of proglottid stopping short of anterior limit of testicular field, uninterrupted by ovary. Excretory
 430 vessels in 2 lateral pairs. Eggs packaged in cocoons; cocoons in form of strands of hundreds of
 431 eggs, or in tandem doublets of eggs with bipolar filaments. Parasites of *Zanobatus* Garman
 432 (Zanobatidae); eastern Atlantic Ocean.

433 Type and only known genus: *Zanobatocestus* Jensen, Mojica and Caira, 2014.

434 Remarks: Zanobatocestidae n. fam. is erected to accommodate what will likely remain a ditypic
 435 genus (see Jensen et al., 2014) given its singular association with the striped panray, *Zanobatus*
 436 *schoenleinii* (Müller & Henle, 1841), which is generally considered to be the only representative of
 437 the family Zanobatidae (see Compagno, 2005; Nelson, 2006). Without exception, across analyses
 438 the two recently described species of *Zanobatocestus* (see Jensen et al., 2014) composed a
 439 monophyletic group, and in all but two cases (e.g., Analysis 1 and 2), grouped independently from
 440 all other genera. The biloculate, rather than uniloculate, configuration of the bothridia serves to
 441 distinguish this family from all others.

442
 443 Clade 4: Tetragonocephalidae Yamaguti, 1959 emended (e.g., Fig. 1Aa)

444 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 445 scolex proper and apical organ. Acetabula in form of suckers. Apical modification of scolex proper
 446 narrower than scolex proper, bearing apical organ. Apical organ extensive, external, non-

447 retractable, not invaginable, muscular, with glandular surface. Cephalic peduncle absent. Worms
 448 euapolytic, apolytic, or rarely anapolytic; often protogynous. Proglottids acraspedote, non-laciniate;
 449 immature proglottids of anterior strobila not laterally expanded; circumcortical longitudinal muscle
 450 bundles present. Testes in 2–4 irregular columns, more than 1 layer deep in cross-section, in single
 451 field anterior to cirrus sac. Vas deferens extending approximately from level of ovary to cirrus sac,
 452 minimal or extensive, expanded to form external seminal vesicle in some; internal seminal vesicle
 453 present or absent. Cirrus sac pyriform; cirrus armed. Genital pores lateral, irregularly alternating;
 454 genital atrium expansive. Ovary compact in dorso-ventral view, C-shaped in cross-section. Vagina
 455 medial, thick-walled, opening into genital atrium posterior to cirrus sac. Seminal receptacle present
 456 or absent. Uterus bisaccate (i.e., constricted at level of genital atrium), medial, extending almost
 457 entire length of proglottid. Vitellarium follicular; vitelline follicles in 3 distinct fields: 1 field
 458 posterior to ovary, 1 field between genital atrium and anterior margin of ovary, and 1 field
 459 consisting of 2 lateral bands (each with 2 or more columns) anterior to cirrus sac; field between
 460 genital atrium and anterior margin of ovary may be absent. Excretory vessels in 2 lateral pairs. Eggs
 461 single, without filaments. Parasites of Dasyatidae (i.e., *Dasyatis* Rafinesque, *Himantura* Müller &
 462 Henle, *Taeniura*, *Urogymnus* Müller & Henle, and possibly *Pastinachus* Rüppell); Arafura Sea,
 463 Gulf of Carpentaria, northern Indian Ocean and western Pacific Ocean.

464 Type and only known genus: *Tetragonocephalum* Shipley & Hornell, 1905.

465 Remarks: This clade consists solely of the genus *Tetragonocephalum*. Although only one of the
 466 four species included in our analyses has been formally described, all four species are fully
 467 consistent with the current concept of the type species, *T. trygonis*, as described by Shipley and
 468 Hornell (1906), and also of the genus as emended by Jensen (2005). Not only did these species
 469 group as a distinct, particularly divergent clade relative to the other lecanicephalidean species in our
 470 molecular analyses, but this family is readily distinguished from all others based on a number of
 471 morphological features: the uterus is bisaccate rather than saccate, the ovary is C-shaped rather than

472 bilobed or tetralobed in cross-section, and the genital atrium is much more expansive than those
 473 seen in any of the other families.

474 As *Tetragonocephalum* is the type of Tetragonocephalidae Yamaguti, 1959, this family name is
 475 appropriate for Clade 4. However, both Yamaguti (1959) and Euzet's (1994) concepts of the family
 476 differed in some respects from that presented here in that both authors also included *Tylocephalum*
 477 in the Tetragonocephalidae; Euzet did so explicitly and Yamaguti did so implicitly in considering
 478 the latter genus to be a synonym of *Tetragonocephalum*. In contrast, our results suggest that
 479 *Tylocephalum* has affinities with members of Clade 8 and thus is treated here as a member of the
 480 Cephalobothriidae.

481
 482 Clade 5: Polypocephalidae Meggitt, 1924 emended (e.g., Fig. 1R–Z)

483 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 484 scolex proper and apical organ. Acetabula in form of suckers or oval, uniloculate bothridia. Apical
 485 modification of scolex proper short (e.g., *Flapocephalus*; Fig. 1T) or extremely elongate
 486 (*Anteropora comicus* n. comb.; Fig. 1Y), partly invaginable (e.g., *Seussapex*; Fig. 1Z) or non-
 487 invaginable (e.g., *Hornellobothrium*; Fig. 1U), with apical aperture; apical aperture expandable or
 488 not. Apical organ extremely variable in form; if external and invaginable, may be divided into
 489 tentacles (*Polypocephalus*, *Anthemobothrium*, New Genus 11; Fig. 1X, S, and V, respectively),
 490 bipartite and telescoping (*Seussapex*; Fig. 1Z), or elongate with large scalloped margins (New
 491 Genus 12; Fig. 1W), primarily muscular or primarily glandular; if internal, ovoid or conoid in form
 492 (*Anteropora*, *Hornellobothrium*; Fig. 1R and U, respectively), primarily glandular or primarily
 493 muscular; if external and non-invaginable, in form of two muscular semicircles (*Flapocephalus*;
 494 Fig. 1T); some also partially retractable (*Seussapex*, New Genus 12; Fig. 1Z and W, respectively);
 495 one or more glandular masses at base of apical organ in some (e.g., *Anthemobothrium*,
 496 *Polypocephalus*, *Seussapex*; Fig. 1S, X and Z, respectively). Cephalic peduncle absent. Worms
 497 hyperapolytic, euapolytic, or apolytic. Proglottids craspedote, rarely acraspedote; immature

498 proglottids rarely laterally expanded (*Hornellobothrium*; Fig. 1U) but never forming trough;
 499 circumcortical longitudinal muscle bundles absent. Testes generally 4 or 6 in number and arranged
 500 in single column, rarely over 15 in number and arranged in 2 or more columns (e.g.,
 501 *Hornellobothrium gerdaae* Mojica, Jensen & Caira, 2014, *Flapocephalus*), 1 layer deep in cross-
 502 section, in single field anterior to ovary. Vas deferens extensive, may be expanded to form external
 503 seminal vesicle. Cirrus sac pyriform or elliptical; cirrus armed or unarmed, thin-walled. Genital
 504 pores lateral or sublateral, irregularly alternating; genital atrium shallow. Ovary essentially H-
 505 shaped in dorso-ventral view (irregular in *Anteropora comicus* n. comb.), generally tetralobed or
 506 bilobed in cross-section (irregular in *Anteropora comicus* n. comb.). Vagina medial or sublateral,
 507 opening into genital atrium posterior to, or at level of, cirrus sac. Seminal receptacle present or
 508 absent. Uterus saccate, medial, variable in extent. Vitellarium follicular; vitelline follicles in 2
 509 lateral bands; each band consisting of 1 to several columns, generally extending entire length of
 510 proglottid, generally interrupted by ovary. Excretory vessels in 2 lateral pairs. Eggs single, with
 511 bipolar filaments (without filaments in *Anteropora comicus* n. comb.). Parasites of batoids
 512 (Dasyatidae, Myliobatidae, Narcinidae Gill, Narkidae Fowler, Rhinidae Müller & Henle,
 513 “Rhinobatidae” Müller & Henle, Rhynchobatidae Garman) and the bamboo shark genus
 514 *Hemiscyllium* Müller & Henle (Hemiscylliidae Gill); western Indo-Pacific Ocean, Red Sea, and
 515 western Atlantic Ocean.

516 Type genus: *Polypocephalus* Braun, 1878.

517 Additional genera: *Anteropora* Subhapradha, 1957; *Anthemobothrium* Shipley & Hornell, 1906;
 518 *Flapocephalus* Deshmukh, 1979; *Hornellobothrium* Shipley & Hornell, 1906; *Seussapex* Jensen &
 519 Russell, 2014; New Genus 11; New Genus 12.

520 Remarks: A clade comprising the seven genera assigned here to the family (i.e., *Anteropora*,
 521 *Anthemobothrium*, *Flapocephalus*, *Hornellobothrium*, *Polypocephalus*, *Sesquipedalapex*,
 522 *Seussapex*, as well as New Genus 11 and New Genus 12), although not recovered in a subset of the
 523 single locus analyses, received strong support in analysis of the concatenated dataset.

524 Morphological grounds for recognition of this suite of genera as a cohesive group are more difficult
 525 to articulate. For the most part, these genera share the following features. Most possess only four or
 526 six testes, two pairs of excretory vessels, and vitelline follicles that are interrupted by the ovary.
 527 Most also exhibit elaborations of the apical modification of the scolex proper and/or apical organ
 528 that are among the most remarkable seen across the Lecanicephalidea. In combination, these
 529 features serve to distinguish most members of the clade from those in other lecanicephalidean
 530 families and thus its recognition as a family appears to be justified. As the group includes both
 531 *Polypocephalus* and *Anteropora*, two candidate family names are available (i.e., Polypocephalidae
 532 Meggitt, 1924 and Anteroporidae Euzet, 1994). As the senior name, Polypocephalidae has priority
 533 and is assigned here to this clade with Anteroporidae as its junior synonym.

534 Our analyses provide some insight into generic monophyly in the six cases in which multiple
 535 congeners were included in analyses. *Flapocephalus*, *Hornellobothrium*, *Seussapex*, and New
 536 Genus 11, were all recovered as monophyletic, but it should be recognized that the former two
 537 genera were represented by only a subset of their known species. *Anteropora* is monophyletic only
 538 if the monotypic *Sesquipedalapex* Jensen, Nikolov & Caira, 2011 is included among its members.
 539 This issue is easily resolved by the transfer of *S. comicus* to *Anteropora* as *Anteropora comicus*
 540 (Jensen, Nikolov & Caira, 2011) n. comb, an action we formally take here. In fact, Jensen et al.
 541 (2011) noted the remarkable similarity between the proglottids of *S. comicus* and those of species of
 542 *Anteropora* when establishing the new genus, but were perhaps overly impressed by the highly
 543 unusual morphology of the scolex of the new species. The most recent generic diagnosis of
 544 *Anteropora* by Mojica et al. (2013) is hereby emended to include species with (i) an apical
 545 modification of the scolex proper that is highly elongated, (ii) a posterior portion of the scolex
 546 posterior to the acetabula that is elongate, containing glandular tissue, (iii) an irregularly lobed
 547 ovary in dorso-ventral view with each lobe consisting of approx. three sublobes, (iv) excretory
 548 ducts that are branched and open to the outside along the length of the scolex and in the velum at

549 the posterior margin of each proglottid, and (v) eggs without bipolar filaments but with numerous
 550 small, regularly spaced protuberances.

551 The paraphyly of *Polypocephalus* is particularly puzzling because their possession of an apical
 552 organ divided into tentacles clearly identifies the three species examined as members of this, the
 553 most recognizable lecanicephalidean genus. Unfortunately, the analyses included only a small
 554 subset of described *Polypocephalus* species; most notably, the type species (*P. granulatus* Braun,
 555 1878 from *Rhinobatus* [sic] *granulatus* Cuvier) and any of the other species described from
 556 guitarfishes were not included. As a consequence, within the family, the phylogenetic position of
 557 the genus, as it was originally defined, is unclear for the presence of an apical organ divided into
 558 tentacles appears to be a feature that is not so unique after all. Supporting this notion is the fact that
 559 the two other genera now known to possess tentacle-like apical organs, although clustering among
 560 the other members of the Polypocephalidae, were supported as lineages or groups without clear
 561 affinities to any of the three *Polypocephalus* species included in the analyses. *Anthemobothrium*,
 562 which exhibits tentacles that taper towards their distal ends and bear with frilled margins (Fig. 1S)
 563 grouped as sister to *Anteropora*. New genus 11 (Fig. 1V), with simple tentacles like *Polypocephalus*
 564 (Fig. 1X), but with conspicuous gladiate spinitriches on the scolex proper not seen in the latter
 565 genus, grouped as sister to *Seussapex* (Fig. 1Z).

566

567 Clade 6: Eniochobothriidae n. fam. (e.g., Fig. 1I)

568 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 569 scolex proper and apical organ. Acetabula in form of suckers. Apical modification of scolex proper
 570 in form of small cone with apical aperture. Apical organ small, internal, non-protrusible, non-
 571 eversible, primarily glandular. Cephalic peduncle absent. Worms apolytic. Proglottids craspedote,
 572 non-laciniate; immature proglottids of anterior strobila laterally expanded to form trough;
 573 circumcortical longitudinal muscle bundles absent. Testes in more than 1 irregular column, 1 layer
 574 deep in cross-section, in single field anterior to ovary. Vas deferens extending from level of ootype

575 to level anterior to cirrus sac, extensive, not expanded to form external seminal vesicle; internal
 576 seminal vesicle present. Cirrus sac recurved or weakly pyriform; cirrus armed, thick-walled. Genital
 577 pores lateral, irregularly alternating; genital atrium shallow. Ovary H-shaped in dorso-ventral view,
 578 bilobed in cross-section, compact to lobulated. Vagina absent. Seminal receptacle present. Uterus
 579 saccate, medial, extending from posterior margin of ovary to level of genital pore. Vitellarium
 580 follicular; vitelline follicles in 2 lateral bands, each band consisting of several columns, extending
 581 from level of genital pore to ovarian bridge, uninterrupted by ovary. Excretory vessels in 2 lateral
 582 pairs. Eggs packaged in cocoons; cocoons containing tens of eggs. Parasites of *Rhinoptera* Cuvier
 583 (Rhinopteridae Jordan & Evermann); Arabian Gulf, eastern Atlantic Ocean Gulf of Mexico,
 584 northern Indian Ocean, Timor Sea.

585 Type and only known genus: *Eniochobothrium* Shipley & Hornell, 1906.

586 Remarks: The name Eniochobothriidae n. fam. is proposed here to house this taxon, which
 587 currently includes only a handful of species. Most previous authors have hesitated to assign the
 588 genus to a family given the uncertainties surrounding its morphology (e.g., Southwell, 1925;
 589 Wardle and McLeod, 1952; Euzet, 1994; Jensen, 2005). Schmidt (1986) was so bold as to place it in
 590 the Lecanicephalidae. However, our results reveal it to be one of the two most molecularly
 591 divergent groups of lecanicephalideans. Given its unique morphology, which includes anterior
 592 immature proglottids that are laterally expanded to form a trough, and the total lack of a vagina, its
 593 recognition as a distinct family appears warranted.

594

595 Clade 7: Lecanicephalidae Braun, 1900 emended (e.g., Fig. 1J–P)

596 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 597 scolex proper and apical organ. Acetabula usually in form of suckers (or oval uniloculate bothridia
 598 in *Rexapex* (Fig. 1O). Apical modification of scolex proper minimal or elongate (*Elicilacunosus*;
 599 Fig. 1K), generally not invaginable, partly invaginable in some (*Elicilacunosus*, *Stoibocephalum*;
 600 Fig. 1K and P, respectively), with apical aperture; apical aperture expandable or not. Apical organ

601 generally external, variable in form, rarely in shape of oval pad (*Collicocephalus*, *Hexacanalís*,
 602 some species of *Stoibocephalum*; Fig. 1J, M and P, respectively), dome (some species of
 603 *Stoibocephalum*; Fig. 1P), highly foldable sheet (*Floriparicapitus*, *Lecanicephalum*; Fig. 1L and N,
 604 respectively), or an inverted cone with papilliform projections (*Rexapex*; Fig. 1O), if internal, may
 605 be ovoid or conoid in form (*Elicilacunosus*; Fig. 1K), primarily muscular or primarily glandular,
 606 fully retractable in most. Cephalic peduncle absent. Worms generally euapolytic, rarely apolytic
 607 (*Stoibocephalum*). Proglottids craspedote, rarely laciniate (*Collicocephalus*) or with dorso-ventral
 608 lappets (some species of *Hexacanalís*), may possess regions of musculo-glandular tissue along
 609 dorsal and ventral medial surfaces manifesting externally as columns of depressions
 610 (*Elicilacunosus*); immature proglottids rarely laterally expanded (*Rexapex*) but never forming
 611 trough; circumcortical longitudinal muscle bundles absent or present. Testes in 1 to several
 612 columns, generally 1 layer deep, rarely 2 or several layers deep (*Hexacanalís* and *Stoibocephalum*),
 613 in single field anterior to ovary. Vas deferens extensive, may be expanded to form external seminal
 614 vesicle. Cirrus sac pyriform; cirrus generally unarmed, rarely armed (*Lecanicephalum* and
 615 *Floriparicapitus*), thin-walled. Genital pores lateral, irregularly alternating; genital atrium shallow.
 616 Ovary H-shaped in dorso-ventral view (in single mass in *Hexacanalís*), bilobed, tetralobed, or C-
 617 shaped in cross-section. Vagina medial or sublateral, opening into genital atrium posterior to cirrus
 618 sac, rarely at the same level or anterior to the cirrus sac (some species of *Floriparicapitus*). Seminal
 619 receptacle absent. Uterus saccate, medial, extending from ovarian bridge to near anterior margin of
 620 proglottid. Vitellarium follicular; vitelline follicles in 2 lateral bands; each band consisting of 2 to
 621 several columns (columns expanding medially in *Hexacanalís*), generally extending entire length of
 622 proglottid, interrupted by ovary or extending to near anterior margin of ovary. Excretory vessels in
 623 1 or 3 lateral pairs. Eggs single, with bipolar filaments or in fibrous mass. Parasites of batoids
 624 (Dasyatidae, Gymnuridae Fowler, Myliobatidae, “Pristidae” Bonaparte, Rhinidae, “Rhinobatidae”,
 625 Rhynchobatidae); western Indo- Pacific Ocean and western Atlantic Ocean.
 626 Type genus: *Lecanicephalum* Linton, 1890.

627 Additional genera: *Collicocephalus* Koch, Jensen & Caira, 2012; *Elicilacunosus* Koch, Jensen &
 628 Caira, 2012; *Floriparicapitus* Cielocha, Jensen & Caira, 2014; *Hexacanalís* Perrenoud, 1931;
 629 *Rexapex* Koch, Jensen & Caira, 2012; *Stoibocephalum* Cielocha & Jensen, 2013.

630 Remarks: The clade comprising species of the seven genera *Elicilacunosus*, *Collicocephalus*,
 631 *Floriparicapitus*, *Hexacanalís*, *Lecanicephalum*, *Rexapex*, and *Stoibocephalum*, was supported to
 632 varying degrees across analyses of all single genes, except *cox1*, and analysis of the concatenated
 633 dataset. As this clade includes *Lecanicephalum* it is the appropriate family to bear the name
 634 Lecanicephalidae. Previous notions of the membership of this family differed substantially from the
 635 concept presented here and also from one another. Euzet (1994) restricted membership to include
 636 only *Lecanicephalum*, and implicitly in considering it a synonym of the former genus, also
 637 *Hexacanalís*. Yamaguti (1959) viewed the family much more broadly; for he considered its
 638 membership to also include *Polypocephalus*, *Cephalobothrium*, *Tylocephalum*, in addition to the
 639 poorly known *Calycobothrium* Southwell, 1911 which remains a *genus inquirendum*. All but
 640 *Calycobothrium*, which was not included in our analyses, have been shown here to have other
 641 affinities—the former with the Polypocephalidae and the latter two with the Cephalobothriidae.

642 Our results prompt reconsideration of the existing generic placements of *Tylocephalum*
 643 *koenneckeorum* and *T. campanulatum* for the specimens of both species were highly supported in
 644 Analysis 4 as close allies of *Stoibocephalum*, rather than of the other species of *Tylocephalum*. It is
 645 worth mentioning that when Cielocha and Jensen (2013) erected *Stoibocephalum*, they did express
 646 some concern that *Stoibocephalum* was somewhat unusual among lecanicephalidean genera in its
 647 possession of three, rather than two, pairs of excretory vessels—a feature it shares only with
 648 *Hexacanalís* and *Floriparicapitus* (see Cielocha et al., 2014). Although noting that *T.*
 649 *koenneckeorum* was generally consistent with the diagnosis of *Stoibocephalum*, Cielocha and
 650 Jensen (2013) refrained from transferring it to *Stoibocephalum* until the condition of its excretory
 651 vessels (originally described as “greater than two pairs” by Jensen, 2005; pg. 164) could be
 652 unambiguously confirmed. Similarly, while Butler (1987) did not include the number of excretory

653 vessels in her original description of *T. campanulatum*, material of this species examined here
 654 confirms the presence of three pairs of excretory vessels. As our understanding of key
 655 morphological features of both species has now converged with our molecular results, both species
 656 are hereby transferred to *Stoibocephalum*, as *S. koenneckeorum* (Jensen, 2005) n. comb. and *S.*
 657 *campanulatum* (Butler, 1987) n. comb. This action requires that Cielocha and Jensen's (2013)
 658 diagnosis of *Stoibocephalum* be emended to include species that are euapolytic and that exhibit an
 659 apical organ that is non-retractable.

660 Again, identifying one or more morphological features to distinguish the Lecanicephalidae from
 661 the seven other lecanicephalidean families is somewhat challenging. Here too, the condition of the
 662 excretory vessels has potential. To our knowledge, *Floriparicapitus*, *Hexacanalisis*, and
 663 *Stoibocephalum* are unique among lecanicephalideans in their possession of three pairs of excretory
 664 ducts. It also appears that *Collicocephalus*, *Elicilacunosis*, *Rexapex* (see Koch et al., 2012), and the
 665 only valid species of *Lecanicephalum* in which the excretory vessels have been examined in detail
 666 (i.e., *L. coangustatum*; see Jensen, 2005), bear only a single pair of excretory vessels. Essentially all
 667 other genera in the order for which the excretory system is known, bear two pairs of vessels. As a
 668 consequence, the Lecanicephalidae is unique in possessing either three or one, but never two, pairs
 669 of excretory vessels. The exception is *Aberrapex* which also possesses only a single pair of
 670 excretory vessels. However, the scolex of members of this genus lacks an apical structure; in this
 671 respect the Aberrapecidae are easily distinguished from the Lecanicephalidae.

672
 673 Clade 8: Cephalobothriidae Pintner, 1928, emended (e.g., Fig. 1B–H)

674 Diagnosis: Scolex with 4 acetabula, and apical structure consisting of apical modification of
 675 scolex proper and apical organ. Acetabula in form of suckers. Apical modification of scolex proper
 676 minimal in most, rarely cylindrical and narrow (*Adelobothrium*; Fig. 1B, C), with apical aperture;
 677 apical aperture expandable or not. Apical organ external, non-invaginable, usually in form of
 678 muscular pad, occasionally in form of muscular dome (*Tylocephalum*, New Genus 13; Fig. 1H and

679 G, respectively) or glandular sphere (*Adelobothrium*; Fig. 1B), retractable (*Cephalobothrium* [Figs.
 680 1D–F], New Genus 13 [Fig. 1G]) or non-retractable (*Adelobothrium* [Figs. 1B and C],
 681 *Tylocephalum* [Fig. 1H]). Cephalic peduncle absent. Worms generally euapolytic. Proglottids
 682 craspedote; immature proglottids of anterior strobila not laterally expanded; circumcortical
 683 longitudinal muscle bundles present. Testes in 2 to several columns, 2 to several layers deep, in
 684 single field anterior to ovary. Vas deferens extensive, expanded to form external seminal vesicle.
 685 Cirrus sac pyriform; cirrus unarmed, thin-walled. Genital pores lateral, alternating irregularly;
 686 genital atrium shallow. Ovary H-shaped in dorso-ventral view, bilobed in cross-section,
 687 conspicuously digitiform. Vagina medial, opening into genital atrium posterior to cirrus sac.
 688 Seminal receptacle absent. Uterus saccate, medial, extending from ovary to anterior margin of
 689 proglottid. Vitellarium follicular; vitelline follicles generally in 2 extensive lateral bands, often
 690 encroaching medially (fully circumcortical in *Tylocephalum*); each band consisting of several
 691 columns, generally extending from near anterior margin of proglottid to ovary, extending posterior
 692 to ovary in some (e.g., *Tylocephalum brooksi* Ivanov & Campbell, 2000). Excretory vessels in 2, or
 693 rarely more (*Tylocephalum*), lateral pairs. Eggs not observed. Parasites of *Aetobatus* Blainville and
 694 *Aetomylaeus* (Myliobatidae), *Rhinoptera* (Rhinopteridae), and possibly *Dasyatis* (Dasyatidae) and
 695 *Squatina* (Squatinidae); western Indo-Pacific Ocean and western Atlantic Ocean.

696 Type genus: *Cephalobothrium* Shipley & Hornell, 1906.

697 Additional genera: *Adelobothrium* Shipley, 1900; *Tylocephalum* Linton, 1890; New Genus 13.

698 Remarks: Inclusion of species of *Cephalobothrium*, *Adelobothrium*, and New Genus 13 in this
 699 family was highly supported by both the ML and BI analyses of the concatenated dataset, as well as
 700 by a subset of the ML analyses focused on individual loci (i.e., Analysis 1 and 2). *Tylocephalum* sp.
 701 1 and *Tylocephalum* sp. 3 placed as the sister group of the other members of the
 702 *Cephalobothrium*+*Adelobothrium*+New Genus 13 clade with a bootstrap value of >70% in trees
 703 resulting from Analysis 2, a posterior probability value of 0.98 in the BI analysis, and with
 704 somewhat less support in trees resulting from Analysis 5. However, because the affinities of these

705 two *Tylocephalum* species with other clades were poorly supported in all other analyses, this
 706 appears to be the most appropriate placement for this genus as this time. Nonetheless, we suspect
 707 that some members of *Tylocephalum* not included here will ultimately be found to have closer
 708 affinities to *Stoibocephalum* than *Tylocephalum*.

709 In terms of morphological cohesiveness, the Cephalobothriidae share the possession of
 710 conspicuous circumcortical muscle bundles, testes arranged in two or more layers, and extensive
 711 lateral bands of vitelline follicles that tend to converge near the median line of the proglottid. The
 712 group is also characterized by the muscular, often pad-like, nature of the apical organ. As the group
 713 includes both *Cephalobothrium* and *Adelobothrium*, two candidate family names are available for
 714 this clade (i.e., Cephalobothriidae Pinter, 1928 and Adelobothriidae Yamaguti, 1959). As the senior
 715 name Cephalobothriidae is assigned here to this clade with Adelobothriidae as its junior synonym.
 716 The diagnosis presented above expands Pinter's (1928) original concept of the family to include the
 717 more detailed morphological information now available on its members.

718 The most interesting result of our analyses involved the affinities of *Cephalobothrium* and
 719 *Adelobothrium*. In trees resulting from most analyses (i.e., Analysis 1–3 and 5), species of these
 720 genera grouped together in a well-supported clade, but the genera were not necessarily mutually
 721 monophyletic. As the type species of both genera (i.e., *C. aetobatidis* Shipley and Hornell, 1906 and
 722 *A. aetobatidis* Shipley, 1900, respectively) were included here, we do not believe this is the result of
 723 taxon misidentification. Although our molecular results call into question existing generic
 724 boundaries, they conflict with morphological features that allow the genera to be readily
 725 distinguished. Whereas the apical suckers of *Adelobothrium* are directed anteriorly, those of
 726 *Cephalobothrium* are directed more laterally; whereas the apical structure of the former is narrow,
 727 feeble, and non-retractable, that of the latter is in the form of an extensive, retractable muscular pad
 728 (Cielocha, J.J., 2013. Contributions to the systematics, comparative morphology, and
 729 interrelationships of selected lecanicephalidean tapeworms. Doctoral dissertation, University of

- 730 Kansas, KS, USA). This conflict requires further investigation before steps to resolve the proposed
 731 non-monophyly of these two genera can be taken.
 732
- 733 Key to lecanicephalidean families:
- 734 1. Acetabula biloculate.....Zanobatocestidae
 735 - Acetabula uniloculate (sucker-like or bothriate).....2
 736 2. Scolex without apical structure3
 737 - Scolex with apical structure4
 738 3. Uterus not extending anterior to genital pore; vitelline follicles not interrupted at level of ovary;
 739 ovary bilobed in cross section..... Paraberrapecidae
 740 - Uterus extending anterior to genital pore; vitelline follicles interrupted at level of ovary;
 741 ovary tetralobed in cross sectionsAberrapecidae
 742 4. Proglottids with expansive genital atrium; ovary C-shaped in cross section;
 743 uterus bisaccate Tetragonocephalidae
 744 - Proglottids with inconspicuous genital atrium; ovary bilobed or tetralobed in cross section;
 745 uterus saccate5
 746 5. Vagina absent; anterior immature proglottids expanded laterally to form trough;
 747 cirrus sac thick-walled Eniochobothriidae
 748 - Vagina present; anterior immature proglottids not laterally expanded; cirrus sac thin-walled6
 749 6. 1 pair of excretory ducts
 750Lecanicephalidae (*Collicocephalus*, *Elicilacunosis*, *Rexapex*, *Lecanicephalum*)
 751 - 2 pairs of excretory ducts (assumed in *Corrugatocephalum*, *Healyum*, *Quadcuspibothrium*).....7
 752 - 3 or more pairs of excretory ducts9
 753 7. Testes 3 in number, arranged in 1 column, 1 or 2 layers deep in cross section.....
 754 *genera incertae sedis* (*Corrugatocephalum*, *Healyum*, *Quadcuspibothrium*)
 755 - Testes 4 or 6 in number, arranged in 1 column, 1 layer deep in cross section

- 756Polypocephalidae (*Anthemobothrium*, *Anteropora*, *Hornellobothrium* in parte,
 757 New Genus 11, New genus 12, *Polypocephalus*, *Seussapex*)
- 758 - Testes greater than 6 in number, arranged in more than 1 column, 1 or more layers deep
 759 in cross section 8
- 760 8. Testes 1 layer deep in cross section Polypocephalidae (*Flapocephalus*)
- 761 - Testes greater than 1 layer deep in cross section 10
- 762 9. Conspicuous circumcortical muscle bundles present; vitelline follicles in extensive lateral
 763 bands often converging on midline of proglottid..... Cephalobothriidae (*Tylocephalum*)
- 764 - Conspicuous circumcortical muscle bundles absent; vitelline follicles in narrow lateral
 765 bands restricted to lateral margins of proglottid
 766 Lecanicephalidae (*Floriparicapitus*, *Hexacanalisis*, *Stoibocephalum*)
- 767 10. Acetabula bothridiate in form Polypocephalidae (*Hornellobothrium* in parte)
- 768 - Acetabula sucker-like in form.....
 769 Cephalobothriidae (*Adelobothrium*, *Cephalobothrium*, New genus 13)

770

771 4.2 Major lecanicephalidean taxa

772 The substantial breadth of the taxon sampling employed here offers a number of insights into
 773 the validity of the majority of lecanicephalidean genera even though exhaustive species
 774 representation was not achieved in all cases. All but three of the genera recognized as valid by
 775 Jensen (2005) have been confirmed to represent independent, and in the cases of non-monotypic
 776 genera, monophyletic, assemblages of taxa. These are *Eniochobothrium*, *Hornellobothrium*,
 777 *Lecanicephalum*, *Paraberrapex*, *Polypocephalus*, and *Tetragonocephalum*. *Aberrapex* also appears
 778 to represent a distinct lineage, but its monophyly remains to be confirmed in future analyses that
 779 include more dense taxon sampling of this genus. *Anteropora* is re-established as a monophyletic
 780 group with *Sesquipedalapex* as a junior synonym. Even after the transfer of two of its species to
 781 *Stoibocephalum*, *Tylocephalum* remains a relatively non-cohesive assemblage of taxa; careful

782 examination of additional species is likely to result in the transfer of at least some to one or more
 783 different genera.

784 The validity of all but one of the eight genera erected since Jensen (2005) (i.e., *Collicocephalus*,
 785 *Elicilacunosus*, *Floriparicapitus*, *Rexapex*, *Seussapex*, *Stoibocephalum*, and *Zanobatocestus*) has
 786 also been confirmed. The exception is *Sesquipedalapex*, which we have now synonymized with
 787 *Anteropora*. The recently resurrected *Hexacanalisis* (see Cielocha and Jensen, 2011), and an
 788 additional two of the nine genera treated as *genera inquirenda* by Jensen (2005) (i.e.,
 789 *Anthemobothrium* and *Flapocephalus*) also appear to be valid. Similarly, in combination,
 790 *Adelobothrium* and *Cephalobothrium* represent an additional independent lineage.

791 Our results also suggest that the taxa tentatively assigned to novel genera do, in fact, represent
 792 unique taxa. Two of these, New Genus 11 and New Genus 12, are members of the
 793 Polypocephalidae. The third, New Genus 13, has affinities with the Cephalobothriidae. Images of
 794 each are provided in Figs. 1V, W and G, respectively, to help guide the future taxonomic work
 795 required to formally erect these genera.

796 The affinities of the three valid lecanicephalidean genera not represented in this study remain
 797 unclear—the monotypic *Corrugatocephalum* and *Quadcuspibothrium*, and the ditypic *Healyum*. All
 798 three exhibit scolex morphologies that are unique among lecanicephalideans (see Caira et al., 1997;
 799 Jensen, 2001, 2005). All three are also distinctive in their possession of a trilobed ovary and only
 800 three testes per proglottid (*Healyum pulvis* Jensen, 2001 is the exception). Even the host
 801 associations of these genera are unusual for all are parasites of planktivorous elasmobranchs
 802 (*Corrugatocephalum* from the megamouth shark, *Megachasma pelagios* Taylor, Compagno &
 803 Struhsaker, and *Quadcuspibothrium* and *Healyum* from the spinetail mobula, *Mobula japanica*
 804 [Müller & Henle]). As a consequence, we have refrained from assigning these genera to families at
 805 this time; all three are considered to be *incertae sedis* within the Lecanicephalidea.

806 Five of the eight families recognized here are monogeneric. The three families determined to
 807 include more than a single genus (i.e., Polypocephalidae, Lecanicephalidae, and Cephalobothriidae)

808 provide opportunities for intergeneric relationships to be assessed. Unfortunately, low support for
 809 most nodes within each of these families limits robust inference of such relationships to a few
 810 generic groupings. Nonetheless, within the Polypocephalidae, the tentacle-bearing New Genus 11
 811 (Fig. 1V) did not group with any of the other tentacle-bearing taxa (i.e., species of
 812 *Anthemobothrium* [Fig. 1S] and *Polypocephalus* [Fig. 1X]). Somewhat unexpectedly, this genus
 813 showed affinities with members of *Seussapex*, which bears a bipartite telescoping apical structure
 814 rather than tentacles (Fig. 1Z). Within the Lecanicephalidae, *Lecanicephalum* grouped with
 815 *Floriparicapitus* and *Hexacanalis* to the exclusion of the other four genera in the family.
 816 Similarities between *Lecanicephalum* and *Floriparicapitus* in proglottid anatomy (see Cielocha et
 817 al., 2014) and even more conspicuously in scolex morphology—in that both possess apical organs
 818 in the form of a highly foldable sheet (Figs. 1N and 1L, respectively)—are evident. Similarities
 819 with *Hexacanalis*, which possesses a robust, muscular apical organ, are less obvious. The
 820 relationships among species of *Adelobothrium* and *Cephalobothrium* are perhaps most problematic;
 821 neither genus was recovered as monophyletic with respect to one another. Despite their dissimilar
 822 scolex morphologies (see comments above), a close relationship is supported by a striking
 823 similarity in proglottid anatomy (see Cielocha, J.J., 2013. Contributions to the systematics,
 824 comparative morphology, and interrelationships of selected lecanicephalidean tapeworms. Doctoral
 825 dissertation, University of Kansas, KS, USA). These discrepancies between morphological and
 826 molecular data regarding their generic boundaries require further study.

827

828 4.3. Host associations

829 Lecanicephalideans are mainly parasites of batoid elasmobranchs (Fig. 5). Among
 830 elasmobranchs, their hosts represent species in three of the four orders in the superorder Batoidea
 831 (i.e., Myliobatiformes [stingrays], Torpediniformes [electric rays], and Rhinopristiformes
 832 [guitarfish and sawfish]); they are conspicuously absent from the fourth, and most speciose, order
 833 (i.e., Rajiformes [skates]). A handful of records also exist from sharks. Some of these are suspect

and require confirmation (i.e., Deshmukh et al., 1982; Sarada et al., 1993; Pramanik and Mana, 2007). Other such records have been verified and come from a heterogeneous assortment of orders: *Paraberrapex manifestus* and *P. atlanticus* Mutti & Ivanov, 2016 are hosted by angelsharks (Squatiniiformes) (Jensen, 2001; Mutti and Ivanov, 2016), *Anteropora leelongi* Jensen, 2005 by an epaulette shark (Orectolobiformes) (Jensen, 2005), and *Corrugatocephalum ouei* Caira, Jensen & Yamane, 1997 by the megamouth shark (Lamniformes) (Caira et al., 1997). It is highly likely that the association with an epaulette shark represents a host switching event because *A. leelongi* was found to be deeply nested among its batoid-parasitizing congeners in trees from essentially all analyses. The angel shark association of *Paraberrapex* is more intriguing as this genus appears to be the earliest diverging group of lecanicephalideans. The cestode order Litobothriidea, an order that exclusively parasitizes lamniform sharks (Caira et al., 2014) has emerged as sister to the acetabulate clade of cestodes, in which the Lecanicephalidea is the earliest diverging group (Waeschenbach et al., 2012). In this context, the phylogenetic position of the lamniform-parasitizing *Corrugatocephalum* would be especially interesting to explore once material becomes available.

Not unexpectedly, the breadth of the host associations of individual lecanicephalidean families generally correlates with the taxon diversity of each family (Fig. 5). Three of the five monogeneric lecanicephalidean families are restricted to host families that are also monogeneric: both species of *Paraberrapecidae* parasitize angelsharks (*Squatina*), both species of *Zanobatocestidae* parasitize panrays (*Zanobatus*), and the approx. six species of *Eniochobothriidae* all parasitize cownose rays (*Rhinoptera*). Members of the remaining two monogeneric families are slightly less catholic in their host associations. Species of *Tetragonocephalidae* parasitize species in four genera (*Dasyatis*, *Himantura*, *Taeniura*, and *Urogymnus*) of *Dasyatidae*. The *Aberrapecidae* parasitize two genera of *Myliobatidae* (*Aetomylaeus*, *Myliobatis*) and one genus of *Dasyatidae* (*Taeniura*).

A surprisingly high degree of host specificity is seen in members of the *Cephalobothriidae*. All members of New Genus 13, *Cephalobothrium*, and *Adelobothrium* parasitize eagle rays (*Myliobatidae*) and species of the latter two genera exhibit an especially high affinity for species of

860 the genus *Aetobatus*. Records for the two species of *Tylocephalum* expand the host associations of
 861 the family to include the myliobatid genus *Rhinoptera*. Hosts of the remaining species of
 862 *Tylocephalum* not included herein are stingrays (*Dasyatis*), angelsharks (*Squatina*), and guitarfish
 863 (*Glaucostegus* Bonaparte, as *Rhinobatos* Linck), but as noted above, the generic and, thus also
 864 familial, placements of most species of *Tylocephalum* are in need of further investigation.

865 With eight and seven genera, respectively, the Polypocephalidae and Lecanicephalidae exhibit
 866 the broadest host associations among lecanicephalidean families. Species in these families have
 867 routinely been reported from the myliobatiform families Dasyatidae (stingrays) and Myliobatidae
 868 (eaglerays), as well as from the rhinopristiform families Rhinidae, “Rhinobatidae,” and
 869 Rhynchobatidae (guitarfish and wedgefish). In addition, some Polypocephalidae parasitize the
 870 torpediniform families Narkidae and Narcinidae (electric rays), and as noted above one epaulette
 871 shark. In addition, some Lecanicephalidae parasitize the myliobatiform family Gymnuridae
 872 (butterfly rays) and the rhinopristiform family “Pristidae” (sawfish).

873 Given their high affinity for batoids overall, the absence of lecanicephalideans from skates is
 874 initially somewhat puzzling—at least until habitat is taken into consideration. The majority of
 875 lecanicephalidean hosts are shallower-water, reef-associated, benthopelagic or demersal species
 876 (see Froese, R. and D. Pauly, Editors, 2015. FishBase. World Wide Web electronic publication.
 877 www.fishbase.org, [04/2015]), which, as noted by Jensen (2005) and Caira and Jensen (2015),
 878 occur in relatively warm regions of the globe. The majority of species in all three families of skates
 879 (Anacanthobatidae von Bonde & Swart, Arhynchobatidae Fowler, and Rajidae) predominantly
 880 occur in relatively much cooler waters, either at higher latitudes or greater ocean depths (e.g., Ebert
 881 and Compagno, 2007). It is thus possible that other factors, such as habitat or intermediate host
 882 availability, rather than some sort of phylogenetic constraint, accounts for the absence of
 883 lecanicephalideans from this speciose order of batoids. For example, it seems feasible that there
 884 may be one or more element of lecanicephalidean life-cycles that requires warmer and/or shallower
 885 water conditions. Only a single lecanicephalidean species, *Lecanicephalum xiamensis* Yang, Lui &

886 Lin, 1995, has been reported from a skates, specifically the yellow-spotted skate, *Okamejei hollandi*
 887 (Jordan & Richardson) (as *Raja holandi* [sic] Jordan & Richardson) (Rajidae Bonaparte); however
 888 this record requires verification.

889

890 4.4. Morphological evolution

891 Most lecanicephalideans are readily distinguished from the majority of the other orders of
 892 elasmobranch-hosted tapeworms in their possession of an apical structure on the adult scolex. The
 893 exceptions are *Aberrapex* and *Paraberapex* both of which entirely lack this feature (Jensen, 2001).
 894 The position of these genera as the earliest diverging groups in the order (Fig. 5) makes it tempting
 895 to consider the adult apical organ as a novel feature uniting the remaining members of the order.
 896 However, caution must be exercised because the Litobothriidea, which as noted above is the sister
 897 taxon to the acetabulate clade of cestodes, in which the Lecanicephalidea take the earliest diverging
 898 position, also bear apical structures in their adult form. In addition, an apical organ in the adult form
 899 is seen in the rhinebothriidean family Echinebothriidae, most cyclophyllideans, and most
 900 freshwater-dwelling onchoproteocephalideans. Thus, even though this feature may unite a subgroup
 901 of lecanicephalideans, it is not clear whether it represents a truly novel morphological innovation
 902 within that order rather than a loss in *Aberrapex* and *Paraberapex*.

903 Also remarkable among lecanicephalidean scolex features is the presence of biloculated
 904 bothridia in both species of *Zanobatocestus*. This feature is likely a synapomorphy for the genus
 905 given that the other two early diverging lineages (*Paraberapex* and *Aberrapex*) and its sister group,
 906 which comprises all other lecanicephalideans, all bear uniloculate acetabula, either in the form of
 907 suckers or bothridia.

908 By far the most striking aspect of the Lecanicephalidea is the remarkable variation seen in the
 909 morphology of the apical structure (i.e., apical modification of the scolex proper and apical organ)
 910 across taxa. Once considered to be of key importance for identifying lecanicephalidean higher taxa
 911 (e.g., Joyeux and Baer, 1961; Schmidt, 1986; Euzet, 1994), its potential in this respect now seems

912 less clear. In fact, if our analyses are correct, the apical structure appears to be among the most
 913 evolutionarily labile morphological feature of the order—the variation seen within at least three
 914 families is truly stunning. In the Lecanicephalidae (Figs. 1J–P) the apical organ can be external and
 915 in the form of a foldable sheet, an oval muscular pad, an inverted cone with papilliform projections,
 916 or an ovoid and entirely internal. The apical organ of the Cephalobothriidae (Figs. 1B–H) can be in
 917 the form of a muscular pad or dome, or a glandular sphere. Perhaps most remarkable are the
 918 Polypocephalidae (Figs. 1R–1Z) in which the apical organ can be divided into tentacles, bipartite
 919 and telescoping, elongate with large scalloped margins, or in the form of two muscular semicircles.
 920 They can be retractable, or not; some are invaginable; most are associated with one or more
 921 glandular masses.

922 Our results suggest that an apical organ in the form of tentacles has arisen at least three
 923 independent times among the Polypocephalidae. An apical organ in the form of a muscular pad is
 924 seen in the members of three unrelated families (Fig. 2). The apical modification of the scolex
 925 proper can also vary among close relatives. Perhaps most extreme is the case of *Anteropora*
 926 *comicus*, which was originally placed in its own genus (*Sesquipedalapex*) owing to the fact that the
 927 apical modification of its scolex proper is almost as long as its strobila (Fig. 1Y), a feature not
 928 shared by any of its congeners (e.g., Fig. 1R).

929 This work also advances our understanding of the conflict between proglottid anatomy and
 930 scolex morphology. Although not universally the case, in a number of instances, proglottid anatomy
 931 was determined to be rather conserved in related taxa that bear widely divergent apical organ
 932 morphologies. Fig. 3, for example, shows proglottids of members of six of the eight genera in the
 933 Polypocephalidae (i.e., *Anthemobothrium*, *Anteropora*, New Genus 11, New Genus 12,
 934 *Polypocephalus* and *Seussapex*). Despite their widely divergent scolex morphologies (Figs. 1S, R
 935 and Y, V, W, X and Z, respectively), the proglottid anatomy in these genera is very uniform (e.g.,
 936 four or six testes, consistent pore position, and presence of post-ovarian vitelline follicles). In
 937 general, proglottid anatomy appears to be much more conserved within clades than scolex

938 morphology. Clade-specific proglottid anatomy was observed in most families suggesting that
 939 scolex morphology is homoplasious across the order. Recent phylogenetic studies of other cestode
 940 orders arrived at similar conclusions regarding scolex morphology. For example, among the
 941 features traditionally used to diagnose proteocephalidean taxa above the species level, de Chambrier
 942 et al. (2015) considered scolex morphology to be homoplasious across the order. Likewise, Kuchta
 943 et al. (2008) and Brabec et al. (2015) considered similarities in scolex morphology within the
 944 Bothriocephalidea to be the result of convergence while characterizing the strobilar morphology as
 945 relatively uniform. Nevertheless, the considerable variation seen in scolex morphology across the
 946 Lecanicephalidea provides a productive source for investigating the evolutionary drivers of this
 947 plasticity in form, such as host use, host-specificity, site of infection and micromorphology of the
 948 mucosal surface of the host's spiral intestine. Continued efforts to collect lecanicephalideans from
 949 localities and host groups not previously sampled for lecanicephalideans are likely to yield yet
 950 additional novelty.

951

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963

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- 1152

1153 **Figure legends**

1154

1155 **Fig. 1.** Scanning electron micrographs of scoleces of lecanicephalidean genera included in the
 1156 analyses arranged in alphabetical order by family. (A) Aberrapecidae n. fam.: *Aberrapex* n. sp. 1 ex
 1157 *Aetomylaeus bovinus* (Geoffroy Saint-Hilaire) (SE-257). (B–H) Cephalobothriidae. (B)
 1158 *Adelobothrium aetiobatidis* ex *Aetobatus ocellatus* (Kuhl) (NT-95). (C) *Adelobothrium* n. sp. 2 ex
 1159 *Aetobatus narutobiei* White, Furumitsu & Yamaguchi (VN-16). (D) *Cephalobothrium* n. sp. 1 ex
 1160 *Aetobatus ocellatus* (TH-19). (E) *Cephalobothrium aetobatidis* ex *Aetobatus ocellatus* (AU-41). (F)
 1161 *Cephalobothrium* n. sp. 2 ex *Aetobatus narinari* (Euphrasen) (FY-5). (G) New Genus 13 sp. ex
 1162 *Aetomylaeus nichofii* (Bloch & Schneider) (BO-180). (H) *Tylocephalum* sp. 3 ex *Rhinoptera*
 1163 *bonasus* (Mitchill) (CH-15). (I) Eniochobothriidae n. fam.: *Eniochobothrium* n. sp. 1 ex *Rhinoptera*
 1164 cf. *steindachneri* Evermann & Jenkins (MS05-49). (J–P) Lecanicephalidae. (J) *Collicocephalus*
 1165 *baggioi* Koch, Jensen & Caira, 2012 ex *Aetomylaeus vespertilio* (Bleeker) (CM03-61). (K)
 1166 *Elicilacunosus* sp. ex *Aetomylaeus nichofii* (BO-180). (L) *Floriparicapitus plicatilis* Cielocha,
 1167 Jensen & Caira, 2014 ex *Glaucostegus typus* (Anonymous) (AU-62). (M) *Hexacanalisis folifer*
 1168 Cielocha & Jensen, 2011 ex *Gymnura zonura* (Bleeker) (KA-437). (N) *Lecanicephalum* sp. ex
 1169 *Dasyatis akajei* (Müller & Henle) (VN-41). (O) *Rexapex nanus* Koch, Jensen & Caira, 2012 ex
 1170 *Aetomylaeus vespertilio* (CM03-61). (P) *Stoibocephalum arafurens* Cielocha & Jensen, 2013 ex
 1171 *Rhina ancylostoma* Bloch & Schneider (NT-103); inset: *Stoibocephalum campanulatum* n. comb.
 1172 ex *Rhina ancylostoma* (NT-111). (Q) Paraberrapexidae n. fam.: *Paraberrapex manifestus* ex
 1173 *Squatina californica* Ayres (BJ-298). (R–Z) Polypocephalidae. (R) *Anteropora* n. sp. 1 ex
 1174 *Pastinachus atrus* (Macleay) (NT-105). (S) *Anthemobothrium* sp. ex *Pastinachus atrus* (SO-19).
 1175 (T) *Flapocephalus* sp. ex *Pastinachus atrus* (CM03-9). (U) *Hornellobothrium najaforme* Mojica,
 1176 Jensen & Caira, 2014 ex *Aetobatus ocellatus* (NT-76). (V) New Genus 11 n. sp. 2 ex *Glaucostegus*
 1177 *typus* (AU-62). (W) New Genus 12 n. sp. 2 ex *Himantura granulata* (Macleay) (SO-24). (X)
 1178 *Polypocephalus* n. sp. ex *Rhinoptera* cf. *steindacheri* (MS05-300). (Y) *Sesquipedalapex comicus* (=

1179 *Anteropora comicus* n. comb.) ex *Narcine maculata* (Shaw) (BO-408). (Z) *Seussapex* sp. ex
 1180 *Himantura uarnacoides* (Bleeker) (KA-206). (Aa) Tetragonocephalidae: *Tetragonocephalum*
 1181 *passeyi* Jensen, 2005 ex *Himantura leoparda* Manjaji-Matsumoto & Last (NT-32). (Ba)
 1182 Zanobatocestidae n. fam.: *Zanobatocestus minor* Jensen, Mojica & Caira, 2014 ex *Zanobatus*
 1183 *schoenleinii* (Müller & Henle) (SE-105). Numbers in parentheses indicate unique host specimen
 1184 Collection Codes and Collection Numbers; host specimen information, including images, are
 1185 available in the Global Cestode Database (<http://elasmobranchs.tapewormdb.uconn.edu>).

1186

1187 **Fig. 2.** Light micrographs of scoleces of genera representing different families exhibiting similar
 1188 apical organ morphology. (A) Lecanicephalidae: *Stoibocephalum koenneckeorum* n. comb. ex
 1189 *Rhynchobatus* cf. *laevis* (Bloch & Schneider) (NT-49). (B) Tetragonocephalidae:
 1190 *Tetragonocephalum* sp. 3 ex *Himantura leoparda* (NT-37). (C) Cephalobothriidae?: *Tylocephalum*
 1191 *pingue* Linon, 1890 (neotype, USNM77631) ex *Rhinoptera bonasus*. Numbers in parentheses
 1192 indicate unique host specimen Collection Codes and Collection Numbers; host specimen
 1193 information, including images, are available in the Global Cestode Database
 1194 (<http://elasmobranchs.tapewormdb.uconn.edu>).

1195

1196 **Fig. 3.** Light micrographs of mature proglottids of selected genera of Polypocephalidae exhibiting
 1197 similar proglottid anatomies. Black arrowheads indicate genital pore position. (A) *Anteropora* n. sp.
 1198 1 ex *Pastinachus atrus* (NT-44). (B) *Anteropora comicus* n. comb. ex *Narcine maculata* (BO-408).
 1199 (C) *Anthemobothrium* sp. 1 ex *Pastinachus atrus* (NT-44). (D) New Genus 11 n. sp. 1 ex
 1200 *Rhynchobatus* cf. *laevis* (NT-49). (E) New Genus 12 n. sp. 2 ex *Himantura granulata* (SO-21). (F)
 1201 *Polypocephalus* sp. ex *Himantura leoparda* (NT-32). (G) *Seussapex* n. sp. 2 ex *Himantura uarnak* 3
 1202 sensu Naylor et al. (2012a) (BO-47). T, testis. Numbers in parentheses indicate unique host
 1203 specimen Collection Codes and Collection Numbers; host specimen information, including images,
 1204 are available in the Global Cestode Database (<http://elasmobranchs.tapewormdb.uconn.edu>).

1205

1206 **Fig. 4.** Phylogenetic tree depicting the eight major clades resulting from maximum likelihood (ML)
 1207 analysis of the concatenated dataset for all 71 specimens (Analysis 5). Constructed using Garli with
 1208 partitioned models (GTR+I+ Γ for complete large subunit [28S] nuclear rDNA [*lsrDNA*], partial
 1209 small subunit [18S] nuclear rDNA [*ssrDNA*] and partial cytochrome c oxidase subunit I [*cox1*], and
 1210 TPM3uf+I+ Γ for partial large mitochondrial ribosomal RNA subunit [*rrnL*]). Nodal support is
 1211 given as bootstrap (BS) values generated from 300 bootstrap replicates (first value) and posterior
 1212 probabilities (PP) (second value); black dots indicate nodes with BS values of 100%; white dots
 1213 indicate nodes with PP values of 1; “–” indicated a node not recovered in the ML analysis. Nodal
 1214 support for major clades from single gene analyses (ML only) is given in the paired squares; nodes
 1215 supported in single gene analyses by BS values of $\geq 70\%$ are represented with grey boxes; nodes
 1216 supported in single gene analyses by BS values of $< 70\%$ are represented with white boxes; N/A
 1217 indicates instances in which missing taxa in single gene analyses prevented comparative inferences.
 1218 Branch length scale bar indicates number of substitutions per site.

1219

1220 **Fig. 5.** Schematic of phylogenetic tree resulting from Analysis 5 (Fig. 4) with monophyletic genera
 1221 collapsed, highlighting the eight lecanicephalidean families and their major elasmobranch host
 1222 group associations. Black dots indicate nodes with bootstrap values of 100%; white dots indicate
 1223 nodes with posterior probabilities of 1. Figure numbers reference representative scoleces shown in
 1224 Fig. 1. Branch length scale bar indicates number of substitutions per site.

1225

1226 **Supplementary figure legends**

1227

1228 **Supplementary Fig. S1.** Phylogenetic tree resulting from maximum likelihood analysis of
1229 complete small subunit (18S) nuclear rDNA (*ssrDNA*) data for all 71 specimens (Analysis 1).
1230 Constructed using Garli under the GTR+I+ Γ model. Nodal support is given as bootstrap values
1231 generated from 300 bootstrap replicates. Branch length scale bar indicates number of substitutions
1232 per site.

1233

1234 **Supplementary Fig. S2.** Phylogenetic tree resulting from maximum likelihood analysis of partial
1235 large subunit (28S) nuclear rDNA (*lsrDNA*) data for all 71 specimens (Analysis 2). Constructed
1236 using Garli under the GTR+I+ Γ model. Nodal support is given as bootstrap values generated from
1237 300 bootstrap replicates. Branch length scale bar indicates number of substitutions per site.

1238

1239 **Supplementary Fig. S3.** Phylogenetic tree resulting from maximum likelihood analysis of partial
1240 large mitochondrial ribosomal RNA subunit (*rrnL*) data for 58 specimens (Analysis 3). Constructed
1241 using Garli under the TPM3uf+I+ Γ model. Nodal support is given as bootstrap values generated
1242 from 300 bootstrap replicates. Branch length scale bar indicates number of substitutions per site.

1243

1244 **Supplementary Fig. S4.** Phylogenetic tree resulting from maximum likelihood analysis of partial
1245 cytochrome c oxidase subunit I (*cox1*) data for 66 specimens (Analysis 4). Constructed using Garli
1246 under the GTR+I+ Γ model. Nodal support is given as bootstrap values generated from 300
1247 bootstrap replicates. Branch length scale bar indicates number of substitutions per site.

Table 1

Table 1. List of lecanicephalidean taxa included in the analyses with details on hosts, localities, and voucher deposition. GenBank numbers in bold indicate sequence data newly generated as part of this study.

Proposed Family	Lecanicephalidea Species	Host Species ¹ (Host No.) (Host Order: Host Family)	Locality	<i>IsrDNA</i>	<i>ssrDNA</i>	<i>rrnL</i>	<i>cox1</i>	Voucher No.	PBI No.; TE No. ⁱ
Aberrapecidae n. fam.	<i>Aberrapex</i> n. sp. 1	<i>Aetomylaeus bovinus</i> (SE-257) (Myliobatiformes: Myliobatidae)	Diogue, Senegal	KU249052	KU249002	–	KU249105	LRP8775	PBI635
Cephalobothriidae	<i>Adelobothrium aetiobatis</i>	<i>Aetobatus ocellatus</i> (AU-57) (Myliobatiformes: Myliobatidae)	Lee Point, NT, Australia	EF095257	EF095249	KU248959	KU249118	LRP8272	PBI903; TE-16B
Cephalobothriidae	<i>Adelobothrium aetiobatis</i>	<i>Aetobatus ocellatus</i> (AU-41) (Myliobatiformes: Myliobatidae)	Buffalo Creek, NT, Australia	KU249060	KU249010	KU248957	KU249116	LRP8756	PBI613
Cephalobothriidae	<i>Adelobothrium aetiobatis</i>	<i>Aetobatus ocellatus</i> (AU-41) (Myliobatiformes: Myliobatidae)	Buffalo Creek, NT, Australia	KU249061	KU249011	KU248958	KU249117	LRP8757	PBI614
Cephalobothriidae	<i>Adelobothrium</i> n. sp. 1	<i>Aetobatus ocellatus</i> (SO-34) (Myliobatiformes: Myliobatidae)	Ghizo, Solomon Islands	KU249063	KU249013	KU248961	KU249120	LRP8783	PBI888
Cephalobothriidae	<i>Adelobothrium</i> n. sp. 2	<i>Aetobatus narutobiei</i> (VN-16) (Myliobatiformes: Myliobatidae)	Cat Ba Island, Vietnam	KU249062	KU249012	KU248960	KU249119	LRP8778	PBI883
Cephalobothriidae	<i>Cephalobothrium aetobatidis</i>	<i>Aetobatus ocellatus</i> (TH-19) (Myliobatiformes: Myliobatidae)	Thailand	KU249066	KU249016	KU248964	KU249123	LRP8776	PBI636
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 1	<i>Aetobatus ocellatus</i> (AU-41) (Myliobatiformes: Myliobatidae)	Buffalo Creek, NT, Australia	KU249058	KU249008	KU248954	KU249113	LRP8759	PBI616
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 1	<i>Aetobatus ocellatus</i> (TH-19) (Myliobatiformes: Myliobatidae)	Bangsaray, Thailand	KF685858	KF685782	KU248955	KU249114	LRP8275	PBI905; TE-21
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 2	<i>Aetobatus narinari</i> (FY-4) (Myliobatiformes: Myliobatidae)	Boot Key, FL, USA	KU249068	KU249018	KU248966	KU249125	LRP8777	PBI637
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 2	<i>Aetobatus narinari</i> (FY-5) (Myliobatiformes: Myliobatidae)	Boot Key, FL, USA	KU249069	KU249019	KU248967	KU249126	LRP8799	PBI926
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 3	<i>Aetobatus ocellatus</i> (AU-41) (Myliobatiformes: Myliobatidae)	Buffalo Creek, NT, Australia	KU249067	KU249017	KU248965	KU249124	LRP8784	PBI890
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 4	<i>Aetobatus ocellatus</i> (SO-34) (Myliobatiformes: Myliobatidae)	Ghizo, Solomon Islands	KU249065	KU249015	KU248963	KU249122	LRP8779	PBI884
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 5	<i>Aetobatus ocellatus</i> (SO-34) (Myliobatiformes: Myliobatidae)	Ghizo, Solomon Islands	KU249059	KU249009	KU248956	KU249115	LRP8780	PBI885
Cephalobothriidae	<i>Cephalobothrium</i> n. sp. 6	<i>Aetobatus ocellatus</i> (VN-38) (Myliobatiformes: Myliobatidae)	Bach Long Vi Island, Vietnam	KU249064	KU249014	KU248962	KU249121	LRP8793	PBI900
Cephalobothriidae	New Genus 13 n. sp. 1	<i>Aetomylaeus nichofii</i> (BO-34) (Myliobatiformes: Myliobatidae)	Mukah, Sarawak, Malaysia	KU249057	KU249007	KU248953	KU249112	LRP8800	PBI929
Cephalobothriidae?	<i>Tylocephalum</i> sp. 1	<i>Rhinoptera bonasus</i> (CH-32) (Myliobatiformes: Rhinopteridae)	Charleston Harbor, SC, USA	KU249084	KU249034	–	KU249141	LRP8802	PBI969
Cephalobothriidae?	<i>Tylocephalum</i> sp. 3	<i>Rhinoptera</i> cf. <i>steindachneri</i> (CH-15) (Myliobatiformes: Rhinopteridae)	Bulls Bay, SC, USA	KU249083	KU249033	–	KU249140	LRP8803	PBI970
Eniochobothriidae n. fam.	<i>Eniochobothrium euaxos</i>	<i>Rhinoptera neglecta</i> (AU-85) (Myliobatiformes: Rhinopteridae)	Dundee Beach, NT, Australia	KF685859	KF685784	–	KU249110	LRP8274	PBI906; TE-18A
Eniochobothriidae n. fam.	<i>Eniochobothrium</i> n. sp. 1	<i>Rhinoptera</i> cf. <i>steindachneri</i> (MS05-49) (Myliobatiformes: Rhinopteridae)	Ship Island, MS, USA	KF685860	KF685785	–	KU249111	LRP8308	PBI907; TE-91
Eniochobothriidae n. fam.	<i>Eniochobothrium</i> n. sp. 2	<i>Rhinoptera</i> sp. (SE-80) (Myliobatiformes: Rhinopteridae)	St. Louis, Senegal	KU249055	KU249005	KU248951	KU249108	LRP8785	PBI891

Eniochobothriidae n. fam.	<i>Eniochobothrium</i> n. sp. 3	<i>Rhinoptera neglecta</i> (AU-85) (Myliobatiformes: Rhinopteridae)	Dundee Beach, NT, Australia	KU249056	KU249006	KU248952	KU249109	LRP8786	PBI892
Lecanicephalidae	<i>Collicocephalus baggioi</i>	<i>Aetomylaeus vespertilio</i> (CM03-61) (Myliobatiformes: Myliobatidae)	Weipa, QLD, Australia	KU249072	KU249022	KU248970	KU249129	LRP8769	PBI626
Lecanicephalidae	<i>Elliclacunosus</i> sp.	<i>Aetomylaeus vespertilio</i> (CM03-61) (Myliobatiformes: Myliobatidae)	Weipa, QLD, Australia	KU249070	KU249020	KU248968	KU249127	LRP8773 ^g	PBI633
Lecanicephalidae	<i>Floriparicapitus</i> n. sp. 2	<i>Glaucostegus thouin</i> (KA-70) (Rhinopristiformes: "Rhinobatidae")	Pagatan, South Kalimantan, Indonesia	KU249075	KU249025	KU248974	KU249132	LRP8801	PBI930
Lecanicephalidae	<i>Floriparicapitus plicatilis</i>	<i>Glaucostegus typus</i> (AU-60) (Rhinopristiformes: "Rhinobatidae")	Dundee Beach, NT, Australia	KU249074	KU249024	KU248972	KU249131	LRP8789	PBI896
Lecanicephalidae	<i>Floriparicapitus plicatilis</i> ^a	<i>Glaucostegus typus</i> (CM03-75) (Rhinopristiformes: "Rhinobatidae")	Weipa, QLD, Australia	KF685866	KF685783	KU248973	–	LRP8305	PBI910; TE-87
Lecanicephalidae	<i>Hexacanalus folifer</i>	<i>Gymnura zonura</i> (KA-437) (Myliobatiformes: Gymnuridae)	Manggar, East Kalimantan, Indonesia	KU249073	KU249023	KU248971	KU249130	LRP8761	PBI618
Lecanicephalidae	<i>Lecanicephalum</i> n. sp. 1	<i>Dasyatis</i> sp. (TW-18) (Myliobatiformes: Dasyatidae)	Penghu Islands, Taiwan	KU249078	KU249028	KU248977	KU249135	LRP8787	PBI894
Lecanicephalidae	<i>Lecanicephalum</i> sp. 1	<i>Dasyatis marmorata</i> (SE-168) (Myliobatiformes: Dasyatidae)	Ouakam, Senegal	KU249076	KU249026	KU248975	KU249133	LRP8781	PBI886
Lecanicephalidae	<i>Lecanicephalum</i> sp. 2	<i>Dasyatis guttata</i> (BE-18) (Myliobatiformes: Dasyatidae)	Gales Point Manatee, Belize	KU249077	KU249027	KU248976	KU249134	LRP8782	PBI887
Lecanicephalidae	<i>Rexapex nanus</i>	<i>Aetomylaeus vespertilio</i> (CM03-61) (Myliobatiformes: Myliobatidae)	Weipa, QLD, Australia	KU249071	KU249021	KU248969	KU249128	LRP8772	PBI632
Lecanicephalidae	<i>Stoibocephalum arafurens</i>	<i>Rhina ancylostoma</i> (NT-91) (Rhinopristiformes: Rhinidae)	Wessel Islands, NT, Australia	KU249080	KU249030	KU248979	KU249137	LRP8762	PBI619
Lecanicephalidae	<i>Stoibocephalum arafurens</i>	<i>Rhina ancylostoma</i> (NT-103) (Rhinopristiformes: Rhinidae)	Wessel Islands, NT, Australia	KU249081	KU249031	KU248980	KU249138	LRP8763	PBI620
Lecanicephalidae	<i>Stoibocephalum campanulatum</i> n. comb.	<i>Rhina ancylostoma</i> (NT-103) (Rhinopristiformes: Rhinidae)	Wessel Islands, NT, Australia	KU249082	KU249032	KU248981	KU249139	LRP8768	PBI625
Lecanicephalidae	<i>Stoibocephalum koenneckeorum</i> n. comb.	<i>Rhynchobatus cf. laevis</i> (NT-49) (Rhinopristiformes: Rhynchobatidae)	Wessel Islands, NT, Australia	KU249079	KU249029	KU248978	KU249136	LRP8770	PBI629
Paraberrapecidae n. fam.	<i>Paraberrapex manifestus</i>	<i>Squatina californica</i> (BJ-298) (Squatiniiformes: Squatinidae)	Santa Rosalia, BCS, Mexico	KF685868	KF685781	KU248948	KU249104	(no voucher)	PBI914; TE-142
Polypocephalidae	<i>Anteropora joannae</i>	<i>Taeniura lymma</i> 1 (BO-86) (Myliobatiformes: Dasyatidae)	Mukah, Sarawak, Malaysia	KF685864	KF685789	KU248993	KU249154	LRP8326	PBI911; TE-122
Polypocephalidae	<i>Anteropora klosmamorphis</i>	<i>Narcine maculata</i> (BO-408) (Torpediniformes: Narcinidae)	Sarawak, Malaysia	KU249095	KU249045	KU248995	KU249157	LRP8758	PBI615
Polypocephalidae	<i>Anteropora leelongi</i>	<i>Hemiscyllium ocellatum</i> (JO-12) (Orectolobiformes: Hemiscylliidae)	Cairns, QLD, Australia	KF685857	KF685787	–	–	LRP8278	PBI904; TE-23A
Polypocephalidae	<i>Anteropora</i> n. sp. 1	<i>Pastinachus atrus</i> (NT-105) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KU249096	KU249046	KU248996	KU249158	LRP8791	PBI898
Polypocephalidae	<i>Anteropora</i> n. sp. 2	<i>Neotrygon cf. kuhlii</i> (BO-322) (Myliobatiformes: Dasyatidae)	Pulau Balambangan, Sabah, Malaysia	KU249097	KU249047	–	KU249159	LRP8798	PBI925
Polypocephalidae	<i>Anteropora patulobothridium</i>	<i>Taeniura lymma</i> 1 (BO-131) (Myliobatiformes: Dasyatidae)	Pulau Mabul, Sabah, Malaysia	KU249092	KU249042	–	KU249155	LRP8796	PBI923
Polypocephalidae	<i>Anteropora pumilionis</i>	<i>Himantura cf. pastinacoides</i> (BO-98) (Myliobatiformes: Dasyatidae)	Kampung Tetabuan, Sabah, Malaysia	KU249093	KU249043	–	–	LRP8797	PBI924

Polypocephalidae	<i>Anteropora comicus</i> n. comb.	<i>Narcine maculata</i> (BO-408) (Torpediniformes: Narcinidae)	Sarawak, Malaysia	KU249094	KU249044	KU248994	KU249156	LRP8794 ^g	PBI902
Polypocephalidae	<i>Anteropora</i> sp. ^b	<i>Taeniura lymma</i> 1 (BO-86) (Myliobatiformes: Dasyatidae)	Semporna, Sabah, Malaysia	KF685863	KF685788	–	–	LRP8307	PBI912; TE-90
Polypocephalidae	<i>Anthemobothrium</i> sp. 1	<i>Pastinachus atrus</i> (NT-44) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KF685870	KF685790	KU248992	KU249153	LRP8292	PBI916; TE-47A
Polypocephalidae	<i>Flapocephalus</i> n. sp. 1	<i>Pastinachus atrus</i> (CM03-79) (Myliobatiformes: Dasyatidae)	Weipa, QLD, Australia	KF685861	KF685795	KU248986	KU249147	LRP8302	PBI908; TE-88
Polypocephalidae	<i>Flapocephalus</i> n. sp. 2	<i>Pastinachus atrus</i> (KA-32) (Myliobatiformes: Dasyatidae)	Sei Kerbau, East Kalimantan, Indonesia	KU249087	KU249037	KU248985	KU249146	LRP8760	PBI617
Polypocephalidae	<i>Hornellobothrium</i> n. sp. 1	<i>Aetobatus ocellatus</i> (NT-76) (Myliobatiformes: Myliobatidae)	Wessel Islands, NT, Australia	KU249090	KU249040	KU248990	KU249151	LRP8795	PBI922
Polypocephalidae	<i>Hornellobothrium</i> n. sp. 2	<i>Aetobatus ocellatus</i> (AU-41) (Myliobatiformes: Myliobatidae)	Buffalo Creek, NT, Australia	KU249089	KU249039	KU248989	KU249150	LRP8774	PBI634
Polypocephalidae	<i>Hornellobothrium najaforme</i> ^c	<i>Aetobatus ocellatus</i> (NT-76) (Myliobatiformes: Myliobatidae)	Wessel Islands, NT, Australia	KF685865	KF685793	–	–	LRP8285	PBI909; TE-32B
Polypocephalidae	New Genus 11 n. sp. 1	<i>Rhynchobatus</i> cf. <i>laevis</i> (NT-49) (Rhinopristiformes: Rhynchobatidae)	Wessel Islands, NT, Australia	KU249098	KU249048	KU248997	KU249161	LRP8771	PBI630
Polypocephalidae	New Genus 11 n. sp. 2	<i>Glaucostegus typus</i> (AU-56) (Rhinopristiformes: "Rhinobatidae")	Dundee Beach, NT, Australia	KU249099	KU249049	KU248998	KU249162	(no voucher)	PBI901
Polypocephalidae	New Genus 12 n. sp. 1	<i>Himantura</i> cf. <i>gerrardi</i> 2 (KA-211) (Myliobatiformes: Dasyatidae)	Sidu, West Kalimantan, Indonesia	KU249091	KU249041	KU248991	KU249152	LRP8790	PBI897
Polypocephalidae	<i>Polypocephalus helmuti</i>	<i>Rhinoptera neglecta</i> (AU-85) (Myliobatiformes: Rhinopteridae)	Dundee Beach, NT, Australia	KF685869	KF685786	KU248988	KU249149	LRP8273	PBI915; TE-17A
Polypocephalidae	<i>Polypocephalus</i> sp. 2	<i>Himantura granulata</i> (CM03-74) (Myliobatiformes: Dasyatidae)	Weipa, QLD, Australia	KF685748	KF685791	–	KU249160	LRP8330	PBI917; TE-133
Polypocephalidae	<i>Polypocephalus</i> sp. 3	<i>Taeniura lymma</i> 1 (BO-86) (Myliobatiformes: Dasyatidae)	Semporna, Sabah, Malaysia	KU249088	KU249038	KU248987	KU249148	LRP8764	PBI621
Polypocephalidae	<i>Seussapex karybares</i> ^d	<i>Himantura uarnak</i> 2 (CM03-24) (Myliobatiformes: Dasyatidae)	Weipa, QLD, Australia	KF685867	KF685792	KU249001	KU249165	LRP8331	PBI913; TE-134
Polypocephalidae	<i>Seussapex</i> n. sp. 2	<i>Himantura uarnak</i> 3 (BO-47) (Myliobatiformes: Dasyatidae)	Mukah, Sarawak, Malaysia	KU249100	KU249050	KU248999	KU249163	LRP8765	PBI622
Polypocephalidae	<i>Seussapex</i> n. sp. 3	<i>Himantura leoparda</i> (NT-32) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KU249101	KU249051	KU249000	KU249164	LRP8792	PBI899
Tetragonocephalidae	<i>Tetragonocephalum passeyi</i>	<i>Himantura leoparda</i> (NT-32) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KF685871	KF685856	KU248984	KU249144	LRP8276 ^h	PBI918; TE-19A
Tetragonocephalidae	<i>Tetragonocephalum</i> n. sp. 1 ^e	<i>Urogymnus asperrimus</i> (CM03-53) (Myliobatiformes: Dasyatidae)	Weipa, QLD, Australia	KF685872	KF685796	–	KU249145	LRP8306	PBI919; TE-89
Tetragonocephalidae	<i>Tetragonocephalum</i> n. sp. 2	<i>Himantura jenkinsii</i> (NT-106) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KU249085	KU249035	KU248982	KU249142	LRP8766	PBI623
Tetragonocephalidae	<i>Tetragonocephalum</i> n. sp. 3	<i>Himantura leoparda</i> (NT-117) (Myliobatiformes: Dasyatidae)	Wessel Islands, NT, Australia	KU249086	KU249036	KU248983	KU249143	LRP8767	PBI624
Zanobatocestidae n. fam.	<i>Zanobatocestus major</i>	<i>Zanobatus schoenleinii</i> (SE-105) (Rhinobatiformes: Zanobatidae?)	Soumbédioune, Senegal	KU249053	KU249003	KU248949	KU249106	LRP8487	PBI628
Zanobatocestidae n. fam.	<i>Zanobatocestus minor</i>	<i>Zanobatus schoenleinii</i> (SE-201) (Rhinobatiformes: Zanobatidae?)	Soumbédioune, Senegal	KU249054	KU249004	KU248950	KU249107	LRP8788	PBI895

^a As New Genus 5 n. sp. 1 in Caira et al. (2014)

^b As *Anteropora patulobothridium* in Caira et al. (2014)

^c As *Hornellobothrium* n. sp. 1 in Caira et al. (2014)

^d as New Genus 6 n. sp. 1 in Caira et al. (2014)

^e as *Tetragonocephalum* sp. 1 in Caira et al. (2014)

^f Host species designations follow Naylor et al. (2012a); host classification follows Naylor et al. (2012b); quotes indicate paraphyletic family

^g photo voucher

^h In error as LRP7276 in Caira et al. (2014)

ⁱ See Caira et al. (2014)

lsrDNA, large subunit (28S) nuclear rDNA; *ssrDNA*, small subunit (18S) nuclear rDNA; *rrnL*, large mitochondrial ribosomal RNA subunit ; *cox1*, cytochrome c oxidase subunit I.

Figure 1

Figure 2 colour

Figure 2 black and white

Figure 3 colour

Figure 3 black and white

Figure 4 revised

Figure 5