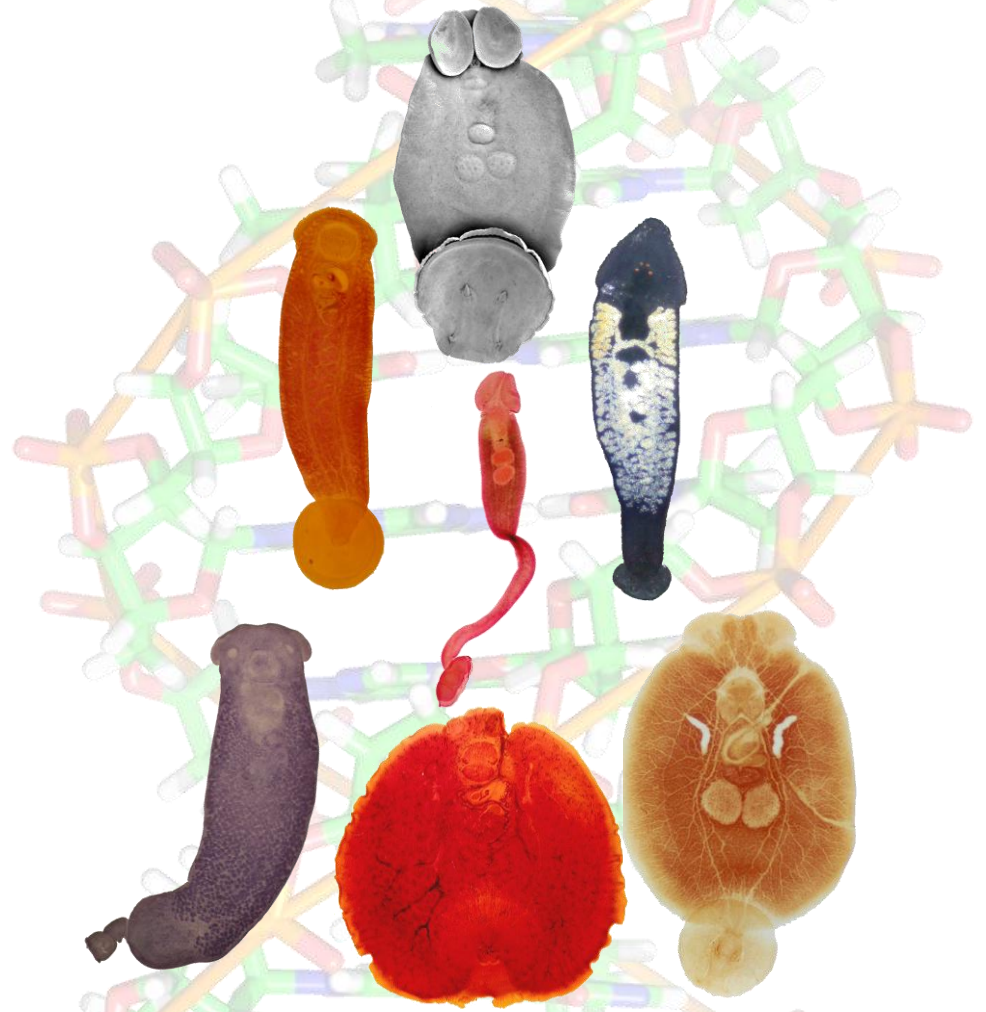


**FAMILY TIES: MOLECULAR
PHYLOGENETICS, EVOLUTION
AND RADIATION OF
FLATWORM PARASITES
(MONOGENEA: CAPSALIDAE)**



ELIZABETH PERKINS

Presented for the degree of Doctor of Philosophy

School of Earth and Environmental Sciences

The University of Adelaide, South Australia

February, 2010

REFERENCES

- Ahlberg PE (2001) 'Major events in early vertebrate evolution: paleontology, phylogeny, genetics, and development.' (Taylor and Francis: London)
- Akaike H (1985) Prediction and entropy. In 'A celebration of statistics'. (Eds AC Atkinson and SE Fienberg) pp. 1–24. (Springer-Verlag: Berlin)
- Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ (2009) Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 13410–13414.
- Antao T, Beja-Pereira A, Fonseca MM, Harris DJ (2007) BACA: a mitochondrial genome retriever, organizer and visualizer. *Molecular Ecology Notes* **7**, 217–220.
- Arevalo E, Davis SK, Sites JW (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among 8 chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology* **43**, 387–418.
- Arratia G (1997) Basal teleosts and teleostean phylogeny. *Palaeo Ichthyologica* **7**, 5–168.
- Arratia G (1999) The monophyly of Teleostei and stem-group teleosts. Consensus and disagreements. In 'Mesozoic fishes 2. Systematics and fossil record.' (Ed. H-P Schultze) pp. 265–334. (Verlag Dr. Friedrich Pfeil: Munchen)
- Arratia G (2004) Mesozoic halecostomes and the early radiation of teleosts. In 'Mesozoic fishes 3: Systematics, paleoenvironments and biodiversity'. (Eds G Arratia and A Tintori) pp. 279–315. (Verlag Dr. Friedrich Pfeil: Munchen)
- Azuma Y, Kumazawa Y, Miya M, Mabuchi K, Nishida M (2008) Mitogenomic evaluation of the historical biogeography of cichlids toward reliable dating of teleostean divergences. *BMC Evolutionary Biology* **8**, Article No. 215.
- Bambach RK, Knoll AH, Wang SC (2004) Origination, extinction, and mass depletions of marine diversity. *Paleobiology* **30**, 522–542.
- Banks JC, Palma RL, Paterson AM (2006) Cophylogenetic relationships between penguins and their chewing lice. *Journal of Evolutionary Biology* **19**, 156–166.
- Banks JC, Paterson AM (2005) Multi-host parasite species in cophylogenetic studies. *International Journal for Parasitology* **35**, 741–746.

- Baverstock PR, Fielke R, Johnson AM, Bray RA, Beveridge I (1991) Conflicting phylogenetic hypotheses for the parasitic Platyhelminthes tested by partial sequencing of 18S ribosomal RNA. *International Journal for Parasitology* **21**, 329–339.
- Benton MJ (1990) 'Vertebrate palaeontology. Biology and evolution.' (Unwin Hyman: London)
- Benton MJ (2003) 'When life nearly died: The greatest mass extinction of all time.' (Thames and Hudson: London)
- Berriman M, Haas BJ, LoVerde PT, Wilson RA, Dillon GP, Cerqueira GC, Mashiyama ST, Al-Lazikani B, Andrade LF, Ashton PD, Aslett MA, Bartholomeu DC, Blandin G, Caffrey CR, Coghlan A, Coulson R, Day TA, Delcher A, DeMarco R, Djikeng A, Eyre T, Gamble JA, Ghedin E, Gu Y, Hertz-Fowler C, Hirai H, Hirai Y, Houston R, Ivens A, Johnston DA, Lacerda D, Macedo CD, McVeigh P, Ning ZM, Oliveira G, Overington JP, Parkhill J, Pertea M, Pierce RJ, Protasio AV, Quail MA, Rajandream MA, Rogers J, Sajid M, Salzberg SL, Stanke M, Tivey AR, White O, Williams DL, Wortman J, Wu WJ, Zamanian M, Zerlotini A, Fraser-Liggett CM, Barrell BG, El-Sayed NM (2009) The genome of the blood fluke *Schistosoma mansoni*. *Nature* **460**, 352–360.
- Boeger WA, Kritsky DC (1993) Phylogeny and a revised classification of the Monogonoidea Bychowsky, 1937 (Platyhelminthes). *Systematic Parasitology* **26**, 1–32.
- Boeger WA, Kritsky DC (1997) Coevolution of the Monogonoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *International Journal for Parasitology* **27**, 1495–1511.
- Boeger WA, Kritsky DC (2001) Phylogenetic relationships of the Monogonoidea. In 'Interrelationships of the Platyhelminthes'. (Eds DTJ Littlewood and RA Bray) pp. 92–102. (Taylor and Francis: London and New York)
- Bohlke EB (1989) Orders Anguilliformes and Saccopharyngiformes, leptocephali. In 'Fishes of the western north Atlantic' pp. 679–681. (Yale University: New Haven)
- Boore JL, Brown WM (2000) Mitochondrial genomes of *Galathealinum*, *Helobdella*, and *Platynereis*: Sequence and gene arrangement comparisons indicate the

- pogonophora is not a phylum and annelida and arthropoda are not sister taxa. *Molecular Biology and Evolution* **17**, 988–988.
- Britz R (2004) Egg structure and larval development of *Pantodon buchholzi* (Teleostei: Osteoglossomorpha), with a review of data on reproduction and early life history in other osteoglossomorphs. *Ichthyological Exploration of Freshwaters* **15**, 209–224.
- Britz R, Bartsch P (2003) The myth of dorsal ribs in gnathostome vertebrates. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, S1–S4.
- Brooks DR (1989) The phylogeny of the Cercomeria (Platyhelminthes, Rhabdocoela) and general evolutionary principles. *Journal of Parasitology* **75**, 606–616.
- Brooks DR, McLennan DA (1993) 'Parascript: parasites and the language of evolution.' (Smithsonian Institution Press: Washington and London)
- Brummitt RK (2002) How to chop up a tree. *Taxon* **51**, 31–41.
- Buchmann K (1999) Immune mechanisms in fish skin against monogeneans - a model. *Folia Parasitologica* **46**, 1–9.
- Bullard SA, Benz GW, Braswell JS (2000) *Dionchus postoncomiracidia* (Monogenea: Dionchidae) from the skin of blacktip sharks, *Carcharhinus limbatus* (Carcharhinidae). *Journal of Parasitology* **86**, 245–250.
- Bychowsky BE (1957) 'Monogenetic trematodes, their systematics and phylogeny.' (Izdatel'suo Akademiya Nauk SSSR: Moscow. In Russian: English translation edited by Hargis, W.J. Jr., 1961)
- Cable J, Harris PD, Tinsley RC, Lazarus CM (1999) Phylogenetic analysis of *Gyrodactylus* spp. (Platyhelminthes : Monogenea) using ribosomal DNA sequences. *Canadian Journal of Zoology* **77**, 1439–1449.
- Campos A, Cummings MP, Reyes JL, Lacleste JP (1998) Phylogenetic relationships of Platyhelminthes based on 18S ribosomal gene sequences. *Molecular Phylogenetics and Evolution* **10**, 1–10.
- Cantino PD, de Queiroz K (2007) International Code of Phylogenetic Nomenclature, version 4b. Available at <http://www.phylocode.org>.
- Carranza S, Baguna J, Riutort M (1997) Are the Platyhelminthes a monophyletic primitive group? An assessment using 18S rDNA sequences. *Molecular Biology and Evolution* **14**, 485–497.

- Castoe TA, de Koning APJ, Kim HM, Gu WJ, Noonan BP, Naylor G, Jiang ZJ, Parkinson CL, Pollock DD (2009) Evidence for an ancient adaptive episode of convergent molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 8986-8991.
- Charleston NA, Perkins SL (2006) Traversing the tangle: Algorithms and applications for cophylogenetic studies. *Journal of Biomedical Informatics* **39**, 62–71.
- Chen WJ, Bonillo C, Lecointre G (2003) Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. *Molecular Phylogenetics and Evolution* **26**, 262–288.
- Chisholm LA, Morgan JAT, Adlard RD, Whittington ID (2001) Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. *International Journal for Parasitology* **31**, 1253–1263.
- Chisholm LA, Whittington ID (2007) Review of the Capsalinae (Monogenea: Capsalidae). *Zootaxa* **1559**, 1–30.
- Clack JA (2002) 'Gaining ground: the origin and evolution of tetrapods.' (Indiana University Press: Bloomington)
- Cloutier R, Ahlberg PE (1996) Morphology, characters, and the interrelationships of basal sarcopterygians. In 'Interrelationships of fishes.' (Eds MLJ Stiassny, LR Parenti and GD Johnson) pp. 445–479. (Academic Press: San Diego)
- Cloutier R, Arratia G (2004) Early diversification of actinopterygians. In 'Recent advances in the origin and early radiation of vertebrates'. (Eds G Arratia and MVH Wilson) pp. 217–270. (Verlag Dr. Friedrich Pfeil: Munchen)
- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46**, 419–437.
- Combes C (2001) 'Parasitism: The ecology and evolution of intimate interactions.' (University of Chicago Press: Chicago)
- Cracraft J, Donoghue MJ (2004) 'Assembling the Tree Of Life.' (Oxford University Press: New York)

- Dalton JP, Skelly P, Halton DW (2004) Role of the tegument and gut in nutrient uptake by parasitic platyhelminths. *Canadian Journal of Zoology* **82**, 211–232.
- Dayrat B, Cantino PD, Clarke JA, De Queiroz K (2008) Species names in the PhyloCode: The approach adopted by the International Society for Phylogenetic Nomenclature. *Systematic Biology* **57**, 507–514.
- de Carvalho MR (1996) Higher-level elasmobranch phylogeny, basal squaleans, and paraphyly. In 'Interrelationships of fishes'. (Eds MLJ Stiassey, LR Parenti and GD Johnson) pp. 35–62. (Academic Press: San Diego)
- de Pinna MCC (1996) Teleostean monophyly. In 'Interrelationships of fishes.' (Eds MLJ Stiasny, LR Parenti and GD Johnson) pp. 147–162. (Academic Press: San Diego)
- de Vienne DM, Giraud T, Martin OC (2007) A congruence index for testing topological similarity between trees. *Bioinformatics* **23**, 3119–3124.
- Delarbre C, Gallut C, Barriol V, Janvier P, Gachelin G (2002) Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: The comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. *Molecular Phylogenetics and Evolution* **22**, 184–192.
- Desdevises Y, Morand S, Jousson O, Legendre P (2002) Coevolution between *Lamellodiscus* (Monogenea : Diplectanidae) and Sparidae (Teleostei): The study of a complex host-parasite system. *Evolution* **56**, 2459–2471.
- Dettai A, Lecointre G (2005) Further support for the clades obtained by multiple molecular phylogenies in the acanthomorph bush. *Comptes Rendus Biologies* **328**, 674–689.
- Deveney M (2002) 'Taxonomy and biology of benedeniine capsalid monogeneans.' PhD Thesis, The University of Queensland.
- Deveney MR, Chisholm LA, Whittington ID (2001) First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Diseases of Aquatic Organisms* **46**, 79–82.
- Didier DA (1995) Phylogenetic systematics of extant chimaeroid fishes (Holocephali, Chimaeroidei). *American Museum Novitates* **3119**, 1–86.
- Dolman G, Hugall AF (2008) Combined mitochondrial and nuclear data enhance resolution of a rapid radiation of Australian rainbow skinks (Scincidae: *Carlia*). *Molecular Phylogenetics and Evolution* **49**, 782–794.

- Douady CJ, Dosay M, Shivji MS, Stanhope MJ (2003) Molecular phylogenetic evidence refuting the hypothesis of Batoidea (rays and skates) as derived sharks. *Molecular Phylogenetics and Evolution* **26**, 215–221.
- Dowton M, Cameron SL, Dowavic JI, Austin AD, Whiting MF (2009) Characterization of 67 mitochondrial tRNA gene rearrangements in the Hymenoptera suggests that mitochondrial tRNA gene position is selectively neutral. *Molecular Biology and Evolution* **26**, 1607–1617.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.
- Dunn CW, Hejnol A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse GW, Obst M, Edgecombe GD, Sorensen MV, Haddock SHD, Schmidt-Rhaesa A, Okusu A, Kristensen RM, Wheeler WC, Martindale MQ, Giribet G (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* **452**, 745–749.
- Duvall MR, Ervin AB (2004) 18S gene trees are positively misleading for monocot/dicot phylogenetics. *Molecular Phylogenetics and Evolution* **30**, 97–106.
- Egger B, Steinke D, Tarui H, De Mulder K, Arendt D, Borgonie G, Funayama N, Gschwentner R, Hartenstein V, Hobmayer B, Hooge M, Hroudá M, Ishida S, Kobayashi C, Kualess G, Nishimura O, Pfister D, Rieger R, Salvenmoser W, Smith J, III, Technau U, Tyler S, Agata K, Salzburger W, Ladurner P (2009) To be or not to be a flatworm: The acoel controversy. *PLoS ONE* **4**, Article No. e5502.
- Egorova TP (1989) A taxonomic analysis of the subfamily Capsalinae (Monogenoidea: Capsalidae). In 'Parazitologicheskie issledovaniya: sbornik nauchnykh trudov' pp. 46–54. (Dal'nevostochnoe otdelenie: Akademiya Nauk SSSR, Vladivostok)
- Egorova TP (1994) A taxonomic review of the subfamily Trochopodinae (Monogenoidea: Capsalidae). *Parazitologiya* **28**, 81–91.
- Egorova TP (1997) A taxonomic review of the subfamily Benedeniinae (Monogenoidea: Capsalidae). *Parazitologiya* **31**, 438–451.
- Egorova TP (1999) Systematics of the subfamily Entobdellinae (Monogenoidea: Capsalidae). *Parazitologiya* **33**, 420–425.

- Egorova TP (2000) New monogeneans of the genus *Dionchus* (Capsalidae: Dionchinae). *Parazitologiya* **34**, 252–258.
- Ehlers U (1985) 'The phylogenetic system of Platyhelminthes.' (G. Fischer: Stuttgart, New York)
- Ernst I, Whittington ID (1996) Hatching rhythms in the capsalid monogeneans *Benedenia lutjani* from the skin and *B. rohdei* from the gills of *Lutjanus carponotatus* at Heron Island, Queensland, Australia. *International Journal for Parasitology* **26**, 1191–1204.
- Euzet L, Combes C (2003) Some controversial questions regarding Monogenea. In 'Taxonomy, ecology and evolution of metazoan parasites Tome I'. (Eds C Combes, J Jourdan, A Ducreux-Modat and J-R Pages) pp. 303–320. (University of Perpignan Press: Perpignan, France)
- Euzet L, Oliver G, Ktari MH (1995) Organization and development of the osmoregulatory system in Monogenea. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 35–51.
- Fahrenholz H (1913) Ectoparasitims and Abstammungslehre. *Zoologischer Anzeiger* **41**, 371–374.
- Filleul A, Lavoue S (2001) Basal teleosts and the question of elopomorph monophyly. Morphological and molecular approaches. *Comptes Rendus de L'Academie des Sciences Serie III - Sciences de la Vie* **324**, 393–399.
- Gerardo NM, Caldera EJ (2007) Labile associations between fungus-growing ant cultivars and their garden pathogens. *Isme Journal* **1**, 373–384.
- Glennon V, Chisholm LA, Whittington ID (2006) Three unrelated species, 3 sites, same host monogenean parasites of the southern fiddler ray, *Trygonorrhina fasciata*, in South Australia: egg hatching strategies and larval behaviour. *Parasitology* **133**, 55–66.
- Grande L, Bemis WE (1998) A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. *Journal of Vertebrate Paleontology* **18**, 1–660.
- Grogan ED, Lund R (2004) The origin and relationships of early Chondrichthyes. In 'Biology of sharks and their relatives'. (Eds JC Carrier, JA Musick and MR Heithaus) pp. 3–31. (CRC Press: Boca Raton, Florida)

- Gursoy HC, Koper D, Benecke BJ (2000) The vertebrate 7S K RNA separates hagfish (*Myxine glutinosa*) and lampreys (*Lampetra fluviatilis*). *Journal of Molecular Evolution* **50**, 456–464.
- Haeberlein S, Haas W (2008) Chemical attractants of human skin for swimming *Schistosoma mansoni* cercariae. *Parasitology Research* **102**, 657–662.
- Hafner MS, Page RDM (1995) Molecular phylogenies and host-parasite cospeciation - gophers and lice as a model system. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **349**, 77–83.
- Halton DW (1997) Nutritional adaptations to parasitism within the Platyhelminthes. *International Journal for Parasitology* **27**, 693–704.
- Hedges SB, Kumar S (2004) Precision of molecular time estimates. *Trends in Genetics* **20**, 242–247.
- Helfman GS, Collette BB, Facey DE (1997) 'The diversity of fishes.' (Blackwell Science: Malden, Massachusetts)
- Ho JS (2001) Why do symbiotic copepods matter? *Hydrobiologia* **453**, 1–7.
- Hoberg EP, Gardner SL, Campbell RA (1999) Systematics of the Eucestoda: advances toward a new phylogenetic paradigm, and observations on the early diversification of tapeworms and vertebrates. *Systematic Parasitology* **42**, 1–12.
- Holcroft NI (2004) A molecular test of alternative hypotheses of tetraodontiform (Acanthomorpha: Tetraodontiformes) sister group relationships using data from the RAG1 gene. *Molecular Phylogenetics and Evolution* **32**, 749–760.
- Horandl E (2006) Paraphyletic versus monophyletic taxa-evolutionary versus cladistic classifications. *Taxon* **55**, 564–570.
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.
- Hurley IA, Mueller RL, Dunn KA, Schmidt EJ, Friedman M, Ho RK, Prince VE, Yang ZH, Thomas MG, Coates MI (2007) A new time-scale for ray-finned fish evolution. *Proceedings of the Royal Society B-Biological Sciences* **274**, 489–498.
- Huyse T, Buchmann K, Littlewood DTJ (2008) The mitochondrial genome of *Gyrodactylus derjavinoidea* (Platyhelminthes: Monogenea) - a mitogenomic approach for *Gyrodactylus* species and strain identification. *Gene* **417**, 27–34.

- Huyse T, Plaisance L, Webster BL, Mo TA, Bakke TA, Bachmann L, Littlewood DTJ (2007) The mitochondrial genome of *Gyrodactylus salaris* (Platyhelminthes: Monogenea), a pathogen of Atlantic salmon (*Salmo salar*). *Parasitology* **134**, 739–747.
- Iglésias SP, Lecointre G, Sellos DY (2005) Extensive paraphyly within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial. *Molecular Phylogenetics and Evolution* **34**, 569–583.
- Inoue JG, Miya M, Tsukamoto K, Nishida M (2001) A mitogenomic perspective on the basal teleostean phylogeny: Resolving higher-level relationships with longer DNA sequences. *Molecular Phylogenetics and Evolution* **20**, 275–285.
- Jamieson BGM (1991) 'Fish evolution and systematics: evidence from spermatozoa.' (Cambridge University Press: Cambridge, England)
- Janvier P (1996a) 'Early vertebrates.' (Oxford University Press: New York)
- Janvier P (1996b) The dawn of the vertebrates: characters versus common ascent in the rise of current vertebrate phylogenies. *Palaeontology* **39**, 259–287.
- Janvier P (2008) Early jawless vertebrates and cyclostome origins. *Zoological Science* **25**, 1045–1056.
- Justine JL (1991) Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony analysis of spermiogenetic and spermatozoal ultrastructural characters. *International Journal for Parasitology* **21**, 821–838.
- Justine JL (1997) The general classification of parasitic Platyhelminthes: recent changes, and the use of ultrastructural characters, particularly those of spermatozoa. *Bulletin de la Société Zoologique de France* **122**, 269–277.
- Justine JL (1998) Non-monophyly of the monogeneans? *International Journal for Parasitology* **28**, 1653–1657.
- Justine JL, Lambert A, Mattei X (1985) Spermatozoon ultrastructure and phylogenetic-relationships in the monogeneans (Platyhelminthes). *International Journal for Parasitology* **15**, 601–608.
- Justine JL, Mattei X (1987) Phylogenetic relationships between the families Capsalidae and Dionchidae (Platyhelminthes, Monogenea, Monopisthocotylea) indicated by the comparative ultrastructural study of spermiogenesis. *Zoologica Scripta* **16**, 111–116.
- Kaneko JJ, Yamada R, Brock JA, Nakamura RM (1988) Infection of tilapia, *Oreochromis mossambicus* (Trewavas), by a marine monogenean,

- Neobenedenia melleni* (Maccallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its treatment. *Journal of Fish Diseases* **11**, 295–300.
- Kearn G (2002) *Entobdella soleae* - pointers to the future. *International Journal for Parasitology* **32**, 367–372.
- Kearn GC (1963) The egg, oncomiracidium and larval development of *Entobdella soleae*, a monogenean skin parasite of the common sole. *Parasitology* **53**, 435–447.
- Kearn GC (1967) Experiments on host-finding and host-specificity in monogenean skin parasite *Entobdella soleae*. *Parasitology* **57**, 585–605.
- Kearn GC (1973) Endogenous circadian hatching rhythm in monogenean skin parasite *Entobdella soleae*, and its relationship to activity rhythm of host (*Solea solea*). *Parasitology* **66**, 101–122.
- Kearn GC (1974) Effects of fish skin mucus on hatching in monogenean parasite *Entobdella soleae* from skin of common sole, *Solea solea*. *Parasitology* **68**, 173–188.
- Kearn GC (1981) Biology of monogeneans. Behaviour of oncomiracidia. *Parasitology* **82**, 57–59.
- Kearn GC (1982) Rapid hatching induced by light intensity reduction in the monogenean *Entobdella diadema*. *Journal of Parasitology* **68**, 171–172.
- Kearn GC (1984) The migration of the monogenean *Entobdella soleae* on the surface of its host, *Solea solea*. *International Journal for Parasitology* **14**, 63–69.
- Kearn GC (1986) The eggs of monogeneans. *Advances in Parasitology* **25**, 175–273.
- Kearn GC (1988) The monogenean skin parasite *Entobdella soleae*: Movement of adults and juveniles from host to host (*Solea solea*). *International Journal for Parasitology* **18**, 313–319.
- Kearn GC (1998) 'Parasitism and the Platyhelminths.' (Chapman and Hall: London)
- Kearn GC, James R, Evans-Gowing R (1993) Insemination and population density in *Entobdella soleae*, a monogenean skin parasite of the common sole, *Solea solea*. *International Journal for Parasitology* **23**, 891–899.
- Kearn GC, Ogawa K, Maeno Y (1992) Hatching patterns of the monogenean parasites *Benedenia seriolae* and *Heteraxine heterocerca* from the skin and gills, respectively, of the same host fish, *Seriola quinqueradiata*. *Zoological Science* **9**, 451–455.

- Kearn GC, Whittington ID (1991) Swimming in a sub-adult monogenean of the genus *Entobdella*. *International Journal for Parasitology* **21**, 739–741.
- Kearn GC, Whittington ID (1992) A response to light in an adult encotyllabine (Capsalid) monogenean from the pharyngeal tooth pads of some marine teleost fishes. *International Journal for Parasitology* **22**, 119–121.
- Kearn GC, Whittington ID (2005) *Neoentobdella* gen nov for species of *Entobdella* Blainville in Lamarck, 1818 (Monogenea: Capsalidae: Entobdellinae) from stingray hosts, with descriptions of two new species. *Acta Parasitologica* **50**, 32–48.
- Kearn GC, Whittington ID, Evans-Gowing R (2007) Revision of *Entobdella* Blainville in Lamarck, 1818, with special emphasis on the nominal (type) species "*Entobdella hippoglossi* (Muller, 1776) Blainville, 1818" (Monogenea : Capsalidae: Entobdellinae) from teleost flatfishes, with descriptions of three new species and a new genus. *Zootaxa* **1659**, 1–53.
- Kritsky DC, Fennessy CJ (1999) *Calicobenedenia polyprioni* n. gen., n. sp. (Monogenoidea: Capsalidae) from the external surfaces of wreckfish, *Polyprion americanus* (Teleostei: Polyprionidae), in the north Atlantic. *Journal of Parasitology* **85**, 192–195.
- Kuraku S, Hoshiyama D, Katoh K, Suga H, Miyata T (1999) Monophyly of lampreys and hagfishes supported by nuclear DNA-coded genes. *Journal of Molecular Evolution* **49**, 729–735.
- Lauder GV, Liem KF (1983) The evolution and interrelationships of the actinopterygian fishes. *Bulletin of the Museum of Comparative Zoology* **150**, 95–197.
- Le TH, Blair D, Agatsuma T, Humair PF, Campbell NJH, Iwagami M, Littlewood DTJ, Peacock B, Johnston DA, Bartley J, Rollinson D, Herniou EA, Zarlenga DS, McManus DP (2000) Phylogenies inferred from mitochondrial gene orders - a cautionary tale from the parasitic flatworms. *Molecular Biology and Evolution* **17**, 1123–1125.
- Le TH, Blair D, McManus DP (2002a) Mitochondrial genomes of parasitic flatworms. *Trends in Parasitology* **18**, 206–213.
- Le TH, Pearson MS, Blair D, Dai N, Zhang LH, McManus DP (2002b) Complete mitochondrial genomes confirm the distinctiveness of the horse-dog and sheep-dog strains of *Echinococcus granulosus*. *Parasitology* **124**, 97–112.

- Le TH, Humair PF, Blair D, Agatsuma T, Littlewood DTJ, McManus DP (2001) Mitochondrial gene content, arrangement and composition compared in African and Asian schistosomes. *Molecular and Biochemical Parasitology* **117**, 61–71.
- Lee MSY (2009) Hidden support from unpromising data sets strongly unites snakes with anguimorph 'lizards'. *Journal of Evolutionary Biology* **22**, 1308–1316.
- Lee MSY, Hugall AF (2003) Partitioned likelihood support and the evaluation of data set conflict. *Systematic Biology* **52**, 15–22.
- Legendre P, Desdevises Y, Bazin E (2002) A statistical test for host-parasite coevolution. *Systematic Biology* **51**, 217–234.
- Legendre P, Legendre L (1998) 'Numerical ecology. Second English edition.' (Elsevier Science B.V.: Amsterdam)
- Li B, Dettai A, Cruaud C, Couloux A, Desoutter-Meniger M, Lecointre G (2009) RNF213, a new nuclear marker for acanthomorph phylogeny. *Molecular Phylogenetics and Evolution* **50**, 345–363.
- Li CH, Orti G, Zhang G, Lu GQ (2007) A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evolutionary Biology* **7**, 44–44.
- Light JE, Hafner MS (2008) Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of the genus *Fahrenholzia* (Phthiraptera: Anoplura). *Systematic Biology* **57**, 449–465.
- Littlewood DTJ, Bray RA, Clough KA (1998) A phylogeny of the Platyhelminthes: towards a total-evidence solution. *Hydrobiologia* **383**, 155–160.
- Littlewood DTJ, Lockyer AE, Webster BL, Johnston DA, Le TH (2006) The complete mitochondrial genomes of *Schistosoma haematobium* and *Schistosoma spindale* and the evolutionary history of mitochondrial genome changes among parasitic flatworms. *Molecular Phylogenetics and Evolution* **39**, 452–467.
- Littlewood DTJ, Olson PD, Telford MJ, Herniou EA, Riutort M (2001) Elongation factor 1-alpha sequences alone do not assist in resolving the position of the Acoela within the Metazoa. *Molecular Biology and Evolution* **18**, 437–442.
- Littlewood DTJ, Rohde K, Bray RA, Herniou EA (1999a) Phylogeny of the Platyhelminthes and the evolution of parasitism. *Biological Journal of the Linnean Society* **68**, 257–287.

- Littlewood DTJ, Rohde K, Clough KA (1999b) The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biological Journal of the Linnean Society* **66**, 75–114.
- Littlewood DTJ, Rohde K, Clough KA (1997) Parasite speciation within or between host species? Phylogenetic evidence from site-specific polystome monogeneans. *International Journal for Parasitology* **27**, 1289–1297.
- Littlewood DTJ, Telford MJ, Bray RA (2004) Protostomes and Platyhelminthes: The worm's turn. In 'Assembling the tree of life'. (Eds J Cracraft and MJ Donoghue) pp. 209–234. (Oxford University Press)
- Litvaitis MK, Rohde K (1999) A molecular test of platyhelminth phylogeny: inferences from partial *28S rDNA* sequences. *Invertebrate Biology* **118**, 42–56.
- Llewellyn J (1970) Taxonomy, genetics and evolution of parasites. Monogenea. *Journal of Parasitology* **56**, 287–504.
- Llewellyn J (1982) Host-specificity and corresponding evolution in monogenean flatworms and vertebrates. *Mémoires du Muséum National D'Histoire Naturelle Nouvelle Série, Série A, Zoologie* **123**, 289–293.
- Lockyer AE, Olson PD, Littlewood DTJ (2003) Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society* **78**, 155–171.
- Long JA (1995) 'The rise of fishes: 500 million years of evolution.' (University of New South Wales Press: Australia)
- Lovtrup S (1977) 'The phylogeny of the Vertebrata.' (Wiley: New York)
- Maddison WP (1997) Gene trees in species trees. *Systematic Biology* **46**, 523–536.
- Maddison WP, Maddison DR (1995) MacClade: analysis of phylogeny and character evolution. Sunderland, MA, Sinauer Associates Inc.
- Maisey JG (1996) 'Discovering fossil fishes. 1st edition.' (Henry Holt & Co.: New York)
- Maisey JG (2001) A primitive chondrichthyan braincase from the middle Devonian of Bolivia. In 'Major events in early vertebrate evolution: Palaeontology, phylogeny, genetics and development'. (Ed. PE Ahlberg) pp. 263–288. (Taylor & Francis: London)

- Maisey JG, Naylor GJP, Ward DJ (2004) Mesozoic elasmobranchs, neoselachian phylogeny and the rise of modern elasmobranch diversity. In 'Mesozoic fishes 3: Systematics, paleoenvironments and biodiversity'. (Eds G Arratia and A Tintori) pp. 17–56. (Verlag Dr. Friedrich Pfeil: Munchen)
- Mallatt J, Sullivan J, Winchell CJ (2001) The relationship of lampreys to hagfishes: a spectral analysis of ribosomal DNA sequences. In 'Major events in early vertebrate evolution: Palaeontology, phylogeny, genetics and development'. (Ed. PE Ahlberg) pp. 106–118. (Taylor & Francis: London)
- Masta SE, Klann AE, Podsiadlowski L (2008) A comparison of the mitochondrial genomes from two families of Solifugae (Arthropoda: Chelicerata): Eremobatidae and Ammotrechidae. *Gene* **417**, 35–42.
- Matejusová I, Cunningham CO (2004) The first complete monogenean ribosomal RNA gene operon: Sequence and secondary structure of the *Gyrodactylus salaris* Malmberg, 1957, large subunit ribosomal RNA gene. *Journal of Parasitology* **90**, 146–151.
- Mathews DH (2006) RNA secondary structure analysis using RNAstructure. *Current Protocols in Bioinformatics*, Supplement 13, Unit 12.6.1–12.6.4.
- McEachran JD, Aschliman N (2004) Phylogeny of Batoidea. In 'Biology of sharks and their relatives.' (Eds JC Carrier, JA Musick and MR Heithaus) pp. 79–113. (CRC Press: Boca Raton, Florida)
- McEachran JD, Dunn KA, Miyake T (1996) Interrelationships of the batoid fishes (Chondrichthyes: Batoidea). In 'Interrelationships of fishes.' (Eds MLJ Stiassny, LR Parenti and GD Johnson) pp. 63–84. (Academic Press: San Diego)
- Meyer M, Stenzel U, Myles S, Prufer K, Hofreiter M (2007) Targeted high-throughput sequencing of tagged nucleic acid samples. *Nucleic Acids Research* **35**.
- Miller TL, Cribb TH (2007) Coevolution of *Retrovarium* n gen (Digenea: Cryptogonimidae) in Lutjanidae and Haemulidae (Perciformes) in the Indo-West Pacific. *International Journal for Parasitology* **37**, 1023–1045.
- Miya M, Nishida M (2000) Use of mitogenomic information in teleostean molecular phylogenetics: A tree-based exploration under the maximum-parsimony optimality criterion. *Molecular Phylogenetics and Evolution* **17**, 437–455.

- Miya M, Satoh TR, Nishida M (2005) The phylogenetic position of toadfishes (order Batrachoidiformes) in the higher ray-finned fish as inferred from partitioned Bayesian analysis of 102 whole mitochondrial genome sequences. *Biological Journal of the Linnean Society* **85**, 289–306.
- Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, Shirai SM, Nishida M (2003) Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **26**, 121–138.
- Mollaret I, Jamieson BGM, Adlard RD, Hugall A, Lecointre G, Chombard C, Justine JL (1997) Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Molecular and Biochemical Parasitology* **90**, 433–438.
- Mollaret I, Jamieson BGM, Justine JL (2000) Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *International Journal for Parasitology* **30**, 171–185.
- Mooney AJ, Ernst I, Whittington ID (2008) Egg-laying patterns and *in vivo* egg production in the monogenean parasites *Heteraxine heterocerca* and *Benedenia seriolae* from Japanese yellowtail *Seriola quinqueradiata*. *Parasitology* **135**, 1295–1302.
- Moore G (1998) A comparison of traditional and phylogenetic nomenclature. *Taxon* **47**, 561–579.
- Morand S, Simkova A, Gourbiere S (2008) Beyond the paradigms of cospeciation and host-switch: Is sympatric speciation an important mode of speciation for parasites? *Vie et Milieu* **58**, 125–132.
- Mordue AJ, Birkett MA (2009) A review of host finding behaviour in the parasitic sea louse, *Lepeophtheirus salmonis* (Caligidae: Copepoda). *Journal of Fish Diseases* **32**, 3–13.
- Munoz G, Grutter AS, Cribb TH (2006) Endoparasite communities of five fish species (Labridae: Cheilinae) from Lizard Island: how important is the ecology and phylogeny of the hosts? *Parasitology* **132**, 363–374.
- Nelson JS (1984) 'Fishes of the world. 2nd edition.' (John Wiley and Sons: New York)

- Nelson JS (1994) 'Fishes of the world. 3rd edition.' (John Wiley and Sons, Inc: New York)
- Nelson JS (2006) 'Fishes of the world. 4th edition.' (John Wiley & Sons, Inc: New York)
- Noble ER, Noble, G.A., Schad, G.A., MacInnes, A.J. (1989) 'Parasitology: The biology of animal parasites.' (Lea and Febiger: Philadelphia and London)
- Olson PD, Caira JN, Jensen K, Overstreet RM, Palm HW, Beveridge I (2010) Evolution of the trypanorhynch tapeworms: parasite phylogeny supports independent lineages of sharks and rays. *International Journal for Parasitology* **40**, 223-242.
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**, 733-755.
- Olson PD, Littlewood DTJ (2002) Phylogenetics of the Monogenea - evidence from a medley of molecules. *International Journal for Parasitology* **32**, 233-244.
- Page RDM (1996) Temporal congruence revisited: Comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. *Systematic Biology* **45**, 151-167.
- Page RDM, Charleston MA (1998) Trees within trees: phylogeny and historical associations. *Trends in Ecology & Evolution* **13**, 356-359.
- Pagel M (1994) Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London Series B-Biological Sciences* **255**, 37-45.
- Pagel M (1997) Inferring evolutionary processes from phylogenies. *Zoologica Scripta* **26**, 331-348.
- Palm HW, Waeschenbach A, Olson PD, Littlewood DTJ (2009) Molecular phylogeny and evolution of the Trypanorhyncha Diesing, 1863 (Platyhelminthes: Cestoda). *Molecular Phylogenetics and Evolution* **52**, 351-367.
- Pariselle A, Euzet L (1995) Gill parasites of the genus *Cichlidogyrus* Paperna, 1960 (Monogenea, Ancyrocephalidae) from *Tilapia guineensis* (Bleeker, 1862), with descriptions of 6 new species. *Systematic Parasitology* **30**, 187-198.
- Park JK, Kim KH, Kang S, Kim W, Eom KS, Littlewood DTJ (2007) A common origin of complex life cycles in parasitic flatworms: evidence from the

- complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes). *BMC Evolutionary Biology* **7**, 11.
- Paterson AM, Banks J (2001) Analytical approaches to measuring cospeciation of host and parasites: through a glass, darkly. *International Journal for Parasitology* **31**, 1012–1022.
- Paterson AM, Poulin R (1999) Have chondracanthid copepods co-specified with their teleost hosts? *Systematic Parasitology* **44**, 79–85.
- Patterson C, Rosen DE (1977) Review of ichthyodectiform and other Mesozoic teleost fishes and the theory and practice of classifying fossils. *Bulletin of the American Museum of Natural History* **158**, 81–172.
- Patterson C, Rosen DE (1989) The Paracanthopterygii revisited: order and disorder. In 'Papers on the systematics of gadiform fishes'. (Ed. DM Cohen) pp. 5–36. (Natural History Museum: Los Angeles)
- Perkins EM, Donnellan SC, Bertozzi T, Chisholm LA, Whittington ID (2009) Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification. *Molecular Phylogenetics and Evolution* **52**, 705–714.
- Peterson KJ, Cotton JA, Gehling JG, Pisani D (2008) The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **363**, 1435–1443.
- Peterson KJ, Lyons JB, Nowak KS, Takacs CM, Wargo MJ, McPeck MA (2004) Estimating metazoan divergence times with a molecular clock. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 6536–6541.
- Plaisance L, Huyse T, Littlewood DTJ, Bakke TA, Bachmann L (2007) The complete mitochondrial DNA sequence of the monogenean *Gyrodactylus thymalli* (Platyhelminthes: Monogenea), a parasite of grayling (*Thymallus thymallus*). *Molecular and Biochemical Parasitology* **154**, 190–194.
- Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Poulin R, Morand S (2000) The diversity of parasites. *Quarterly Review of Biology* **75**, 277–293.

- Rambaut A (1996) Se-AL: Sequence Alignment Editor. Available at <http://evolve.zoo.ox.ac.uk/>. Institute of Evolutionary Biology, University of Edinburgh.
- Rambaut A (2009) FigTree v1.2.2. Available at <http://tree.bio.ed.ac.uk/>. Institute of Evolutionary Biology, University of Edinburgh.
- Rambaut A, Drummond AJ (2007) Tracer v1.4, Available from <http://beast.bio.ed.ac.uk/Tracer>.
- Rambaut A, Drummond AJ (2008) TreeAnnotator v1.4.8, Available from <http://beast.bio.ed.ac.uk/TreeAnnotator>.
- Raup DM, Sepkoski JJ (1982) Mass extinctions in the marine fossil record. *Science* **215**, 1501–1503.
- Rohde K (1994) The origins of parasitism in the Platyhelminthes. *International Journal for Parasitology* **24**, 1099–1115.
- Rohde K (2001) The Aspidogastrea, an archaic group of Platyhelminthes. In 'Interrelationships of the Platyhelminthes'. (Eds DTJ Littlewood and RA Bray) pp. 159–167. (Taylor and Francis: London and New York)
- Rohde K, Hefford C, Ellis JT, Baverstock PR, Johnson AM, Watson NA, Dittmann S (1993) Contributions to the phylogeny of Platyhelminthes based on partial sequencing of 18S ribosomal DNA. *International Journal for Parasitology* **23**, 705–724.
- Romer AS (1945) 'Vertebrate Paleontology.' (The University of Chicago Press: Chicago)
- Rosen DE (1973) Interrelationships of higher euteleostean fishes. In 'Interrelationships of fishes'. (Eds PH Greenwood, RS Miles and C Patterson) pp. 397–513. (Academic Press: New York)
- Rosen DE, Forey PL, Gardiner BG, Patterson C (1981) Lungfishes, tetrapods, paleontology, and plesiomorphy. *Bulletin of the American Museum of Natural History* **167**, 163–275.
- Ruiz-Trillo M, Riutort M, Fourcade HM, Baguna J, Boore JL (2004) Mitochondrial genome data support the basal position of Acoelomorpha and the polyphyly of the Platyhelminthes. *Molecular Phylogenetics and Evolution* **33**, 321–332.
- Rusk N (2009) Cheap third-generation sequencing. *Nature* **6**, 244–245.
- Rychlik W (1992) The importance of oligonucleotide stability at its 3'-terminus in sequencing and PCR primer selection. *Biophysical Journal* **61**, A76.

- Santini F, Harmon LJ, Carnevale G, Alfaro ME (2009) Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. *BMC Evolutionary Biology* **9**, 194.
- Schuh RT (2003) The Linnaean system and its 250-year persistence. *Botanical Review* **69**, 59–78.
- Schultze H-P, Cloutier R (1996) Comparison of the Escuminac formation ichthyofauna with other late Givetian/early Frasnian ichthyofaunas. In 'Devonian fishes and plants of Miguasha, Quebec, Canada.' (Eds HP Schultze and R Cloutier) pp. 348–368. (Verlag Dr. Friedrich Pfeil: Munchen)
- Sepkoski Jr. JJ (1982) A compendium of fossil marine families. *Milwaukee Public Museum Contributions in Biology and Geology* **51**, 1–125.
- Shimodaira H, Hasegawa M (2001) CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* **17**, 1246–1247.
- Shirai SM (1996) Phylogenetic interrelationships of neoselachians (Chondrichthyes: Euselachii). In 'Interrelationships of Fishes'. (Eds MLJ Stiassny, LR Parenti and GD Johnson) pp. 9–34. (Academic Press: New York)
- Simmons MP, Miya M (2004) Efficiently resolving the basal clades of a phylogenetic tree using Bayesian and parsimony approaches: a case study using mitogenomic data from 100 higher teleost fishes. *Molecular Phylogenetics and Evolution* **31**, 351–362.
- Smith WL, Craig MT (2007) Casting the percomorph net widely: The importance of broad taxonomic sampling in the search for the placement of serranid and percoid fishes. *Copeia* **2007**, 35–55.
- Smith WL, Wheeler WC (2004) Polyphyly of the mail-cheeked fishes (Teleostei: Scorpaeniformes): evidence from mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution* **32**, 627–646.
- Smith WL, Wheeler WC (2006) Venom evolution widespread in fishes: A phylogenetic road map for the bioprospecting of piscine venoms. *Journal of Heredity* **97**, 206–217.
- Smyth JD, Halton DW (1983) 'The physiology of trematodes 2nd Edition.' (Cambridge University Press: New York)
- Springer VG, Johnson GD (2004) Study of the dorsal gill-arch musculature of teleostome fishes, with special reference to the Actinopterygii. *Bulletin of the Biological Society of Washington* **11**, 1–260.

- Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690.
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**, 758–771.
- Takezaki N, Figueroa F, Zaleska-Rutczynska Z, Klein J (2003) Molecular phylogeny of early vertebrates: Monophyly of the Agnathans as revealed by sequences of 35 genes. *Molecular Biology and Evolution* **20**, 287–292.
- Telford MJ, Herniou EA, Russell RB, Littlewood DTJ (2000) Changes in mitochondrial genetic codes as phylogenetic characters: Two examples from the flatworms. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 11359–11364.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**, 4876–4882.
- Thoney DA, Hargis WJ (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* **1**, 133–153.
- Timofeeva TA (1990) Phylogenetic relationships of capsalids and dionchids and the position of the latter in the system of monogeneans (Monogenea, Monopisthocotylea). *Trudy Zoologicheskii Institut, Akademii Nauk SSSR*, 3–16.
- Tingbao Y, Kritsky DC, Yuan S (2004) Revision of *Allobenedenia* Yamaguti, 1963 (Monogenoidea: Capsalidae) with the description of *A. zhangii* n. sp. from *Epinephelus fasciatus* (Teleostei: Serranidae) in the South China Sea. *Systematic Parasitology* **59**, 223–233.
- Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56**, 1–15.
- Underwood CJ (2006) Diversification of the Neoselachii (Chondrichthyes) during the Jurassic and Cretaceous. *Paleobiology* **32**, 215–235.
- Upeniece I (2001) The unique fossil assemblage from the Lode Quarry (Upper Devonian, Latvia). *Mitteilungen aus dem Museum fuer Naturkunde in Berlin Geowissenschaftliche Reihe* **4**, 101–119.

- Venkatesh B, Erdmann MV, Brenner S (2001) Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 11382–11387.
- Wang CH, Kuo CH, Mok HK, Lee SC (2003) Molecular phylogeny of elopomorph fishes inferred from mitochondrial 12S ribosomal RNA sequences. *Zoologica Scripta* **32**, 231–241.
- Weckstein JD (2004) Biogeography explains cophylogenetic patterns in toucan chewing lice. *Systematic Biology* **53**, 154–164.
- Whittington ID (1998) Diversity "down under": monogeneans in the Antipodes (Australia) with a prediction of monogenean biodiversity worldwide. *International Journal for Parasitology* **28**, 1484–1493.
- Whittington ID (2004) The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. *Folia Parasitologica* **51**, 109–122.
- Whittington ID (2005) Monogenea Monopisthocotylea (ectoparasitic flukes). In 'Marine Parasitology'. (Ed. K Rohde) pp. 63–72. (CSIRO Publishing: Melbourne, Australia)
- Whittington ID, Chisholm LA (2003) Biodiversity of marine parasites in Australia: More than just a list of largely invisible creatures. *Records of the South Australian Museum Monograph Series* **7**, 51–60.
- Whittington ID, Chisholm LA (2008) Diseases caused by Monogenea. In 'Fish diseases'. (Eds JC Eiras, H Segner, T Wahli and BG Kapoor) pp. 683–816. (Science Publishers: Enfield (NH), USA)
- Whittington ID, Chisholm LA, Rohde K (2000) The larvae of Monogenea (Platyhelminthes). *Advances in Parasitology* **44**, 139–232.
- Whittington ID, Corneillie S, Talbot C, Morgan JAT, Adlard RD (2001) Infections of *Seriola quinqueradiata* Temminck & Schlegel and *S. dumerili* (Risso) in Japan by *Benedenia seriolae* (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. *Journal of Fish Diseases* **24**, 421–425.
- Whittington ID, Deveney MR, Morgan JAT, Chisholm LA, Adlard RD (2004) A preliminary phylogenetic analysis of the Capsalidae (Platyhelminthes: Monogenea: Monopisthocotylea) inferred from large subunit rDNA sequences. *Parasitology* **128**, 511–519.

- Whittington ID, Horton MA (1996) A revision of *Neobenedenia* Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of *N. melleni* (MacCallum, 1927) Yamaguti, 1963. *Journal of Natural History* **30**, 1113–1156.
- Whittington ID, Kearn GC (1992) The eggs and oncomiracidia of *Encotyllabe* spp and the relationship between encotyllabines and other capsalid monogeneans. *Parasitology* **104**, 253–261.
- Whittington ID, Kearn GC (2005) The precocious oncomiracidium of *Neoentobdella parvtesticulata* (Monogenea: Capsalidae: Entobdellinae). *Parasitology Research* **96**, 331–334.
- Whittington ID, Kearn GC (2009) Two new species of entobdelline skin parasites (Monogenea, Capsalidae) from the blotched fantail ray, *Taeniura meyeni*, in the Pacific Ocean, with comments on spermatophores and the male copulatory apparatus. *Acta Parasitologica* **54**, 12–21.
- Wiley EO (1979) Ventral gill arch muscles and the phylogenetic relationships of *Latimeria*. *Occasional Papers California Academy of Sciences*, 56–67.
- Wiley EO, Johnson GD, Dimmick WW (2000) The interrelationships of Acanthomorph fishes: A total evidence approach using molecular and morphological data. *Biochemical Systematics and Ecology* **28**, 319–350.
- Winchell CJ, Martin AP, Mallatt J (2004) Phylogeny of elasmobranchs based on LSU and SSU ribosomal RNA genes. *Molecular Phylogenetics and Evolution* **31**, 214–224.
- Xie Q, Tian Y, Zheng LY, Bu WJ (2008) 18S rRNA hyper-elongation and the phylogeny of Euhemiptera (Insecta: Hemiptera). *Molecular Phylogenetics and Evolution* **47**, 463–471.
- Yamaguti S (1963) 'Systema Helminthum.' (InterScience Publishers: New York)
- Yamaguti S (1965) New monogenetic trematodes from Hawaiian fishes I. *Pacific Science* **19**, 55–95.
- Yamaguti S (1968) 'Monogenetic trematodes of Hawaiian fishes.' (University of Hawaii press: Hawaii)
- Yamanoue Y, Miya M, Matsuura K, Miyazawa S, Tsukamoto N, Doi H, Takahashi H, Mabuchi K, Nishida M, Sakai H (2009) Explosive speciation of *Takifugu*: another use of fugu as a model system for evolutionary biology. *Molecular Biology and Evolution* **26**, 623–629.

- Yoder JI (1997) A species-specific recognition system directs haustorium development in the parasitic plant *Triphysaria* (Scrophulariaceae). *Planta* **202**, 407–413.
- Zardoya R, Garrido-Pertierra A, Bautista JM (1995) The complete nucleotide sequence of the mitochondrial DNA genome of the rainbow trout, *Oncorhynchus mykiss*. *Journal of Molecular Evolution* **41**, 942–951.
- Zhou Y, Zheng HJ, Chen YY, Zhang L, Wang K, Guo J, Huang Z, Zhang B, Huang W, Jin K, Dou TH, Hasegawa M, Wang L, Zhang Y, Zhou J, Tao L, Cao ZW, Li YX, Vinar T, Brejova B, Brown D, Li M, Miller DJ, Blair D, Zhong Y, Chen Z, Hu W, Wang ZQ, Zhang QH, Song HD, Chen SJ, Xu XN, Xu B, Ju C, Huang YC, Brindley PJ, McManus DP, Feng Z, Han ZG, Lu G, Ren SX, Wang YZ, Gu WY, Kang H, Chen J, Chen XY, Chen ST, Wang LJ, Yan J, Wang BY, Lv XY, Jin L, Wang BF, Pu SY, Zhang XL, Zhang W, Hu QP, Zhu GF, Wang J, Yu J, Yang HM, Ning ZM, Berriman M, Wei CL, Ruan YJ, Zhao GP, Wang SY, Liu F (2009) The *Schistosoma japonicum* genome reveals features of host-parasite interplay. *Nature* **460**, 345–351.
- Zhu M, Zhao WJ, Jia LT, Lu J, Qiao T, Qu QM (2009) The oldest articulated osteichthyan reveals mosaic gnathostome characters. *Nature* **458**, 469–474.
- Zwickl DJ (2006) 'Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.' Ph.D. dissertation thesis, The University of Texas, Austin.

APPENDIX I

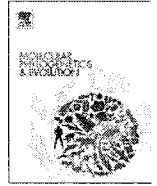
Reprint of published paper.

Chapter II



Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification

Elizabeth M. Perkins^{a,*}, Steve C. Donnellan^{b,c}, Terry Bertozzi^b, Leslie A. Chisholm^a, Ian D. Whittington^{a,d}

^a Marine Parasitology Laboratory, School of Earth and Environmental Sciences (DX 650 418), The University of Adelaide, North Terrace, Adelaide, SA 5005, Australia

^b Evolutionary Biology Unit, The South Australian Museum, North Terrace, Adelaide, SA 5000, Australia

^c Australian Centre for Evolutionary Biology and Biodiversity, The University of Adelaide, North Terrace, Adelaide, SA 5005, Australia

^d Monogenean Research Laboratory, Parasitology Section, The South Australian Museum, North Terrace, Adelaide, SA 5000, Australia

ARTICLE INFO

Article history:

Received 13 October 2008

Revised 30 April 2009

Accepted 12 May 2009

Available online 18 May 2009

Keywords:

Monogenea

Phylogeny

Capsalidae

Parasite

Fish

Classification

ABSTRACT

The morphological based taxonomy of highly derived parasite groups is likely to poorly reflect their evolutionary relationships. The taxonomy of the monogenean family Capsalidae, which comprises approximately 180 species of flatworm parasites that predominantly attach to external surfaces of chondrichthyan and teleost fishes, is based mainly on six morphological characters. The phylogenetic history of the family is largely unknown. We reconstructed the phylogenetic relationships of 47 species in 20 genera from eight of the nine subfamilies, from nucleotide sequences of three unlinked nuclear genes, 28S ribosomal RNA, Histone 3 and Elongation Factor 1 α . Our phylogeny was well corroborated, with 75% of branches receiving strong support from both Bayesian posterior probabilities and maximum likelihood bootstrap proportions and all nodes showed positive partitioned likelihood support for each of the three genes. We found that the family was monophyletic, with the Gyrodactylidae and Udonellidae forming the sister group. The Capsalinae was monophyletic, however, our data do not support monophyly for the Benedeniinae, Entobdellinae and Trochopodinae. Monophyly was supported for *Capsala*, *Entobdella*, *Listrocephalos*, *Neobenedenia* and *Tristoma*, but *Benedenia* and *Neoentobdella* were polyphyletic. Comparisons of the distribution of character states for the small number of morphological characters on the molecular phylogeny show a high frequency of apparent homoplasy. Consequently the current morphological classification shows little correspondence with the phylogenetic relationships within the family.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

The Platyhelminthes is a diverse phylum of aquatic and terrestrial organisms that are classified into mostly free-living 'turbellarians' and the wholly parasitic Neodermata (see Kearn, 1998). The Neodermata comprises three classes, the Cestoda (tapeworms), Trematoda (internal flukes) and Monogenea (principally ectoparasitic flukes of teleosts and chondrichthyans). Monogenea have a direct life cycle and tend to be highly host specific, i.e. species commonly infect a single host species. The Monogenea is divided into two subclasses, the Monopisthocotylea that feed on epithelial cells and the Polyopisthocotylea that are exclusively blood feeders.

The Capsalidae (Monopisthocotylea) include parasitic flatworms that attach predominantly to external surfaces of marine fish. Capsalids are distributed worldwide and some are among the largest monogenean species known (up to 3 cm long) (Whittington, 2004). Some can be site specific and different species parasitise different sites including the: epithelium covered lamina of

teleost scales; smooth external ventral epithelium of batoids; gill lamellae, arches and rakers; fins; branchiostegal membranes; lip folds and pharyngeal tooth pads (Whittington, 2004). While capsalids generally parasitise 'modern' marine teleosts, some parasitise 'primitive' anadromous and freshwater teleosts, like acipenserids and also marine elasmobranchs (sharks and rays) (Whittington, 2004). Some capsalids are important pathogens in aquaculture and public aquaria e.g. *Benedenia seriola*, *Neobenedenia 'melleni'* and have been responsible for significant losses of fish stocks (Deveney et al., 2001). The current taxonomic classification, which comprises nine subfamilies, 45 genera and approximately 180 species (Whittington, 2004, Table 1), is based on very few morphological characters (e.g. attachment organ characteristics, testis number and arrangement). Within the Capsalidae, some subfamilies and genera are considered ill-defined and require taxonomic revision (Whittington et al., 2004). Four subfamilies contain only a single genus and many capsalid genera are monotypic.

Whittington et al. (2004) conducted a preliminary phylogenetic study of the Capsalidae which used partial 28S ribosomal DNA (28S rDNA) nucleotide sequences, and included only 17 species, representing seven genera and five of the nine subfamilies. Monophyly

* Corresponding author. Fax: +61 8 8303 4364.

E-mail address: elizabeth.perkins@adelaide.edu.au (E.M. Perkins).

for the Capsalidae was supported as was monophyly for the Encotyllabinae and Entobdellinae. Benedeniinae was paraphyletic with *Neobenedenia* species failing to fall within the subfamily. *Capsala* was not monophyletic due to the inclusion of *Tristoma integrum*. While this is the only phylogenetic analysis of the family to date, it emphasises the need to establish phylogenetic relationships to assess the substance of the current systematic classification. Far greater taxon sampling and use of multiple genes will be required to infer and resolve relationships within the Capsalidae robustly (Whittington, 2004).

Other than the preliminary phylogenetic hypothesis by Whittington et al. (2004), phylogenetic relationships among capsalids remain unexplored. Currently there are too few morphological characters adequate to establish evolutionary relationships for the entire group. The paucity of phylogenetically useful morphological characters is due largely to the fact that parasites tend to have simplified and conserved body plans compared to free-living relatives (Brooks and McLennan, 1993). Homology is another critical consideration when establishing a morphological dataset for phylogenetic analyses. If relationships between taxa are unknown, homology is usually inferred by developmental, structural and positional similarity (Brooks and McLennan, 1993). Such an approach can be problematic in relation to parasites and may lead to inaccurate assumptions about homology, an issue of concern for capsalid morphological characters (Whittington, 2004). A molecular phylogenetic hypothesis will allow an examination of the issue of homology in these key morphological characters and an assessment of the frequency and the potential impacts of homoplasy.

Our study extends the preliminary work of Whittington et al. (2004) by increasing taxon and gene sampling. We base our analyses on 47 capsalid species in 20 genera representing eight of the nine subfamilies and also include 15 outgroup taxa (in nine families) from the Monopisthocotylea and Polyopisthocotylea. Presently the sister taxon of the Capsalidae is unknown. Our analyses combine partial sequence data for 28S rDNA, Histone 3 (H3) and Elongation Factor 1 α (EF1 α) and is the first molecular phylogeny of a monogenean family to include multiple unlinked nuclear markers. Six morphological characters commonly used in higher level capsalid classifications were assessed relative to the molecular phylogenetic hypothesis for their utility as phylogenetically informative characters.

2. Materials and methods

2.1. Sample collection

Specimens (preserved in 95% AR grade ethanol) were collected or obtained from various sources between 1993 and 2007 from 47 capsalid and 15 outgroup taxa (see Appendix 1). Table 1 shows the current taxonomic classification of the capsalids. Trees were rooted with *Microcotylodes incisa* (Polyopisthocotylea: Microcotylidae), the most distant outgroup included in the analyses. The other 14 outgroup taxa belong to the subclass Monopisthocotylea and represent eight families (Acanthocotylidae, Amphibdellatidae, Calceostomatidae, Dactylogyridae, Gyrodactylidae, Microbothriidae, Monocotylidae and Udonellidae).

2.2. DNA preparation, PCR amplification and sequencing

DNA was extracted according to the Genra Kit (Genra Systems) protocol for animal tissues preserved in ethanol. Extracted DNA was stored in hydration solution at 4 °C. PCR amplification of partial 28S rDNA, H3 and EF1 α sequence was carried out with published primers and additional primers designed using OLIGO

Table 1
Current capsalid subfamilies and included genera, listed alphabetically.

Subfamilies*	Included genera**
Benedeniinae (13)	<i>Allometabenedeniella</i> (1), <i>Ancyrocotyle</i> (2), ^b <i>Benedenia</i> (21), <i>Benedeniella</i> (2), <i>Calicobenedenia</i> (1), <i>Dioncopsudobenedenia</i> (1), <i>Lagenivaginopseudobenedenia</i> (2), <i>Menziesia</i> (5), <i>Metabenedeniella</i> (2), <i>Neobenedenia</i> (6), <i>Oligoncobenedenia</i> (1), <i>Pseudallobenedenia</i> (2), <i>Trimusculotrema</i> (5)
⁹ Capsalinae (4)	^b <i>Capsala</i> (22), <i>Capsaloides</i> (7), <i>Nasicola</i> (3), <i>Tristoma</i> (4)
Dioncinae (1)	<i>Dioncus</i> ^{b,c} (11)
Encotyllabinae (2)	<i>Alloencotyllabe</i> (1), ^b <i>Encotyllabe</i> (17)
Entobdellinae (5)	<i>Branchobdella</i> (1), ^b <i>Entobdella</i> (7), <i>Listrocephalos</i> (4), <i>Neoentobdella</i> (10), <i>Pseudoentobdella</i> (1)
Internilocolinae (1)	^b <i>Internilocolus</i> (2)
Nitzschiinae (1)	^b <i>Nitzschia</i> (2)
Pseudonitzschiinae (1)	^b <i>Pseudonitzschia</i> (1)
Trochopodinae (17)	<i>Allobenedenia</i> (8), <i>Allomegalocotyla</i> (2), <i>Macrophyllida</i> (1), <i>Mediavogina</i> (2), <i>Megalobenedenia</i> (2), <i>Megalocotyle</i> (6), <i>Pseudobenedenia</i> (3), <i>Pseudobenedeniella</i> (1), <i>Pseudobenedenioides</i> (2), <i>Pseudomegalocotyla</i> (1), <i>Sessilorbis</i> (1), <i>Sprostonia</i> (27) ^d , <i>Sprostoniella</i> (3), <i>Tetrasepta</i> (1), <i>Trilobiodiscus</i> (1), <i>Trochopelta</i> (1), ^b <i>Trochopus</i> (15)

* Number of genera in bold.

** Approximate number of species in parentheses; genera in bold denotes those with species that parasitise elasmobranchs.

^a Subfamily contains type species (*Capsala martinieri*) for the Capsalidae.

^b Type genus for each subfamily.

^c *Dioncus* postoncomiracidia are reported from skin of blacktip sharks (*Carcharhinus limbatus*) (Carcharhinidae), adult specimens of *Dioncus* occur on teleosts of the families Carangidae, Echeneidae and Rachycentridae (see Bullard et al., 2000).

^d Host associations in *Sprostonia* require re-evaluation because according to Egorova (1994a), the host of the type species, *S. squatinae*, is the angel shark *Squatina squatina* (Squatinae) but the host of *S. longiphallus* is the teleost, *Epinephelus tauvina* (Serranidae). Table based on Whittington (2004) and updated from Tingbao et al. (2004), Chisholm and Whittington (2007), Kearns et al. (2007) and Whittington and Kearns (2009).

4.0 (Rychlik, 1992) listed in Table 2. For amplification of the 28S rDNA dataset, primer combinations used were C1/D2 (approx. 800 bp), LSU5/EC-D2 (approx. 800 bp) and G904/G905 (approx. 400 bp). For amplification of the H3 dataset, primer combinations used were H3aF/H3R2 (approx. 350 bp) and G926/G927 (approx. 300 bp). For amplification of the EF1 α dataset, primer combina-

Table 2
Primers used for PCR amplification.

Gene	Primer ID	Sequence (5'-3')	Forward/Reverse	Source
28S rDNA	C1	ACCCGCTGAATTTAAGCAT	F	^a
	D2	TGGTCCGTGTTTCAAGAC	R	^a
	LSU5	TAGGTCGACCCCGCTGAAYTTAAGCA	F	^b
	EC-D2	CCTGGTCCGTGTTTCAAGACGGG	R	^b
	G904	GATTCTCTAGTAAACKGGCAGTG	F	^c
G905	GTTTAACTCYCAWGTGTTTCA	R	^c	
H3	H3aF	ATGGCTCGTACCAAGCAGACVGC	F	^d
	H3R2	ATRTCTGGGCATGATTGTTAC	R	^d
	G926	GACCCGCYGYAAAAGYAC	F	^c
	G927	AGCRTGRATDGCRCACAA	R	^c
EF1 α	G959	GATTTTATTAARAAYATGATYACTGG	F	^c
	G960	CRGGATGRTTCATAAYRATAAC	R	^c
	G1050	CTGGWACYAGYCARCGTGA	F	^c
	G1051	CATACCATACCACGYTTKA	R	^c

^a Chisholm et al. (2001).

^b Littlewood et al. (1997).

^c This study.

^d Colgan et al. (1998).

tions used were G959/G960 (approx. 800 bp) and G1050/G1051 (approx. 800 bp). Primers used for PCR were also used for sequencing. PCR amplifications were performed in 25 μ L reactions using the following cycle conditions: denaturation at 94 °C for 45 s, annealing at a minimum 50 °C and maximum 65 °C (dependent on primers being used) for 45 s and extension at 72 °C for 1 min; this was repeated for 34 cycles and increased to 38–40 cycles when PCR product yield was low. Each 25 μ L PCR contained a final concentration of: 0.5 U AmpliTaq Gold[®] (5 U/ μ L), 0.2 μ M of each primer, 200 μ M of each dNTPs, 2–4 μ M MgCl₂, 1 \times AmpliTaq Gold[®] buffer. Annealing temperature and MgCl₂ concentration were varied to produce optimal amplification.

PCR products were cleaned using Agencourt[®] AMPure[®] PCR purification kit and were cycle sequenced using the BigDye Terminator v3.1 cycle-sequencing kit (Applied Biosystems). The cycling protocol consisted of 25 cycles of denaturation at 96 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for 4 min. All samples were sequenced on an Applied Biosystems 3730 DNA sequencer.

2.3. Phylogenetic analyses and hypothesis testing

Sequence chromatograms were edited using SeqEd version 1.0.3 and aligned initially using Clustal X (Thompson et al., 1997). Adjustments to alignments were made manually in SeAl version 2.0a11 (Rambaut, 1996) using inferred amino acid sequences where applicable (*H3* and *EF1 α*). For the 28S rDNA sequence data, we tried to align our sequences to the predicted RNA structure for *Gyrodactylus salaris* (see Matejusová and Cunningham, 2004). All sequences have been deposited on GenBank (Accession Nos. FJ971962–FJ972138). Voucher specimens (most mounted on slides but some are specimens or part specimens stored in 95% AR grade ethanol) of each monogean species are deposited in the Australian Helminthological Collection (AHC) of the South Australian Museum (SAMA), Parasitology Section, North Terrace, Adelaide, South Australia 5000, Australia or in the Muséum National d' Histoire Naturelle (MNHN), Paris, France.

Monte Carlo Markov Chain (MCMC) Bayesian phylogenetic analyses were run using MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001). This analysis method allowed the data to be partitioned and optimal models of nucleotide substitution applied to each partition. The model of nucleotide substitution for each partition was assessed using the Akaike Information Criteria (AIC – Akaike, 1985) in ModelTest version 3.7 (Posada and Crandall, 1998). The General Time Reversible (GTR) model with a proportion of invariable sites and a gamma distribution for rates across sites was selected. To determine an optimal partitioning strategy, preliminary Bayesian analyses (1 million generations) using each possible partitioning strategy were run and then the AIC for each partitioning strategy calculated. The final MCMC analyses were run for 10,000,000 generations with a sample frequency of every 100 generations. Tracer v1.4 (Rambaut and Drummond, 2007) was used (to plot the generation number against the log likelihood value) to identify the point at which log likelihood values became stable and all trees generated before this point were discarded. A 50% majority rule consensus tree of the remaining trees was computed.

Maximum likelihood (ML) analyses were run in RAxML (Stamatakis, 2006; Stamatakis et al., 2008) using the default rapid hill climbing algorithm. Adjusting the values of distinct rate categories and rearrangement settings did not improve the likelihood scores so the defaults were used in each case. The model of nucleotide substitution chosen was GTRMIX. These analyses were run for 200 replicates and the best tree chosen from those runs. Bootstrap proportions were estimated under the same conditions for 100 pseudoreplicates. Two constraint analyses (with monophyly en-

forced for all subfamilies and genera in ingroup and outgroup taxa and Acanthocotylidae and Gyrodactylidae forced to be sister taxa following Boeger and Kritsky (2001)) were also run under the same criteria for use in hypothesis testing.

The 50% majority rule consensus tree from the Bayesian analyses was used to view the distribution of six morphological characters in relation to the phylogenetic hypothesis produced. Description of these characters (haptoral septa, haptoral accessory sclerites, haptoral hamuli, vagina and number of testes) follows Whittington (2004) and elaboration of the anterior attachment organ morphology is shown in Fig. 1.

Partitioned Likelihood Support (PLS – Lee and Hugall, 2003) determines whether the different data partitions are in support or disagreement with each node of the tree derived from the combined data matrix. PLS was assessed for all nodes found in the best ML tree produced in RAxML. PLS was analysed for the three different genes: 28S rDNA, *H3* and *EF1 α* . The log likelihood values for the three different genes for this tree were calculated in PAUP* using the site log likelihood function. The constraint trees necessary for PLS were constructed in MacClade v 4.0 (Maddison and Maddison, 1995). As reverse constraint analyses could not be run in RAxML, all analyses for the different nodes were run in GARLI v0.95 (Zwickl, 2006). The GTR model with a proportion of invariable sites and a gamma distribution for rates across sites was used. Termination conditions were set at 10,000 (genthreshfortopterm) and 0.01 (significanttopchange). The remaining default settings were used as it has been shown that altering these generally has little effect on the likelihood scores (Zwickl, 2006). Bootstrap analyses in GARLI were run using 100 pseudoreplicates.

The approximately unbiased (AU) test is a multi-scale bootstrap technique developed for general hypothesis testing and provides a procedure to assess the confidence of tree selection. In the AU test, several sets of bootstrap replicates are generated by changing sequence length, with the number of times the hypothesis is supported by replicates counted for each set to obtain bootstrap probability values for different sequence lengths. The log likelihood values for each site (generated in PAUP*) for the ML tree without constraints, the monophyly constrained ML tree (monophyly constrained for all families, subfamilies and genera) and the ML tree with the Acanthocotylidae/Gyrodactylidae constraint (Acanthocotylidae and Gyrodactylidae were constrained to be sister taxa) were used in CONSEL version 0.1i (Shimodaira and Hasegawa, 2001) to run the AU test to determine in which trees to have confidence. Monophyly constrained for all families, subfamilies and genera was used to test the current hypothesis of capsalid classification. Acanthocotylidae and Gyrodactylidae were constrained to be sister taxa to test the hypothesis of Boeger and Kritsky (2001) who suggested that the Acanthocotylidae and Gyrodactylidae may be sister groups.

3. Results

3.1. DNA sequence characteristics

There were no premature stop codons within the coding regions of the protein coding nuclear genes. The secondary structure of the 28S rDNA sequence for *Gyrodactylus salaris* could not be used to align our sequence data. Parts of the 28S rDNA sequence data span a highly variable section of 28S rDNA so areas where the model predicted stems did not correspond to conserved regions in the sequence data and so the model was not used to infer an alignment. The three loci for 47 ingroup taxa and 15 outgroup taxa were concatenated for a total alignment of 1528 characters of sequence including: 430 characters 28S rDNA, 292 characters *H3* and 806 characters *EF1 α* . This included 104 parsimony informative

sites for 28S rDNA, 141 parsimony informative sites for H3 and 348 parsimony informative sites for EF1 α . We were unable to obtain sequence for H3 for *Udonella* sp. and EF1 α for the following taxa: *Benedenia anticavaginata*, *Capsala* sp. 1, *Encotyllabe caranxi*, *Internilocus chilensis*, *Neontobdella diadema*, *Tristoma integrum*, *Tristoma* sp., and *Trochopodinae* sp. 3 (Appendix 1). These taxa were included in analyses as missing data for this gene. The EF1 α sequence spanned an intron of variable length (approx. 50–100 bp), which we excluded from our analyses because it could not be aligned unambiguously due to high variability. Some primer pairs for 28S rDNA generated larger sequence fragments (approx. 800 bp) but because alignment at the 3' end of this sequence was ambiguous, only approximately 400 bp were included in analyses. Other areas of 28S rDNA and EF1 α sequence, where alignment was also ambiguous, were excluded from analyses reducing the final number of characters used in the analyses to 1280. Indels occurred at 29 sites in the 28S rDNA sequence data (20 of which occurred only in *Udonella* sp.) and 14 sites in the EF1 α sequence data. Sequencing of some 28S rDNA, H3, and EF1 α sequences revealed heterozygotes, indicated by overlapping signals for two kinds of bases in the sequence chromatograms data. These sites were scored with the IU-PAC ambiguity codes for dimorphic sites.

3.2. Phylogenetic analyses

The preliminary Bayesian analyses and AIC showed that seven partitions (28S rDNA, H3 1st codon position, H3 2nd codon position, H3 3rd codon position, EF1 α 1st codon position, EF1 α 2nd codon position and EF1 α 3rd codon position) were optimal for the data (Fig. 2).

We present the Bayesian 50% majority rule consensus tree in Fig. 3 along with posterior probabilities and because the ML tree was so similar in topology, the ML bootstrap proportions (BS). For comparison, we present the ML tree in Supplementary Fig. 1. Bayesian and ML analyses of the combined data (Fig. 3) yielded some interesting relationships that were recovered consistently and some were strongly supported as indicated by Bayesian posterior probabilities (PP) and non-parametric bootstrap proportions (BS). Monophyly of the Capsalidae was supported strongly (PP 100%, BS 99%) and consistently in all analyses. A clade comprising three *Gyrodactylus* species (*Gyrodactylidae*) and a *Udonella* sp. (*Udonellidae*) (Fig. 3, Clade 3) formed the sister group to the family (PP 97%, BS 63%). Of the three outgroup families where two or more taxa were represented, two formed well supported clades: *Gyrodactylidae* (*Gyrodactylus* spp.; PP 100%, BS 100%) and the *Microrobothriidae* (*Asthenocotyle*, *Dermophthirus* spp. and *Pseudoleptobothrium*; PP 100%, BS 93%). The *Monocotylidae* represented by a *Calicotyle* sp. and *Dendromonocotyle bradsmithi* were not monophyletic.

Capsalids were split into two major clades (Fig. 3). Clade 1 comprised species currently in five subfamilies (*Benedeniinae*, *Encotyllabinae*, *Internilocolinae*, *Pseudonitzschiinae* and *Trochopodinae*) and nine genera. Clade 1 is further divided into two subclades (Clade 1a and Clade 1b) but while consistently recovered, these clades were not strongly supported (PP 64% for both, BS 10% and 12%, respectively). Clade 1a comprises species currently in *Neobenedenia*, *Pseudonitzschiinae* and other representatives of the *Benedeniinae*, *Trochopodinae* and seven undescribed capsalid species not yet assigned to a genus. Clade 1b consists of species currently in *Benedeniinae*, *Encotyllabinae*, *Internilocolinae*, *Trochopodinae* and one undescribed capsalid species unassigned to a genus. Clade 2 comprised species currently in five subfamilies: *Benedeniinae* (*Benedeniella posterocolpa*), *Capsalinae*, *Entobdellinae*, *Nitzschiinae* and *Trochopodinae* (*Macrophyllida* sp.) and ten genera. Clade 2 has a strongly supported subclade (PP 100%, BS 93%) within it (Clade 2a) containing all included species of *Capsal-*

inae that are the strongly supported sister group to *Nitzschia sturionis* (*Nitzschiinae*). The remainder of Clade 2 comprises species currently in *Benedeniinae*, *Entobdellinae* and *Trochopodinae* and one species unassigned to either subfamily or genus. Eight of the nine capsalid subfamilies were represented in our analyses but

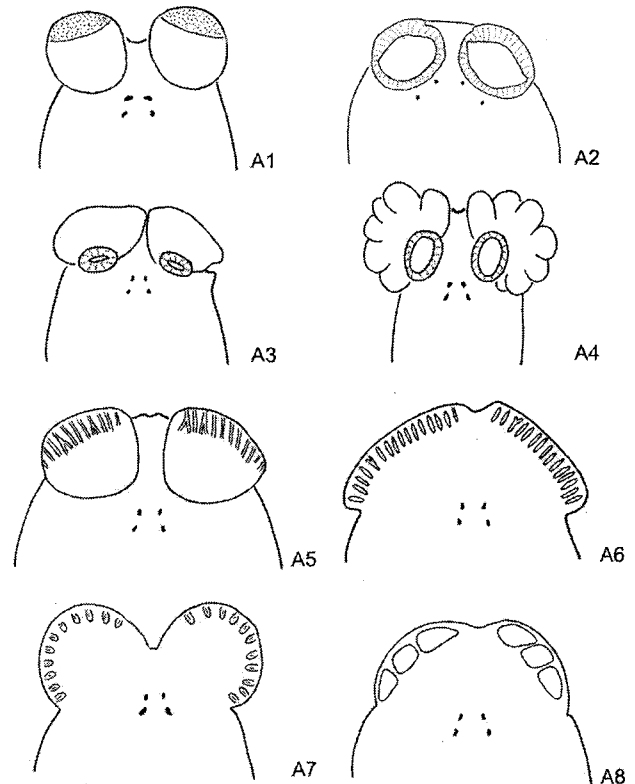


Fig. 1. Diagrammatic representations of the variation in anterior attachment organ morphology among the Capsalidae. A1—paired circular discs, A2—paired circular discs with anterior glandular and posterior muscular regions, A3—paired circular suckers, A4—paired structure with convoluted edges and muscular suckers, A5—paired circular discs with anterolateral ridges, A6—paired diadems, A7—paired anterolateral adhesive areas with ventral columns of multiple raised ovoid structures, A8—paired anterolateral adhesive pads each with three separate areas.

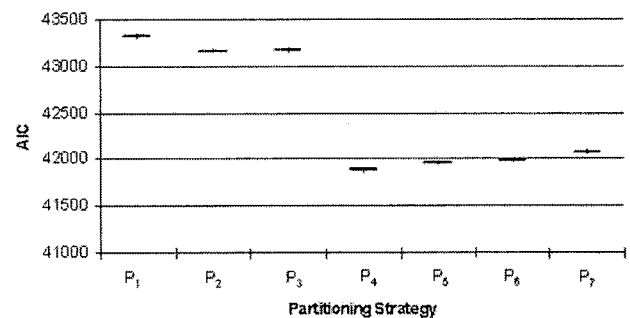


Fig. 2. AIC values for the different partitioning strategies. P₁—All data combined (1 partition), P₂—28S; H3; EF1 α (3 partitions), P₃—28S; H3 and EF1 α combined (2 partitions), P₄—28S; H3 codon positions; EF1 α codon positions (7 partitions), P₅—28S; H3 and EF1 α codon positions combined (4 partitions), P₆—28S; H3 codon position 1 and 2; H3 3rd codon position; EF1 α codon position 1 and 2; EF1 α 3rd codon position (5 partitions), P₇—28S; H3 and EF1 α codon positions 1 and 2; H3 and EF1 α 3rd codon positions (3 partitions).

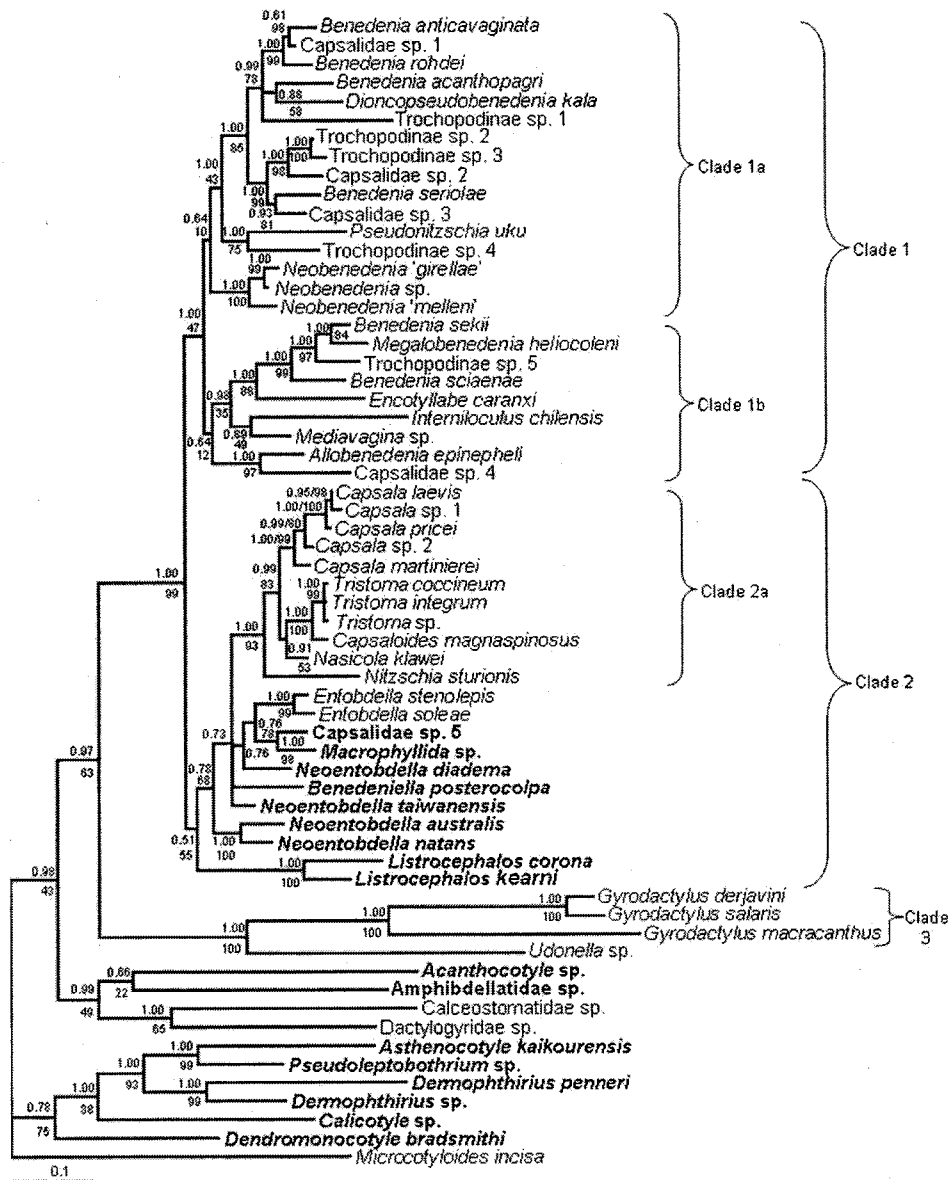


Fig. 3. A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear sequence data for the Capsalidae and 15 outgroup taxa representing 9 families and 2 subclasses. Posterior probabilities and maximum likelihood bootstrap proportions are indicated above and below each node, respectively, or, in some cases in Clade 2a before and after a /, respectively. Taxa in bold parasitise elasmobranch hosts. See Table 1 for current capsalid classification, Fig. 4 for subfamily status of capsalid taxa studied and Appendix 1 for outgroup families.

monophyly was only tested for four of those (Benedeniinae, Capsalinae, Entobdellinae and Trochopodinae) as three of the remaining subfamilies (Encotyllabinae, Internilocolinae, Nitzschiinae) were each represented by a single taxon and Pseudonitzschiinae is monotypic. The only capsalid subfamily not represented was the Dioncinae. Of the subfamilies tested, only the Capsalinae was found to be monophyletic (PP 99%, BS 83%). Of the 20 genera included, only seven (*Benedenia*, *Capsala*, *Entobdella*, *Listrocephalos*, *Neobenedenia*, *Neoentobdella* and *Tristoma*) were represented by multiple species to test generic monophyly. Of these, only five genera (*Capsala*, *Entobdella*, *Listrocephalos*, *Neobenedenia*, and *Tristoma*) were monophyletic and all with strong support (Fig. 3).

Despite poor support at some nodes, these phylogenetic hypotheses are strongly supported. Both Bayesian inference and

ML produce concordant topologies and there is strong PP support and BS support for 75% of nodes. Positive PLS for each gene at every node (data not shown) indicates that all genes are contributing to the phylogenetic signal at all nodes, including those with poor PP and BS support, therefore supporting their usefulness as markers in analyses of phylogenetic relationships of capsalid parasites. The PLS values did not vary significantly with the depth in the tree indicating they are contributing to all levels of the phylogeny. The large number of outgroup taxa included also allows for a better estimation of the root position.

We carried out AU tests of whether our data can reject a number of alternate hypotheses proposed in previous studies. The ML analysis produced a tree with a log likelihood of -31045.52 . The ML analysis with monophyly constrained for subfamilies and gen-

era of both ingroup and outgroup taxa produced a tree with a log likelihood of -32281.56 . The results of the AU test are as follows: the ML tree without any topological constraints had a p -value ($\alpha = 0.05$) of 0.87, the ML tree with monophyly enforced had a p -value ($\alpha = 0.05$) of 0.00, indicating confidence in the ML tree produced without monophyly constraints. In the ML tree in which Acanthocotylidae and Gyrodactylidae were constrained to be sister taxa following Boeger and Kritsky (2001), the p -value ($\alpha = 0.05$) was 0.131 indicating confidence in both this tree and the ML tree where no topological constraints were enforced.

The distribution of six key morphological characters that are used commonly in combination to distinguish capsalid subfamilies and genera (e.g. Whittington, 2004) were assessed relative to the

Bayesian hypothesis generated (Fig. 3) to examine the instance and frequency of homoplasy (Fig. 4). Haptorial septa are found in the Capsalinae, Encotyllabinae, Interniloculinae and Trochopodinae. In our study, septa were identified also in *Pseudonitzschia uku* (Pseudonitzschiinae) but were neither described nor illustrated by Yamaguti (1965, 1968). Accessory sclerites were absent in only one species, *P. uku* (Fig. 4). Hamuli are absent in the Capsalinae (represented by ten species), *Dioncopsudobenedenia kala* (Benedeniinae), Interniloculinae (represented in our study by one species) and Pseudonitzschiinae (monotypic) (Fig. 4). The vagina is absent only in *Neobenedenia* species (Benedeniinae). Anterior attachment organ morphology, not previously considered in detail, was the most complex morphological character included here with

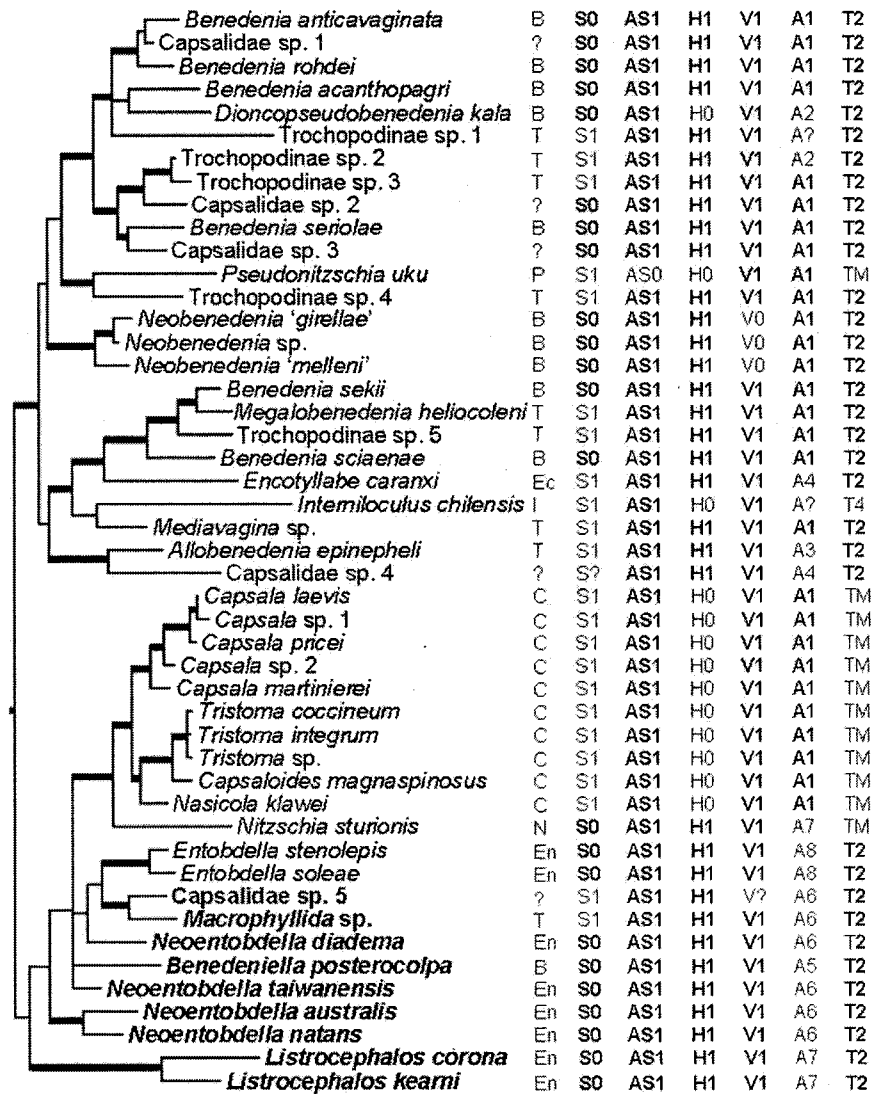


Fig. 4. A 50% majority rule consensus tree produced from Bayesian inference analyses (from Fig. 3) of the combined nuclear sequence data with current subfamily designations and distributions of key morphological characters displayed beside it. Thicker internal branches indicate those with strong support (PP > 90%). Column 1—subfamilies: Benedeniinae (B), Capsalinae (C), Encotyllabinae (Ec), Entobdellinae (En), Interniloculinae (I), Nitzschiinae (N), Pseudonitzschiinae (P) and Trochopodinae (T); column 2—haptorial septa (S): absent (S0), present (S1), unknown (S?); column 3—haptorial accessory sclerites (AS): absent (AS0), present (AS1); column 4—haptorial hamuli (H): absent (H0), present (H1); column 5—vagina: absent (V0), present (V1), unknown (V?); column 6—anterior attachment organ morphology (A; see Fig. 1): paired circular discs (A1), paired circular discs with anterior glandular and posterior muscular regions (A2), paired circular discs with muscular suckers (A3), paired structures with convoluted edges and muscular suckers (A4), paired circular discs with anterolateral ridges (A5), paired diadems (A6), paired anterolateral adhesive areas with ventral columns of multiple raised ovoid structures (A7), paired anterolateral adhesive pads each with three separate areas (A8), morphology unknown (A?), column 7—number of testes: two (T2), four (T4) or multiple (TM). Characters in bold denote the most frequently occurring state. Taxa in bold parasitise elasmobranch hosts.

eight states present in the family (plus one uncharacterised state (A?)). Character state A1 (see Fig. 1) was predominant in both Clade 1 and Clade 2 (Fig. 4). Character states A2, A3 and A4 (see Fig. 1) were only found in Clade 1 (Fig. 4) and character states A5, A6, A7 and A8 (see Fig. 1) were only found in Clade 2 (Fig. 4). Indeed the most diverse anterior attachment organ variation is displayed in capsalid taxa infecting elasmobranchs (Fig. 4, taxa in bold) with three separate character states identified among the nine included taxa (Clade 2). Multiple testes occur only in the Capsalinae and Pseudonitzschiinae but some Trochopodinae species not available for our analyses apparently also have multiple testes (Egorova, 1994). The only species included in the analyses with four testes was *Internilocus chilensis* but some described Trochopodinae species also have four testes (Egorova, 1994; Whittington, 2004). Benedeniinae (11 species), Entobdellinae (eight species) and all remaining Trochopodinae species included (nine species) had two testes. Most species in the analyses have two juxtaposed testes with the exception of *Macrophyllida* sp. and *Mediavagina* sp. where they are in tandem.

4. Discussion

4.1. Monophyly of the Capsalidae

Our study is the first molecular phylogeny of the Capsalidae with comprehensive taxon sampling (30 described species, seven species assigned to genus, five species assigned to subfamily and five species assigned to family) and multiple loci. Monophyly of the Capsalidae has been questioned and its composition has been changed multiple times and continues to be unstable (Yamaguti, 1963; Timofeeva, 1990; Egorova, 1999, 2000). The Dioncinae was considered previously to have familial status and to be the sister group to the Capsalidae (Bychowsky, 1957). *Dioncus* has since been incorporated into the family, based on haptor characteristics and reproductive morphology (Timofeeva, 1990). Inclusion of the Dioncinae provides a unique morphological synapomorphy for the family (Whittington, 2004): the presence of accessory sclerites on the haptor (Kearn, 1963). Accessory sclerites are absent only in two capsalid species (*Pseudonitzschia uku*; Fig. 3, Clade 1a) and *Calicobenedenia polyprioni* (not represented in our study) which presumably represent secondary losses (Whittington, 2004). The perforated bead shape of the spermatid mitochondrion and the progressive disappearance of the microtubules of the zone of differentiation have also been suggested as synapomorphies with the inclusion of *Dioncus* into the Capsalidae (see Justine and Mattei, 1987). The Capsalidae was shown to be monophyletic by Mollaret et al. (1997) and by Whittington et al. (2004). However, as the Dioncinae was not included in their or in our analyses, a rigorous test of capsalid monophyly in future studies should include a representative taxon. Boeger and Kritsky (2001) suggested that those microbothriids which as adults lack haptor sclerites and have two testes (e.g. *Dermophthirus penneri*, see Fig. 3) may actually be capsalids but this is not supported by our analyses because the four investigated microbothriids were monophyletic, forming a strongly supported clade (PP 100%, BS 93%) distantly related to capsalids.

4.2. Sister group to the Capsalidae

Phylogenetic hypotheses based on morphology have suggested that sister groups to the Capsalidae are the Loimoidae and Monocotylidae (see Boeger and Kritsky, 2001) while previous molecular analyses based on RNA only showed that the Gyrodactylidae and Udonellidae are closest (Olson and Littlewood, 2002). The latter is a scenario strongly supported (PP 97%, BS 63%) in our analyses

(see Fig. 3, Clade 3). It has also been hypothesised that the Acanthocotylidae is closely related to Gyrodactylidae based on multiple morphological synapomorphies (Boeger and Kritsky, 1997). While this relationship was not found in our analyses (Fig. 3), an AU test showed that our data could not reject it. More monopisthocotylean outgroups could be included to examine this relationship further.

4.3. The subfamily classification

Within the Capsalidae, the revision of some genera and species has required an ongoing reassessment of subfamilial classifications (Whittington and Horton, 1996; Egorova, 1999; Whittington, 2004). Many of these revisionary works have been done by Egorova, particularly with subfamilial and generic classifications in the Capsalinae, Trochopodinae, Benedeniinae, Entobdellinae and Dioncinae (Egorova, 1989, 1994, 1997, 1999, 2000). Of the four subfamilies for which we tested monophyly (Benedeniinae, Capsalinae, Entobdellinae and Trochopodinae), only the Capsalinae is monophyletic. This subfamily has recently undergone significant revision by rigorous evaluation of original descriptions and type material. Chisholm and Whittington (2007) identified many synonymous species and reduced the seven genera and 60 species to four genera and 36 species. Interestingly, Nitzschiinae, species of which parasitise acipenserids, is sister to the Capsalinae in our analyses (Fig. 3). Capsaline species generally parasitise highly mobile pelagic species like tuna and marlin so this infers a host switching event between euryhaline sturgeons and cosmopolitan oceanic pelagic fish.

The Benedeniinae and Trochopodinae are both large subfamilies comprising 13 and 17 genera, respectively, and approximately 51 and 52 species each (Table 1; Whittington, 2004). Together they contain >50% of capsalid diversity but based on traditional morphological characters, differ principally by possession of an aseptate (Benedeniinae) or septate (Trochopodinae) haptor (Whittington, 2004). Our study demonstrates that polyphyly in the Benedeniinae is extensive indicating that relationships are widely misunderstood in this subfamily. Whittington et al. (2004) suggested that *Neobenedenia* could be placed in a separate subfamily and this is strongly supported (PP 100%, BS 100%) in our analyses since the three *Neobenedenia* species form a monophyletic group (Fig. 3). Monophyly is also supported by the unique character, absence of a vagina (Fig. 4). The loss of the vagina may be an evolutionary innovation related to a specific mating behaviour or strategy among the species of *Neobenedenia* and this deserves further investigation. Insemination is likely achieved by sperm being introduced via the common genital pore (Whittington and Horton, 1996). A single specimen of *Neobenedenia* has been observed with its penis directed into its own uterus indicating they may self-inseminate (Whittington and Horton, 1996). With the confused composition of the Benedeniinae, it is currently unreasonable to erect a new subfamily without first re-examining the subfamily to which *Neobenedenia* presently belongs.

The Trochopodinae has been considered previously a “dumping ground” for capsalid species that are not assignable to other subfamilies and shows most morphological variation in testes number (Whittington, 2004). Its unsatisfactory definition is only further highlighted in our analyses. Whittington (2004) predicted that members of the Interniloculinae and Pseudonitzschiinae could be moved to the Trochopodinae on further study. While they do appear to be closely related to some so-called species of Trochopodinae, the extreme polyphyletic state of species currently assigned to this subfamily as shown in our analyses precludes inclusion of *Internilocus* and *Pseudonitzschia* at this stage.

The Entobdellinae has undergone recent revision (Kearn and Whittington, 2005; Kearn et al., 2007) and is considered currently to comprise 23 species in five genera (see Table 1; *Entobdella*,

Branchobdella, *Listrocephalos*, *Neoentobdella* and *Pseudoentobdella*). Our analyses, however, show paraphyly among this group of capsalids that parasitise both elasmobranchs and teleosts. In our hypothesis, a *Macrophyllida* sp. (currently considered to be a Trochopodinae) and *Benedeniella posterocolpa* (currently in the Benedeniinae) group with entobdellines and two *Listrocephalos* species group together in a separate but closely related clade. The positions of *Benedeniella posterocolpa* (Benedeniinae) and *Macrophyllida* (Trochopodinae) within the Entobdellinae (Fig. 3) are consistent with the host range and these species share some morphological characteristics with other entobdellines (e.g. anterior attachment organ morphology, see Figs. 1 and 4). Species of *Trimusculotrema* (Benedeniinae) and *Sprostonia* (Trochopodinae), which were not included in our study, also infect elasmobranch hosts and will be valuable additions to future analyses.

No representative from Dioncinae was available. Dioncinae infect remoras of the Echeneidae such as *Echeneis* and *Remora* but species are also recorded from carangids and rachycentrids (Table 1). Remoras can be 'carried' on larger organisms such as sharks, rays, teleosts, turtles and cetaceans. *Dioncus* attach their eggs to the gills of remoras and therefore these teleosts may provide a vector for host switching from chondrichthyans to teleost fish groups or perhaps in the other direction (Whittington, 2004). With capsalid parasites from sharks and rays grouping together, it is possible that remoras have been the means of transmission for ancestral capsalids on elasmobranchs to a diversity of teleost hosts.

4.4. Generic classifications

Of the 46 capsalid genera recognised, some remain poorly defined (Whittington, 2004). Five (*Capsala*, *Entobdella*, *Listrocephalos*, *Neobenedenia* and *Tristoma*) of the seven genera for which we had more than one representative were monophyletic. Genera represented by large numbers of species in our analyses such as *Benedenia* (six of 21 species included) were not monophyletic and were spread throughout Clade 1 of the tree. Unexpectedly, *Neoentobdella* was also not monophyletic in the Bayesian analyses (Fig. 3). It was monophyletic in the ML analyses but with very weak support (BS 11%, see Supplementary Fig. 1). The genus was erected recently based on morphological characters and host association and comprises 10 species infecting rays (Whittington and Kearn, 2009). Our analyses included four described *Neoentobdella* species (Whittington and Kearn, 2009). Our analyses indicate that further revision of *Benedenia* is needed but monophyly for *Neoentobdella* cannot be rejected and further work incorporating faster evolving genes is required. The confused state of capsalid subfamilial classification is further complicated by poor generic definitions. Adding a mitochondrial dataset may also help to tease out some of the shallower relationships in the tree and further test support in these areas. This, along with broader taxon representation, will further elucidate relationships within the Capsalidae.

4.5. Systematic utility of morphological characters

In parasites, molecular genetic data have been viewed more favourably than morphological data for phylogenetic analyses due to the apparent lack of stability of morphological based hypotheses and the lack of available morphological characters (Littlewood et al., 1999). However, morphological analyses are important as they allow the identification of synapomorphies and lead to the development of a robust set of characters with which to describe taxa. Examination of the distribution of defining morphological characters relative to our molecular phylogenetic hypotheses generated shows that some of the character states (haptoral septa, haptoral hamuli, anterior attachment organ morphology and testis number) show apparent homoplasious evolution in the Capsalidae.

These morphological characters may be homoplastic due to convergent evolution which is considered highly likely in parasites given the similar life history challenges they face (Poulin and Morand, 2000). A parasite must find its host, attach to it and then derive nutrition from it. Similarities in the type of host and specific microhabitat parasitised may elicit morphological adaptations by parasites that impose phylogenetic constraints on character evolution (Whittington, 2004). Homoplasy may also be an artefact of poor or insufficient character state definitions. While capsalid morphology is considered conserved, there is variation within some of these characters. The usefulness of morphological characters is thought to increase with the complexity with which they are described (Littlewood et al., 1999). Currently five of the subfamilies have haptoral septa but the haptors are divided in very different ways. The Capsalinae haptor is divided into a series of peripheral compartments surrounding a central loculus. This arrangement is not seen in the haptoral septa of the other subfamilies (Whittington, 2004). Many of these characters, at the detail to which they are described, are also not unique to the Capsalidae. Septate haptors occur in other monogenean families (e.g. Monocotylidae) but there has been no assessment about whether these structures are homologous (Whittington, 2004).

Individual characters will only contribute to relationships at certain levels of a tree. Many of the anterior attachment organ morphologies only apply to species in a single genus and so provide no information on relationships at higher levels. Some combinations of these characters appear to define some subfamilies and genera relative to the molecular phylogenetic analysis. The Capsalinae are defined morphologically as having haptoral septa, presence of accessory sclerites, absence of haptoral hamuli, presence of a vagina, paired anterior circular discs and multiple testes (Fig. 4). There are no other taxa in these analyses that have this combination. Similarly, *Neobenedenia* can be defined as lacking haptoral septa and a vagina, but possessing accessory sclerites, haptoral hamuli, paired anterior circular discs and two juxtaposed testes, a combination unique to this genus (Fig. 4). However, with only six morphological characters commonly used to distinguish capsalid subfamilies and genera, it is inconceivable that these characters can comprehensively define the relationships at all levels between the approximately 180 described capsalid taxa. More morphological characters are needed to provide phylogenetic information throughout all levels of the tree.

These simplistic definitions and paucity of morphological characters provide little information on relationships at any level and this is reflected in the disparity between the molecular phylogenetic hypothesis and morphological taxonomy. Perhaps these characters need examining at an ultrastructural level to identify informative structural differences. New characters need exploring, such as larval characters, as they are believed to be less modified by parasitism and better reflect ancestry (Whittington, 2004). Care must be taken when examining and inferring further characters and states. Biological and environmental variables such as parasite and host age, host species and water temperature can also induce changes in morphology making characters problematic when used in phylogenetic analyses due to phenotypic plasticity and low heritability (Brooks and McLennan, 1993). The phylogenetic framework presented here provides a basis to explore further morphological characters.

The Linnaean ranks used for classification of taxa within the Capsalidae are subjective because they are not based on phylogenetic hypotheses. Furthermore our analyses show they are also poor estimates of relationships within the family likely due to homoplasy. As a consequence of the small number of informative adult morphological characters in these parasites and the logistical problems associated with documenting variation in larval or gamete characters, it is unlikely that a morphological dataset robust

enough to establish a comprehensive phylogenetic hypothesis will be compiled any time soon. While molecular data are providing new and valuable insights into the relationships of these parasites, by themselves they are no more useful in defining Linnaean ranks. This is not an uncommon dilemma and there has been much debate in the literature about how to combine traditional taxonomy with phylogenetic relationships (Moore, 1998; Brummitt, 2002; Schuh, 2003; Horandl, 2006). The PhyloCode has been proposed as a means for governing nomenclature in a phylogenetic context (Cantino and de Queiroz, 2007). Since its inception, one of the biggest criticisms has been a failure to develop a means to deal with species ranks. However, there is now a system proposed whereby Linnaean binomials can be used in a way that is consistent with phylogenetic nomenclature (Dayrat et al., 2008). Such a system, that can bridge the legacy of the extensive use of Linnaean ranks with the principles of phylogenetic nomenclature based on molecular phylogenies, is perhaps where the answer lies for producing a classification that both conveys biological information and the phylogenetic history of these organisms.

Acknowledgments

We are exceptionally grateful to those colleagues and collaborators who have collected and provided us with specimens, without which this study would not have been possible (Appendix 1). We also thank Gaynor Dolman for her continual support and advice throughout this project. This study was funded by an Australian Research Council Discovery Grant (DP0556780) awarded to I.D.W. and S.C.D. E.M.P. was supported by an Australian Postgraduate Award during PhD candidature in the School of Earth and Environmental Sciences at the University of Adelaide.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.008.

References

- Akaike, H., 1985. Prediction and entropy. In: Atkinson, A.C., Fienberg, S.E. (Eds.), *A Celebration of Statistics*. Springer-Verlag, Berlin, pp. 1–24.
- Boeger, W.A., Kritsky, D.C., 1997. Coevolution of the Monogeneoidea (Platyhelminthes) based on a revised hypothesis of parasite phylogeny. *Int. J. Parasitol.* 27, 1495–1511.
- Boeger, W.A., Kritsky, D.C., 2001. Phylogenetic relationships of the Monogeneoidea. In: Littlewood, D.T.J., Bray, R.A. (Eds.), *Interrelationships of the Platyhelminthes*. Taylor & Francis, London and New York.
- Brooks, D.R., McLennan, D.A., 1993. *Parascript: Parasites and the Language of Evolution*. Smithsonian Institution Press, Washington and London.
- Brummitt, R.K., 2002. How to chop up a tree. *Taxon* 51, 31–41.
- Bullard, S.A., Benz, G.W., Braswell, J.S., 2000. *Dioncus postoncomiracidia* (Monogenea: Dionchidae) from the skin of blacktip sharks, *Carcharhinus limbatus* (Carcharhinidae). *J. Parasitol.* 86, 245–250.
- Bychowsky, B.E., 1957. Monogenetic trematodes, their systematics and phylogeny. *Izdatel'stvo Akademii Nauk SSSR, Moscow* (In Russian: English translation edited by Hargis Jr., W.J., 1961), 627 pp.
- Cantino, P.D., de Queiroz, K., 2007. International Code of Phylogenetic Nomenclature, version 4b. Available from: <http://www.phylocode.org>.
- Chisholm, L.A., Morgan, J.A.T., Adlard, R.D., Whittington, I.D., 2001. Phylogenetic analysis of the Monocotylidae (Monogenea) inferred from 28S rDNA sequences. *Int. J. Parasitol.* 31, 1253–1263.
- Chisholm, L.A., Whittington, I.D., 2007. Review of the Capsalinae (Monogenea: Capsalidae). *Zootaxa*, 1–30.
- Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.* 46, 419–437.
- Dayrat, B., Cantino, P.D., Clarke, J.A., De Queiroz, K., 2008. Species names in the PhyloCode: the approach adopted by the international society for phylogenetic nomenclature. *Syst. Biol.* 57, 507–514.
- Deveney, M.R., Chisholm, L.A., Whittington, I.D., 2001. First published record of the pathogenic monogenean parasite *Neobenedenia melleni* (Capsalidae) from Australia. *Dis. Aquat. Org.* 46, 79–82.
- Egorova, T.P., 1989. A Taxonomic Analysis of the Subfamily Capsalinae (Monogeneoidea: Capsalidae). *Parazitologicheskii issledovaniya: sbornik nauchnykh trudov. Dal'nevostochnoe otdelenie, Akademiya Nauk SSSR, Vladivostok*, pp. 46–54.
- Egorova, T.P., 1994. A taxonomic review of the subfamily Trochopodinae (Monogeneoidea: Capsalidae). *Parazitologiya (St. Petersburg.)* 28, 81–91.
- Egorova, T.P., 1997. A taxonomic review of the subfamily Benedeniinae (Monogeneoidea: Capsalidae). *Parazitologiya (St. Petersburg.)* 31, 438–451.
- Egorova, T.P., 1999. Systematics of the subfamily Entobdellinae (Monogeneoidea: Capsalidae). *Parazitologiya (St. Petersburg.)* 33, 420–425.
- Egorova, T.P., 2000. New monogeneans of the genus *Dionchus* (Capsalidae: Dionchinae). *Parazitologiya (St. Petersburg.)* 34, 252–258.
- Horandl, E., 2006. Paraphyletic versus monophyletic taxa—evolutionary versus cladistic classifications. *Taxon* 55, 564–570.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Justine, J.L., Mattei, X., 1987. Phylogenetic relationships between the families Capsalidae and Dionchidae (Platyhelminthes, Monogenea, Monopisthocotylea) indicated by the comparative ultrastructural study of spermiogenesis. *Zool. Scr.* 16, 111–116.
- Kearn, G.C., 1963. The egg, oncomiracidium and larval development of *Entobdella soleae*, a monogenean skin parasite of the common sole. *Parasitology* 53, 435–447.
- Kearn, G.C., 1998. *Parasitism and the Platyhelminths*. Chapman and Hall, London.
- Kearn, G.C., Whittington, I.D., 2005. *Neoentobdella* gen nov for species of *Entobdella* Blainville in Lamarck, 1818 (Monogenea: Capsalidae: Entobdellinae) from stingray hosts, with descriptions of two new species. *Acta Parasitol.* 50, 32–48.
- Kearn, G.C., Whittington, I.D., Evans-Gowing, R., 2007. A revision of *Entobdella* Blainville in Lamarck, 1818, with special emphasis on the nominal (type) species “*Entobdella hippoglossi* (Mueller, 1776) Blainville, 1818” (Monogenea: Capsalidae: Entobdellinae) from teleost flatfishes, with descriptions of three new species and a new genus. *Zootaxa* 1659, 1–54.
- Lee, M.S.Y., Hugall, A.F., 2003. Partitioned likelihood support and the evaluation of data set conflict. *Syst. Biol.* 52, 15–22.
- Littlewood, D.T.J., Rohde, K., Clough, K.A., 1997. Parasite speciation within or between host species? Phylogenetic evidence from site-specific polystome monogeneans. *Int. J. Parasitol.* 27, 1289–1297.
- Littlewood, D.T.J., Rohde, K., Clough, K.A., 1999. The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. *Biol. J. Linn. Soc.* 66, 75–114.
- Maddison, W.P., Maddison, D.R., 1995. *MacClade: Analysis of Phylogeny and Character Evolution*. Sinauer Associates Inc., Sunderland, MA.
- Matejusová, I., Cunningham, C.O., 2004. The first complete monogenean ribosomal RNA gene operon: sequence and secondary structure of the *Gyrodactylus salaris* Malmberg, 1957, large subunit ribosomal RNA gene. *J. Parasitol.* 90, 146–151.
- Mollaret, I., Jamieson, B.G.M., Adlard, R.D., Hugall, A., Lecointre, G., Chombard, C., Justine, J.L., 1997. Phylogenetic analysis of the Monogenea and their relationships with Digenea and Eucestoda inferred from 28S rDNA sequences. *Mol. Biochem. Parasitol.* 90, 433–438.
- Moore, G., 1998. A comparison of traditional and phylogenetic nomenclature. *Taxon* 47, 561–579.
- Olson, P.D., Littlewood, D.T.J., 2002. Phylogenetics of the Monogenea—evidence from a medley of molecules. *Int. J. Parasitol.* 32, 233–244.
- Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Poulin, R., Morand, S., 2000. The diversity of parasites. *Q. Rev. Biol.* 75, 277–293.
- Rambaut, A., 1996. Se-Al: Sequence Alignment Editor. Available from: <http://evolve.zoo.ox.ac.uk/>.
- Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. Available from: <http://beast.bio.ed.ac.uk/Tracer>.
- Rychlik, W., 1992. The importance of oligonucleotide stability at its 3'-terminus in sequencing and PCR primer selection. *Biophys. J.* 61, A76.
- Schuh, R.T., 2003. The Linnaean system and its 250-year persistence. *Bot. Rev.* 69, 59–78.
- Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17, 1246–1247.
- Stamatakis, A., 2006. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A Rapid Bootstrap Algorithm for the RAXML Web-Servers. *Syst. Biol.* 57, 758–771.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876–4882.
- Timofeeva, T.A., 1990. Phylogenetic Relationships of Capsalids and Dionchids and the Position of the Latter in the System of Monogeneans (Monogenea, Monopisthocotylea). *Trudy Zoologicheskii Institut, Akademii Nauk SSSR*, pp. 3–16.
- Tingbao, Y., Kritsky, D.C., Yuan, S., 2004. Revision of *Allobenedenia* Yamaguti, 1963 (Monogeneoidea: Capsalidae) with the description of *A. zhangii* n. sp. from *Epinephelus fasciatus* (Teleostei: Serranidae) in the South China Sea. *Syst. Parasitol.* 59, 223–233.
- Whittington, I.D., 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. *Folia Parasitol.* 51, 109–122.
- Whittington, I.D., Deveney, M.R., Morgan, J.A.T., Chisholm, L.A., Adlard, R.D., 2004. A preliminary phylogenetic analysis of the Capsalidae (Platyhelminthes:

- Monogenea: Monopisthocotylea) inferred from large subunit rDNA sequences. *Parasitology* 128, 511–519.
- Whittington, I.D., Horton, M.A., 1996. A revision of *Neobenedenia* Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of *N. melleni* (MacCallum, 1927) Yamaguti, 1963. *J. Nat. Hist.* 30, 1113–1156.
- Whittington, I.D., Kearns, G.C., 2009. Two new species of entobdelline skin parasites (Monogenea, Capsalidae) from the blotched fantail ray, *Taeniura meyeni*, in the Pacific Ocean, with comments on spermatophores and the male copulatory apparatus. *Acta Parasitol.* 54, 12–21.
- Yamaguti, S., 1963. *Systema Helminthum*. InterScience Publishers, New York.
- Yamaguti, S., 1965. New monogenetic trematodes from Hawaiian fishes I. *Pac. Sci.* 19, 55–95.
- Yamaguti, S., 1968. *Monogenetic trematodes of Hawaiian fishes*. University of Hawaii press, Hawaii.
- Zwickl, D.J., 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. The University of Texas at Austin, Austin.

APPENDIX II

Copyright transfer statement.

ELSEVIER LICENSE TERMS AND CONDITIONS

This is a License Agreement between Elizabeth M Perkins ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier	Elsevier Limited The Boulevard, Langford Lane Kidlington, Oxford, OX5 1GB, UK
Registered Company Number	1982084
Customer name	Elizabeth M Perkins
Customer address	Earth and Environmental Sciences Adelaide, other 5005
License Number	2275131065684
License date	Sep 23, 2009
Licensed content publisher	Elsevier
Licensed content publication	Molecular Phylogenetics and Evolution
Licensed content title	Looks can deceive: Molecular phylogeny of a family of flatworm ectoparasites (Monogenea: Capsalidae) does not reflect current morphological classification
Licensed content author	Elizabeth M. Perkins, Steve C. Donnellan, Terry Bertozzi, Leslie A. Chisholm and Ian D. Whittington
Licensed content date	September 2009
Volume number	52
Issue number	3
Pages	10
Type of Use	Thesis / Dissertation
Portion	Full article
Format	Both print and electronic
You are an author of the Elsevier article	Yes
Are you translating?	No
Order Reference Number	
Expected publication	Nov 2009

date

Elsevier VAT number GB 494 6272 12

Permissions price 0.00 USD

Value added tax 0.0% 0.00 USD

Total 0.00 USD

Terms and Conditions

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at <http://myaccount.copyright.com>).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

“Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER].” Also Lancet special credit - “Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier.”

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment

terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation:** This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website:** The following terms and conditions apply to electronic reserve and author websites:

Electronic reserve: If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:

This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,

All content posted to the web site must maintain the copyright information line on the bottom of each image,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> or the Elsevier homepage for books at <http://www.elsevier.com> , and

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and

the permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version,

A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx> , As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect (www.sciencedirect.com). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:

Authors are permitted to place a brief summary of their work online only.

A hyper-text must be included to the Elsevier homepage at <http://www.elsevier.com>

All content posted to the web site must maintain the copyright information line on the

bottom of each image

You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.

Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at <http://www.sciencedirect.com/science/journal/xxxxx>. or for books to the Elsevier homepage at <http://www.elsevier.com>

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

21. **Other Conditions**None

v1.6

Gratis licenses (referencing \$0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

APPENDIX III

Parasite taxa used in analyses in Chapter II.

Appendix III

Taxa used in analyses in Chapter II

Museum	Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29644		28SrRNA – FJ971962 H3 – FJ972078 EF1a – FJ972024	<i>Acanthocotyle</i> sp. (A)	<i>Urolophus cruciatus</i>	S	Tasmania	L.A.C. & I.D.W.
AHC 29645		28SrRNA – FJ971963 H3 – FJ972079 EF1a – FJ972025	<i>Allobenedenia epinepheli</i> (C)	<i>Epinephelus coioides</i>	G	Northern Arabian Gulf	J. Abdul-Salam & B.S. Sreelatha
AHC 29646		28SrRNA – FJ971964 H3 – FJ972080 EF1a – FJ972026	Amphibdellatidae sp. (Am)	<i>Narcine tasmaniensis</i>	G	Tasmania	L.A.C. & I.D.W.
AHC 29647		28SrRNA – FJ971965 H3 – FJ972081 EF1a – FJ972027	<i>Asthenocotyle kaikourensis</i> (M)	<i>Proscymnodon plunketi</i>	SD	New Zealand	C.J. Healy & E. Burreson
AHC 29648		28SrRNA – FJ971967 H3 – FJ972083 EF1a – FJ972029	<i>Benedenia acanthopagri</i> (C)	<i>Sparidentex hasta</i>	S	Culture cages, Kuwait	A. Al-Marzouk
AHC 45061		28SrRNA – FJ971968 H3 – FJ9872084	<i>Benedenia anticavaginata</i> (C)	<i>Acanthopagrus australis</i>	BPF	Stradbroke Island, QLD	M. Deveney & I.D.W.
AHC 29649		28SrRNA – FJ971969 H3 – FJ972085 EF1a – FJ972030	<i>Benedenia rohdei</i> (C)	<i>Lutjanus carponotatus</i>	BM	Heron Island, QLD	T.H. Cribb
AHC 29650		28SrRNA – FJ971970 H3 – FJ972086 EF1a – FJ972031	<i>Benedenia sciaenae</i> (C)	<i>Argyrosomus japonicus</i>	S	Port Augusta, SA	I. Ernst & C. Chambers
AHC 29651		28SrRNA – FJ971971 H3 – FJ972087 EF1a – FJ972032	<i>Benedenia sekii</i> (C)	<i>Chrysophrys auratus</i>	F	Pisces Fish Farm, NSW	A. Mooney
AHC 29181		28SrRNA – FJ971972 H3 – FJ972088 EF1a – FJ972033	<i>Benedenia seriolae</i> (C)	<i>Seriola hippos</i>	S	Greenly Island, SA	K. Hutson

Museum	Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
	AHC 29652	28SrRNA – FJ971975	<i>Benedeniella posterocolpa</i> (C)	<i>Rhinoptera bonasus</i>	S	New York Aquarium (originally ex Virginia), USA	A.M. Dove
		H3 – FJ972091 EF1a – FJ972036					
	AHC 29653	28SrRNA – FJ971977	Calceostomatidae sp. (Cs)	<i>Eugerres axillaris</i>	G	La Paz, Mexico	L.A.C. & I.D.W.
		H3 – FJ972093 EF1a – FJ972038					
	AHC 29654	28SrRNA – FJ971978	<i>Calicotyle</i> sp. (Mo)	<i>Aptychotrema rostrata</i>	C	Stradbroke Island, QLD	L.A.C. & I.D.W.
		H3 – FJ972094 EF1a – FJ972039					
	AHC 29655	28SrRNA – FJ971979	<i>Capsala laevis</i> (C)	<i>Tetrapterus audax</i>	G	Port Stephens, NSW	L.A.C. & I.D.W.
		H3 – FJ972095 EF1a – FJ972040					
	AHC 45062	28SrRNA – FJ971980	<i>Capsala martinieri</i> (C)	<i>Mola mola</i>	S	Two Oceans Aquarium, South Africa	K. Christison
		H3 – FJ972096 EF1a – FJ972041					
	AHC 29656	28SrRNA – FJ971982	<i>Capsala pricei</i> (C)	<i>Tetrapterus audax</i>	S	Port Stephens, NSW	L.A.C. & I.D.W.
		H3 – FJ972098 EF1a – FJ972043					
	AHC 29657	28SrRNA – FJ971983	<i>Capsala</i> sp. 1 (C)	<i>Tetrapterus belone</i>	S	Mediterranean Sea, Italy	F. Garibaldi
		H3 – FJ972099 EF1a – FJ972042					
	AHC 29658	28SrRNA – FJ971981	<i>Capsala</i> sp. 2 (C)	<i>Makaira nigricans</i>	S	Port Stephens, NSW	L.A.C. & I.D.W.
		H3 – FJ972097 EF1a – FJ972042					
MNHN JNC1559 B1		28SrRNA – FJ971974	Capsalidae sp. 1 (C)	<i>Plectorhinchus chaetodonoides</i>	G	Noumea, New Caledonia	J.-L. Justine
		H3 – FJ972090 EF1a – FJ972035					
	AHC 29659	28SrRNA – FJ971976	Capsalidae sp. 2 (C)	<i>Nemadactylus valenciennesi</i>	Pf	Port MacDonnell, SA	K. Hutson
		H3 – FJ972092 EF1a – FJ972037					
	AHC 29660	28SrRNA – FJ971973	Capsalidae sp. 3 (C)	<i>Scarus ghobban</i>	S	Heron Island, QLD	T.H. Cribb

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
	H3 – FJ972089 EF1a – FJ972034 28SrRNA – FJ971966	Capsalidae sp. 4 (C)	<i>Paralabrax maculatofasciatus</i>	G & PT	Bahia Santa Rosaliita, Mexico	M. Carmen
AHC 29661	H3 – FJ972082 EF1a – FJ972028 28SrRNA – FJ971993	Capsalidae sp. 5 (C)	<i>Triakis megalopterus</i>	S	Ushaka Marine World, Durban, South Africa	K. Christison
AHC 28913-16	H3 – FJ972109 EF1a – FJ972052 28SrRNA – FJ971984	<i>Capsaloides magnaspinosus</i> (C)	<i>Tetrapterus audax</i>	N	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 29662	H3 – FJ972100 EF1a – FJ972044 28SrRNA – FJ971985	Dactylogyridae sp. (D)	<i>Nemadactylus valenciennesi</i>	G	Port MacDonnell, SA	K. Hutson
AHC 28777-93	H3 – FJ972101 EF1a – FJ972045 28SrRNA – FJ971984	<i>Dendromonocotyle bradsmithi</i> (Mo)	<i>Myliobatis australis</i>	DS	Outer Harbour, Adelaide, SA	B. Smith, K. Hutson, L.A.C. & I.D.W.
AHC 29663	H3 – FJ972102 EF1a – FJ972046 28SrRNA – FJ971987	<i>Dermophthirius penneri</i> (M)	<i>Carcharhinus limbatus</i>	SD	Northern Gulf of Mexico, Mississippi, USA	S.A. Bullard
AHC 29664	H3 – FJ972103 EF1a – FJ972047 28SrRNA – FJ971988	<i>Dermophthirius</i> sp. (M)	<i>Rhinobatos typus</i>	SD	Cairns Marine Aquarium Supply, Cairns, QLD	J. Caira & K. Jensen
MNHJNC1552 A1	H3 – FJ972104 EF1a – FJ972048 28SrRNA – FJ971989	<i>Dioncospseudobenedenia kala</i> (C)	<i>Naso unicornis</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 29665	H3 – FJ972105 EF1a – FJ972049 28SrRNA – FJ971990	<i>Encotyllabe caranxi</i> (C)	<i>Pseudocaranx dentex</i>	PT	Heron Island, QLD	M. Deveney
AHC 29666	H3 – FJ972106 28SrRNA – FJ971992	<i>Entobdella soleae</i> (C)	<i>Solea solea</i>	S	Aquarium, Univ. of East Anglia, UK	G.C. Kearns

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 45063	H3 – FJ972108 EF1a – FJ972051 28SrRNA – FJ971991	<i>Entobdella stenolepis</i> (C)	<i>Hippoglossus stenolepis</i>	S	British Columbia, Canada	M. Kent
AHC 45064	H3 – FJ972107 EF1a – FJ972050 28SrRNA – FJ971994	<i>Gyrodactylus derjavini</i> (G)	<i>Oncorhynchus mykiss</i>	S	Experimental culture, Frederiksberg, Denmark	K. Buchmann
AHC 45065	H3 – FJ972110 EF1a – FJ972053 28SrRNA – FJ971995	<i>Gyrodactylus macracanthus</i> (G)	<i>Misgurnus anguillicaudatus</i>	S	Uni of Melbourne, experimental fish	J. Kearns
AHC 45066	H3 – FJ972111 EF1a – FJ972054 28SrRNA – FJ971996	<i>Gyrodactylus salaris</i> (G)	<i>Salmo salar</i>	F	Laerdalselva, Norway	K. Buchmann
AHC 45067	H3 – FJ972112 EF1a – FJ972055 28SrRNA – FJ971997	<i>Interniloculus chilensis</i> (C)	<i>Sebastes capensis</i>	G	Aysén Channels, Chile	M.T. Gonzalez
AHC 45068	H3 – FJ972113 28SrRNA – FJ971998	<i>Listrocephalos corona</i> (C)	<i>Dasyatis sabina</i>	S	Mississippi Sound, off Biloxi, Mississippi, USA	S.A. Bullard
AHC 29667	H3 – FJ972114 EF1a – FJ972056 28SrRNA – FJ971999	<i>Listrocephalos kearni</i> (C)	<i>Dasyatis brevis</i>	S	La Paz, Mexico	L.A.C. & I.D.W.
AHC 29668	H3 – FJ972115 EF1a – FJ972057 28SrRNA – FJ972000	<i>Macrophyllida</i> sp. (C)	<i>Hemigaleus microstoma</i>	G	Moreton Bay, QLD	T. Turner
AHC 29669	H3 – FJ972116 EF1a – FJ972058 28SrRNA – FJ972019	<i>Mediavagina</i> sp. (C)	<i>Nemadactylus valenciennesi</i>	G	Port MacDonnell, SA	K. Hutson
AHC 29670	H3 – FJ972135 EF1a – FJ972074 28SrRNA – FJ972001	<i>Megalobenedenia helicoleni</i> (C)	<i>Helicolenus percoides</i>	G	Port MacDonnell, SA	K. Hutson
AHC 45069	H3 – FJ972117 EF1a – FJ972059 28SrRNA – FJ972002	<i>Microcotyloides incisa</i> (Mi)	<i>Lutjanus argentiventris</i>	G	La Paz, Mexico	L.A.C. & I.D.W.

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 28962-67	H3 – FJ972118 EF1a – FJ972060 28SrRNA – FJ972003	<i>Nasicola klawei</i> (C)	<i>Thunnus albacares</i>	N	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 29671	H3 – FJ972119 EF1a – FJ972061 28SrRNA – FJ972004	<i>Neobenedenia 'girellae'</i> (C)	<i>Verasper variegatus</i>	S	Aquarium, Nippon Suisan Kaisha, Oita, Japan	N. Hirazawa
AHC 29672	H3 – FJ972120 EF1a – FJ972062 28SrRNA – FJ972005	<i>Neobenedenia 'melleni'</i> (C)	<i>Sphoeroides annulatus</i>	S	Aquaria, CIAD, Mazatlan, Sinoloa, Mexico	I.D.W.
AHC 29673	H3 – FJ972121 EF1a – FJ972063 28SrRNA – FJ972006	<i>Neobenedenia</i> sp. (C)	<i>Seriola rivoliana</i>	S	Aquaculture, Guayaquil, Ecuador	E. Blacio
AHC 29555-59	H3 – FJ972122 EF1a – FJ972064 28SrRNA – FJ972010	<i>Neoentobdella taiwanensis</i> (C)	<i>Taeniura meyeni</i>	S	Public Aquarium, Taiwan	M.H. Chen
AHC 29674	H3 – FJ972126 EF1a – FJ972067 28SrRNA – FJ972007	<i>Neoentobdella australis</i> (C)	<i>Neotrygon kuhlii</i>	VS	Stradbroke Island, QLD	I.D.W., L.A.C. & V. Glennon
AHC 29675	H3 – FJ972123 EF1a – FJ972065 28SrRNA – FJ972008	<i>Neoentobdella diadema</i> (C)	<i>Pteroplatytrygon violacea</i>	S	Mediterranean Sea, off Italy	A. Gustinelli
AHC 29676	H3 – FJ972124 28SrRNA – FJ972009	<i>Neoentobdella natans</i> (C)	<i>Pastinachus sephen</i>	VS	Heron Island, QLD	T.H. Cribb
AHC 45070	H3 – FJ972125 EF1a – FJ972066 28SrRNA – FJ972011	<i>Nitzschia sturionis</i> (C)	<i>Huso huso</i>	OC	Caspian Sea near mouth of River Kuma, Dagestan	M.R. Deveney
AHC 28917	H3 – FJ972127 EF1a – FJ972068 28SrRNA – FJ972012	<i>Pseudoleptobothrium</i> sp. (M)	<i>Aptychotrema rostrata</i>	SD	Stradbroke Island, QLD	B.W. Cribb
	H3 – FJ972128					

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
MNHN JNC1557 A1	EF1a – FJ972069 28SrRNA – FJ972013	<i>Pseudonitzschia uku</i> (C)	<i>Aprion virescens</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 45071	H3 – FJ972129 EF1a – FJ972070 28SrRNA – FJ972014	<i>Tristoma coccineum</i> (C)	<i>Xiphias gladius</i>	G	Mediterranean	S. Mattiucci
AHC 45072	H3 – FJ972130 EF1a – FJ972071 28SrRNA – FJ972015	<i>Tristoma integrum</i> (C)	<i>Xiphias gladius</i>	G	Mediterranean	S. Mattiucci
AHC 45073	H3 – FJ972131 28SrRNA – FJ972016	<i>Tristoma</i> sp. (C)	<i>Xiphias gladius</i>	G	Azores, Atlantic Ocean	S. Mattiucci
MNHN JNC1281 A5	H3 – FJ972132 28SrRNA – FJ972020	Trochopodinae sp. 1 (C)	<i>Pseudobalistes fuscus</i>	G	Noumea, New Caledonia	J.-L. Justine
MNHN JNC1391 B1	H3 – FJ972136 EF1a – FJ972075 28SrRNA – FJ972017	Trochopodinae sp. 2 (C)	<i>Plectropomus leopardus</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 45074	H3 – FJ972133 EF1a – FJ972072 28SrRNA – FJ972022	Trochopodinae sp. 3 (C)	<i>Plectropomus leopardus</i>	G	Heron Island, QLD	M. Deveney
MNHN JNC1379 B2	H3 – FJ972138 28SrRNA – FJ972018	Trochopodinae sp. 4 (C)	<i>Epinephelus fuscoguttatus</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 29677	H3 – FJ972134 EF1a – FJ972073 28SrRNA – FJ972021	Trochopodinae sp. 5 (C)	<i>Chelidonichthys capensis</i>	S	Two Oceans Aquarium, Cape Town, South Africa	K. Christison
AHC 45075	H3 – FJ972137 EF1a – FJ972076 28SrRNA – FJ972023 EF1a – FJ972077	<i>Udonella</i> sp. (U)	<i>Urolophus halleri</i>	CCS	La Paz, Mexico	L.A.C. & I.D.W.

Abbreviations used in Appendix III.

Museum

AHC = Australian Helminthological Collection of the South Australian Museum, Adelaide, South Australia, Australia

MNHN = Muséum National d'Histoire Naturelle, Paris, France

Monogenean family

A = Acanthocotylidae

Am = Amphibdellatidae

C = Capsalidae

Cs = Calceostomatidae

D = Dactylogyridae

G = Gyrodactylidae

M = Microbothriidae

Mi = Microcotylidae

Mo = Monocotylidae

U = Udonellidae

Collectors

I.D.W. - I.D. Whittington

L.A.C. - L.A. Chisholm

Site Parasitised

BM = Branchiostegal membranes

BPF = Base of pectoral fin

C = Cloaca

CCS = Copepod carapace ex skin

DS = Dorsal skin

F = Fins

G = Gills

N = Nares
OC = Oral cavity
Pf = Pectoral fins
PT = Pharyngeal teeth
S = Skin
SD = Skin denticles
VS = Ventral skin

APPENDIX IV

The maximum likelihood tree derived from analysis of the combined nuclear sequence data for the Capsalidae in Chapter II.

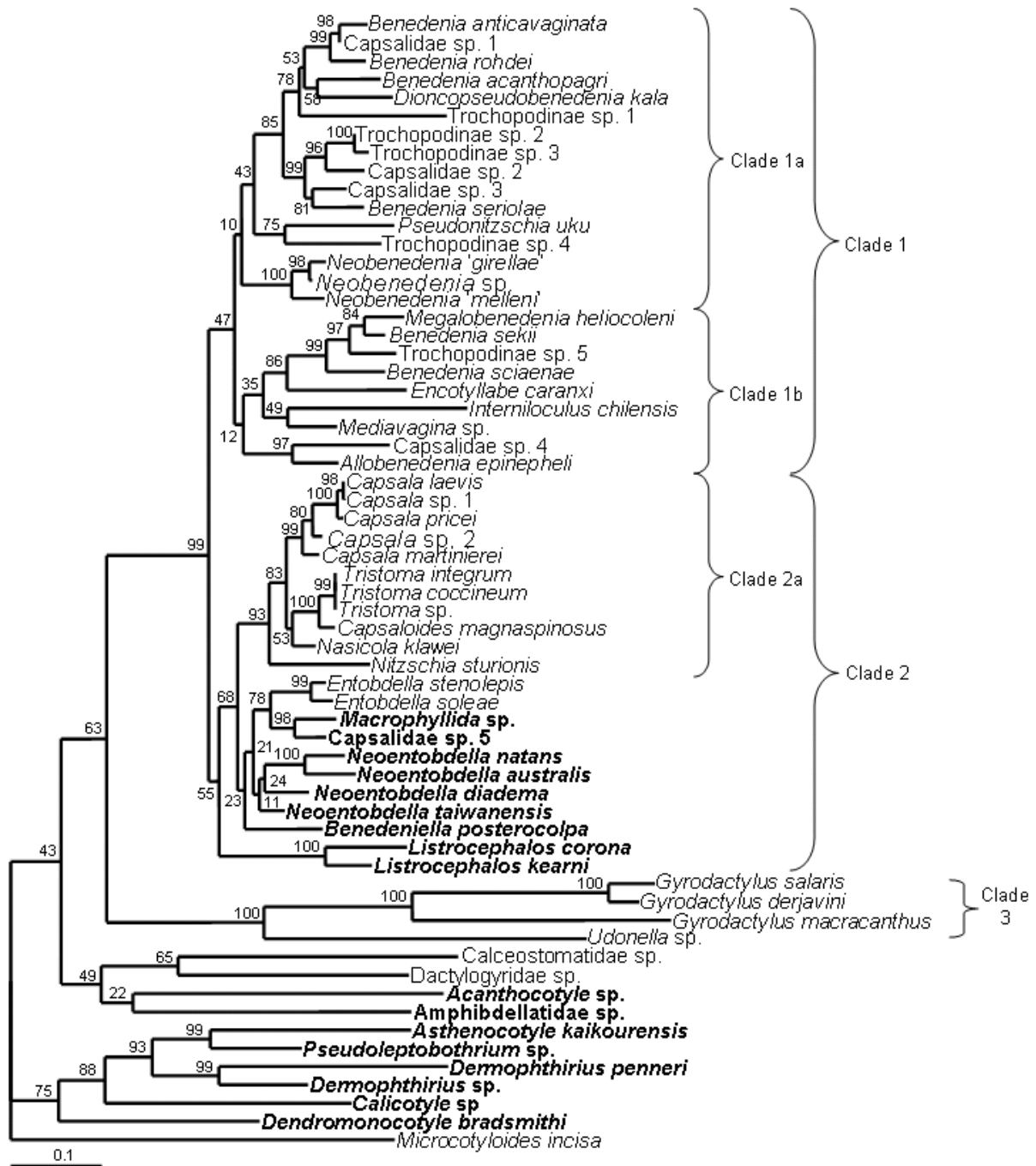


Fig. 1. The maximum likelihood tree derived from analyses of the combined nuclear sequence data for the Capsalidae. Non-parametric bootstrap support proportions are indicated at each node. Log likelihood score -31045.52. Taxa in bold parasitise elasmobranch hosts. See Appendix III for outgroup families.

APPENDIX V

Parasite taxa used in analyses in Chapter IV.

Appendix V

Taxa used in analyses in Chapter IV

Museum	Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
	AHC 29644	28SrRNA – FJ971962 H3 – FJ972078 EF1 α – FJ972024	<i>Acanthocotyle</i> sp. (A) ¹	<i>Urolophus cruciatus</i>	S	Tasmania	L.A.C. & I.D.W.
	AHC 29645	28SrRNA – FJ971963 H3 – FJ972079 EF1 α – FJ972025 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Allobenedenia epinepheli</i> (C) ^{1,2,3}	<i>Epinephelus coioides</i>	G	Northern Arabian Gulf	J. Abdul-Salam & B.S. Sreelatha
	AHC 29727	28SrRNA – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Allomegalocotyla johnstoni</i> (C) ^{1,2}	<i>Latris lineata</i>	G	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
MNHN JNC2125 A7		28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Allometabenedeniella</i> sp. (C) ^{1,2}	<i>Platax teira</i>	S	Noumea, New Caledonia	J.-L. Justine
	AHC 29646	28SrRNA – FJ971964 H3 – FJ972080 EF1 α – FJ972026 CYTB – XXXXXXXXX	Amphibdellatidae sp. (Am) ^{1,2}	<i>Narcine tasmaniensis</i>	G	Tasmania	L.A.C. & I.D.W.
	AHC 29704	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Anoplodiscus</i> sp. (An) ^{1,2}	<i>Chrysoblephus gibbiceps</i>	S	Two Oceans Aquarium, South Africa	D. Vaughan
	AHC 29647	28SrRNA – FJ971965 H3 – FJ972081 EF1 α – FJ972027 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Asthenocotyle kaikourensis</i> (M) ^{1,2,3}	<i>Proscymnodon plunketi</i>	Sd	New Zealand	C.J. Healy & E. Burreson
	AHC 29705	28SrRNA – XXXXXXXXX	<i>Asthenocotyle taranakiensis</i> (M) ^{1,2}	<i>Oxynotus bruniensis</i>	S	New Zealand	C.J. Healy

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29648	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971967 H3 – FJ972083 EF1 α – FJ972029	<i>Benedenia acanthopagri</i> (C) ^{1,2,3}	<i>Sparidentex hasta</i>	S	Culture cages, Kuwait	A. Al-Marzouk
AHC 45061	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971968 H3 – FJ9872084	<i>Benedenia anticavaginata</i> (C) ^{1,2}	<i>Acanthopagrus australis</i>	Bpf	Stradbroke Island, QLD	M. Deveney & I.D.W.
AHC 29649	28SrRNA – FJ971969 H3 – FJ972085 EF1 α – FJ972030	<i>Benedenia rohdei</i> (C) ^{1,2,3}	<i>Lutjanus carponotatus</i>	Bm	Heron Island, QLD	T.H. Cribb
AHC 29650	28SrRNA – FJ971970 H3 – FJ972086 EF1 α – FJ972031	<i>Benedenia sciaenae</i> (C) ^{1,2,3}	<i>Argyrosomus japonicus</i>	S	Port Augusta, SA	I. Ernst & C. Chambers
AHC 29651	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971971 H3 – FJ972087 EF1 α – FJ972032	<i>Benedenia sekii</i> (C) ^{1,2,3}	<i>Chrysophrys auratus</i>	F	Pisces Fish Farm, NSW	A. Mooney
AHC 29181	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971972 H3 – FJ972088 EF1 α – FJ972033	<i>Benedenia seriolae</i> (C) ^{1,2,3}	<i>Seriola hippos</i>	S	Greenly Island, SA	K. Hutson
AHC 29652	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971975 H3 – FJ972091 EF1 α – FJ972036 COX3 – XXXXXXXXX	<i>Benedeniella posterocolpa</i> (C) ^{1,2,3}	<i>Rhinoptera bonasus</i>	S	New York Aquarium (originally ex Virginia), USA	A.M. Dove

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29653	CYTB – XXXXXXXXX 28SrRNA – FJ971977 H3 – FJ972093 EF1 α – FJ972038	Calceostomatidae sp. (Cs) ^{1,2}	<i>Eugerres axillaris</i>	G	La Paz, Mexico	L.A.C. & I.D.W.
AHC 29736	EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Calicobenedenia polyprioni</i> (C) ^{2,3}	<i>Polyprion americanus</i>	Cf	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 29654	28SrRNA – FJ971978 H3 – FJ972094 EF1 α – FJ972039 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Calicotyle</i> sp. (Mo) ^{1,2,3}	<i>Aptychotrema rostrata</i>	C	Stradbroke Island, QLD	L.A.C. & I.D.W.
AHC 45376	28SrRNA – XXXXXXXXX	<i>Callorhynchicola multitesticulatus</i> (Ch) _{1,2}	<i>Callorhynchus capensis</i>	G	Two Oceans Aquarium, South Africa	D. Vaughan
AHC 29655	H3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971979 H3 – FJ972095 EF1 α – FJ972040 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Capsala laevis</i> (C) ^{1,2,3}	<i>Tetrapterus audax</i>	G	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 45062	28SrRNA – FJ971980 H3 – FJ972096 EF1 α – FJ972041 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Capsala martinieri</i> (C) ^{1,2,3}	<i>Mola mola</i>	S	Two Oceans Aquarium, South Africa	K. Christison
AHC 29656	28SrRNA – FJ971982 H3 – FJ972098 EF1 α – FJ972043 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Capsala pricei</i> (C) ^{1,2,3}	<i>Tetrapterus audax</i>	S	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 29657	28SrRNA – FJ971983	<i>Capsala</i> sp. 1 (C) ^{1,2}	<i>Tetrapterus belone</i>	S	Mediterranean Sea, Italy	F. Garibaldi

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29658	H3 – FJ972099 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971981	<i>Capsala</i> sp. 2 (C) ^{1,2,3}	<i>Makaira nigricans</i>	S	Port Stephens, NSW	L.A.C. & I.D.W.
MNHN JNC1559 B1	H3 – FJ972097 EF1 α – FJ972042 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971974	Capsalidae sp. 1 (C) ^{1,2,3}	<i>Plectorhinchus chaetodonoides</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 29659	H3 – FJ972090 EF1 α – FJ972035 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971976	Capsalidae sp. 2 (C) ^{1,2,3}	<i>Nemadactylus valenciennesi</i>	Pf	Port MacDonnell, SA	K. Hutson
AHC 29660	H3 – FJ972092 EF1 α – FJ972037 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971973	Capsalidae sp. 3 (C) ^{1,2,3}	<i>Scarus ghobban</i>	S	Heron Island, QLD	T.H. Cribb
None	H3 – FJ972089 EF1 α – FJ972034 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971966	Capsalidae sp. 4 (C) ^{1,2,3}	<i>Paralabrax maculatofasciatus</i>	G & Pt	Bahia Santa Rosaliita, Mexico	M. Carmen
AHC 29661	H3 – FJ972082 EF1 α – FJ972028 CYTB – XXXXXXXXX 28SrRNA – FJ971993	Capsalidae sp. 5 (C) ^{1,2,3}	<i>Triakis megalopterus</i>	S	Ushaka Marine World, Durban, South Africa	K. Christison
	H3 – FJ972109 EF1 α – FJ972052					

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
None	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	Capsalidae sp. 6 (C) ^{1,2}	<i>Lutjanus russellii</i>	Bw	Noumea, New Caledonia	J.-L. Justine
AHC 29707	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX EF1 α – XXXXXXXXX	Capsalidae sp. 7 (C) ^{1,2,3}	<i>Lutjanus vitta</i>	Bm	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29708	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Capsalidae sp. 8 (C) ^{1,2}	<i>Gymnocranius grandoculis</i>	Bm	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 45379	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Capsalidae sp. 9 (C) ^{1,2}	<i>Naso unicornis</i>	Bm	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
None	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	Capsalidae sp. 10 (C) ^{1,2}	<i>Lethrinus miniatus</i>	S	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29711	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Capsalidae sp. 11 (C) ^{1,2}	<i>Lethrinus rubrioperculatus</i>	H	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
None	28SrRNA – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Capsalidae sp. 12 (C) ^{1,2,3}	<i>Cephalopholis urodeta</i>	Cf	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29707	28SrRNA – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Capsalidae sp. 13 (C) ^{1,2}	<i>Nemipterus furcosus</i>	Bm	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 45380	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX EF1 α – XXXXXXXXX	Capsalidae sp. 14 (C) ^{1,2,3}	<i>Cephalopholis urodeta</i>	G	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29709	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	Capsalidae sp. 15 (C) ^{1,2}	<i>Cephalopholis miniata</i>	G	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29715	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX EF1 α – XXXXXXXXX	Capsalidae sp. 16 (C) ^{1,2,3}	<i>Lethrinus miniatus</i>	Bw	Noumea, New Caledonia	J.-L. Justine
None	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX	Capsalidae sp. 17 (C) ^{1,2}	<i>Pristipomoides argyrogrammicus</i>	H	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29710	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	Capsalidae sp. 18 (C) ^{1,2}	<i>Epinephelus maculatus</i>	G	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29716	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	Capsalidae sp. 19 (C) ^{1,2}	<i>Latris lineata</i>	Pf	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 28913-16	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971984 H3 – FJ972100 EF1 α – FJ972044	<i>Capsaloides magnaspinosus</i> (C) ^{1,2,3}	<i>Tetrapterus audax</i>	N	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 45378	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX	<i>Capsaloides</i> sp. 1 (C) ^{1,2,3}	<i>Tetraptures belone</i>	G	Mediterranean	F. Garibaldi

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 45377	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Capsaloides</i> sp. 2 (C) ^{1,2}	<i>Tetrapterus belone</i>	N	Mediterranean	F. Garibaldi
AHC 29662	28SrRNA – FJ971985 H3 – FJ972101 EF1 α – FJ972045	Dactylogyridae sp. (D) ^{1,2}	<i>Nemadactylus valenciennesi</i>	G	Port MacDonnell, SA	K. Hutson
AHC 28777-93	28SrRNA – FJ971984 H3 – FJ972102 EF1 α – FJ972046 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Dendromonocotyle bradsmithi</i> (Mo) ^{1,2,3}	<i>Myliobatis australis</i>	Ds	Outer Harbour, Adelaide, SA	B. Smith, K. Hutson, L.A.C & I.D.W
AHC 45381	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Dendromonocotyle californica</i> (Mo) ^{1,2}	<i>Myliobatis californica</i>	S	New York Aquarium	A. Dove
AHC28577-93	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Dendromonocotyle lasti</i> (Mo) ^{1,2}	<i>Himantura</i> sp.	S	Stradbroke Island, QLD	L.A.C., V. Glennon & I.D.W.
	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX EF1 α – XXXXXXXXX	<i>Dermophthirius maccallumi</i> (M) ^{1,2}	<i>Carcharhinus leucas</i>	S	Ushaka Marine World, Durban, South Africa	D. Vaughan
AHC 29663	28SrRNA – FJ971987 H3 – FJ972103 EF1 α – FJ972047 CYTB – XXXXXXXXX	<i>Dermophthirius penneri</i> (M) ^{1,2,3}	<i>Carcharhinus limbatus</i>	Sd	Northern Gulf of Mexico, Mississippi, USA	S.A. Bullard
AHC 29664	28SrRNA – FJ971988 H3 – FJ972104 EF1 α – FJ972048	<i>Dermophthirius</i> sp. (M) ^{1,2,3}	<i>Rhinobatos typus</i>	Sd	Cairns Marine Aquarium Supply, Cairns, QLD	J. Caira & K. Jensen
AHC 29718	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Dioncus remorae</i> (C) ^{1,2}	<i>Echeneis naucrates</i>	G	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
MNHN JNC1552 A1	28SrRNA – FJ971989	<i>Dioncoseudobenedenia kala</i> (C) ^{1,2,3}	<i>Naso unicornis</i>	G	Noumea, New Caledonia	J.-L. Justine
	H3 – FJ972105 EF1 α – FJ972049 COX3 – XXXXXXXXX CYTB – XXXXXXXXX					
AHC 29719	28SrRNA – XXXXXXXXX	<i>Encotyllabe caballeri</i> (C) ^{1,2}	<i>Lethrinus miniatus</i>	Pt	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
	COX3 – XXXXXXXXX CYTB – XXXXXXXXX					
AHC 29665	28SrRNA – FJ971990	<i>Encotyllabe caranxi</i> (C) ^{1,2}	<i>Pseudocaranx dentex</i>	Pt	Heron Island, QLD	M. Deveney
	H3 – FJ972106					
AHC 29722	28SrRNA – XXXXXXXXX	<i>Encotyllabe chironemi</i> (C) ^{1,2,3}	<i>Nemadactylus macropterus</i>	Pt	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
	H3 – XXXXXXXXX EF1 α – XXXXXXXXX					
AHC 45382	28SrRNA – XXXXXXXXX	<i>Encotyllabe</i> sp. 1 (C) ^{1,2,3}	<i>Lethrinus rubrioperculatus</i>	Pt	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
	H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX					
AHC 29720	28SrRNA – XXXXXXXXX	<i>Encotyllabe</i> sp. 2 (C) ^{1,2,3}	<i>Gymnocranius grandoculis</i>	Pt	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
	H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX					
AHC 29721	28SrRNA – XXXXXXXXX	<i>Encotyllabe</i> sp. 3 (C) ^{1,2,3}	<i>Gymnocranius euanus</i>	Pt	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
	H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX					
AHC 29666	28SrRNA – FJ971992	<i>Entobdella soleae</i> (C) ^{1,2,3}	<i>Solea solea</i>	S	Aquarium, Univ. of East Anglia, UK	G.C. Kearn
	H3 – FJ972108 EF1 α – FJ972051					

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29723	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX EF1 α – XXXXXXXXX	<i>Entobdella squamula</i> (C) ^{1,2,3}	<i>Paralichthys californicus</i>	S	Dana Point, Orange County, CA., U.S.A.	J. Kalman
AHC 45063	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971991 H3 – FJ972107 EF1 α – FJ972050	<i>Entobdella stenolepis</i> (C) ^{1,2,3}	<i>Hippoglossus stenolepis</i>	S	British Columbia, Canada	M. Kent
AHC 29725	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Erpocotyle antarctica</i> (H) ^{1,2}	<i>Mustelus antarcticus</i>	G	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 29730	CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Eurysorchis manteri</i> (Di) ^{1,2}	<i>Hyperoglyphe antarctica</i>	G	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 29732	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Gotocotyla elagatis</i> (Go) ^{1,2}	<i>Elagatis bipinnulata</i>	G	Sir John Young Banks, NSW	K. Hutson
AHC 45064	28SrRNA – FJ971994 H3 – FJ972110 EF1 α – FJ972053	<i>Gyrodactylus derjavini</i> (G) ^{1,2,3}	<i>Oncorhynchus mykiss</i>	S	Experimental culture, Frederiksberg, Denmark	K. Buchmann
AHC 45065	28SrRNA – FJ971995 H3 – FJ972111 EF1 α – FJ972054	<i>Gyrodactylus macracanthus</i> (G) ^{1,2,3}	<i>Misgurnus anguillicaudatus</i>	S	Uni of Melbourne, experimental fish	J. Kearns
AHC 45066	28SrRNA – FJ971996 H3 – FJ972112 EF1 α – FJ972055 COX3 – NC_008815 CYTB – NC_008815	<i>Gyrodactylus salaris</i> (G) ^{1,2,3}	<i>Salmo salar</i>	F	Laerdalselva, Norway	K. Buchmann
AHC 29724	28SrRNA – XXXXXXXXX	<i>Heterocotyle chinensis</i> (Mo) ^{1,2}	<i>Dasyatis fluviatorum</i>	G	Stradbroke Island, QLD	L.A.C. & V. Glennon

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 45067	CYTB – XXXXXXXXX 28SrRNA – FJ971997 H3 – FJ972113	<i>Interniloculus chilensis</i> (C) ^{1,2}	<i>Sebastes capensis</i>	G	Aysén Channels, Chile	M.T. Gonzalez
MNHN JNC2448 A1	28SrRNA – XXXXXXXXX	<i>Lagenivaginopseudobenedenia</i> sp. (C) _{1,2}	<i>Etelis coruscans</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 45068	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ971998	<i>Listrocephalos corona</i> (C) ^{1,2,3}	<i>Dasyatis sabina</i>	S	Mississippi Sound, off Biloxi, Mississippi, USA	S.A. Bullard
AHC 29667	H3 – FJ972114 EF1 α – FJ972056 28SrRNA – FJ971999 H3 – FJ972115	<i>Listrocephalos kearni</i> (C) ^{1,2,3}	<i>Dasyatis brevis</i>	S	La Paz, Mexico	L.A.C. & I.D.W.
AHC 29668	EF1 α – FJ972057 28SrRNA – FJ972000 H3 – FJ972116 EF1 α – FJ972058	<i>Macrophyllida</i> sp. (C) ^{1,2,3}	<i>Hemigaleus microstoma</i>	G	Moreton Bay, QLD	T. Turner
AHC 29669	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972019 H3 – FJ972135 EF1 α – FJ972074	<i>Mediavagina</i> sp. (C) ^{1,2,3}	<i>Nemadactylus valenciennesi</i>	G	Port MacDonnell, SA	K. Hutson
AHC 29670	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972001 H3 – FJ972117 EF1 α – FJ972059	<i>Megalobenedenia helicoleni</i> (C) ^{1,2,3}	<i>Helicolenus percoides</i>	G	Port MacDonnell, SA	K. Hutson
AHC 29712	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Metabenedeniella parva</i> (C) ^{1,2}	<i>Plectorhinchus lineatus</i>	Bm	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29714	CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Metabenedeniella</i> sp. (C) ^{1,2}	<i>Lutjanus argentimaculatus</i>	Pf	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29729	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Microcotyle nemadactylus</i> (Mi) ^{1,2}	<i>Nemadactylus macropterus</i>	G	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 29731	28SrRNA – XXXXXXXXX CYTB – XXXXXXXXX	<i>Microcotyle neozealanicus</i> (Mi) ^{1,2}	<i>Helicolenus percoides</i>	G	Port MacDonnell, SA	K. Hutson, E.M.P. & I.D.W.
AHC 45069	28SrRNA – FJ972002 H3 – FJ972118 EF1 α – FJ972060 CYTB – XXXXXXXXX	<i>Microcotyloides incisa</i> (Mi) ^{1,2,3}	<i>Lutjanus argentiventris</i>	G	La Paz, Mexico	L.A.C. & I.D.W.
AHC 28962-67	28SrRNA – FJ972003 H3 – FJ972119 EF1 α – FJ972061	<i>Nasicola klawei</i> (C) ^{1,2,3}	<i>Thunnus albacares</i>	N	Port Stephens, NSW	L.A.C. & I.D.W.
AHC 29671	28SrRNA – FJ972004 H3 – FJ972120 EF1 α – FJ972062 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Neobenedenia 'girellae'</i> (C) ^{1,2,3}	<i>Verasper variegatus</i>	S	Aquarium, Nippon Suisan Kaisha, Oita, Japan	N. Hirazawa
AHC 29672	28SrRNA – FJ972005 H3 – FJ972121 EF1 α – FJ972063	<i>Neobenedenia 'melleni'</i> (C) ^{1,2,3}	<i>Spherooides annulatus</i>	S	Aquaria, CIAD, Mazatlan, Sinoloa, Mexico	I.D.W.
AHC 29673	28SrRNA – FJ972006 H3 – FJ972122 EF1 α – FJ972064 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Neobenedenia</i> sp. (C) ^{1,2,3}	<i>Seriola rivoliana</i>	S	Aquaculture, Guayaquil, Ecuador	E. Blacio

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
AHC 29674	28SrRNA – FJ972007 H3 – FJ972123 EF1 α – FJ972065 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Neoentobdella australis</i> (C) ^{1,2,3}	<i>Neotrygon kuhlii</i>	Vs	Stradbroke Island, QLD	I.D.W., L.A.C. & V. Glennon
AHC 29675	28SrRNA – FJ972008 H3 – FJ972124	<i>Neoentobdella diadema</i> (C) ^{1,2}	<i>Pteroplatytrygon violacea</i>	S	Mediterranean Sea, off Italy	A. Gustinelli
AHC 29676	28SrRNA – FJ972009 H3 – FJ972125 EF1 α – FJ972066 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Neoentobdella natans</i> (C) ^{1,2,3}	<i>Pastinachus sephen</i>	Vs	Heron Island, QLD	T.H. Cribb
AHC 29555-59	28SrRNA – FJ972010 H3 – FJ972126 EF1 α – FJ972067 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Neoentobdella taiwanensis</i> (C) ^{1,2,3}	<i>Taeniura meyeni</i>	S	Public Aquarium, Taiwan	M.H. Chen
AHC 45070	28SrRNA – FJ972011 H3 – FJ972127 EF1 α – FJ972068 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Nitzschia sturionis</i> (C) ^{1,2,3}	<i>Huso huso</i>	Oc	Caspian Sea near mouth of River Kuma, Dagestan	M.R. Deveney
AHC 29728	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Nitzschia sturionis</i> 2 (C) ^{1,2,3}	<i>Acipenser stellatus</i>	Gr	Ramsar area, Caspian Sea, Iran	S. Tavakol & B. Jalali
AHC 28917	28SrRNA – FJ972012 H3 – FJ972128 EF1 α – FJ972069	<i>Pseudoleptobothrium</i> sp. (M) ^{1,2,3}	<i>Aptychotrema rostrata</i>	Sd	Stradbroke Island, QLD	B.W. Cribb

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
MNHN JNC1557 A1	COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972013	<i>Pseudonitzschia uku</i> (C) ^{1,2,3}	<i>Aprion virescens</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 29713	H3 – FJ972129 EF1 α – FJ972070 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX	<i>Trilobiodiscus lutiani</i> (C) ^{1,2}	<i>Lutjanus argentimaculatus</i>	G	Noumea, New Caledonia	E.M.P., I.D.W. & J.-L. Justine
AHC 29733	H3 – XXXXXXXXX EF1 α – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX	<i>Triloculotrema japonicae</i> (Mo) ^{1,2}	<i>Mustelus antarcticus</i>	N	Port MacDonnell, SA	K. Hutson & E.M.P.
AHC 29734	H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – XXXXXXXXX	<i>Trimusculotrema</i> sp. (C) ^{1,2}	<i>Trygonoptera mucosa</i>	Vs	Kangaroo Island, SA	E.M.P., I.D.W & T. Bertozzi
AHC 45071	H3 – FJ972130 EF1 α – FJ972071 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972014	<i>Tristoma coccineum</i> (C) ^{1,2,3}	<i>Xiphias gladius</i>	G	Mediterranean	S. Mattiucci
AHC 45072	H3 – FJ972131 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972015	<i>Tristoma integrum</i> (C) ^{1,2}	<i>Xiphias gladius</i>	G	Mediterranean	S. Mattiucci
AHC 45073	H3 – FJ972132 COX3 – XXXXXXXXX CYTB – XXXXXXXXX 28SrRNA – FJ972016	<i>Tristoma</i> sp. (C)	<i>Xiphias gladius</i>	G	Azores, Atlantic Ocean	S. Mattiucci

Museum Voucher No.	GenBank No.	Monogenean species	Host species	Site	Locality	Source
MNHN JNC1281 A5	28SrRNA – FJ972020 H3 – FJ972136 EF1 α – FJ972075	Trochopodinae sp. 1 (C) ^{1,2,3}	<i>Pseudobalistes fuscus</i>	G	Noumea, New Caledonia	J.-L. Justine
MNHN JNC1379 B2	28SrRNA – FJ972018 H3 – FJ972134 EF1 α – FJ972073 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Trochopodinae sp. 4 (C) ^{1,2,3}	<i>Epinephelus fuscoguttatus</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 29677	28SrRNA – FJ972021 H3 – FJ972137 EF1 α – FJ972076 COX3 – XXXXXXXXX CYTB – XXXXXXXXX	Trochopodinae sp. 5 (C) ^{1,2,3}	<i>Chelidonichthys capensis</i>	S	Two Oceans Aquarium, Cape Town, South Africa	K. Christison
MNHN JNC1391 B1	28SrRNA – FJ972017 H3 – FJ972133 EF1 α – FJ972072	<i>Trochopus plectropomi</i> (C)* ^{1,2,3}	<i>Plectropomus leopardus</i>	G	Noumea, New Caledonia	J.-L. Justine
AHC 45383	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX COX3 – XXXXXXXXX CYTB – XXXXXXXXX	<i>Troglocephalus rhinobatidis</i> (Mo) ^{1,2}	<i>Rhinobatus typus</i>	G	Stradbroke Island, QLD	I.D.W., L.A.C. & V. Glennon
AHC 45075	28SrRNA – FJ972023 EF1 α – FJ972077	<i>Udonella</i> sp. (U) ^{1,2,3}	<i>Urolophus halleri</i>	Ccs	La Paz, Mexico	L.A.C. & I.D.W.
AHC 29735	28SrRNA – XXXXXXXXX H3 – XXXXXXXXX	<i>Zeuxapta seriolae</i> (He) ^{1,2}	<i>Seriola lalandi</i>	G	Sir John Young Banks, NSW	K. Hutson

Abbreviations used in Appendix V.

Museum

AHC = Australian Helminthological Collection of the South Australian Museum, Adelaide, South Australia, Australia

MNHN = Muséum National d'Histoire Naturelle, Paris, France

Monogenean family

A = Acanthocotylidae

Am = Amphibdellatidae

An = Anoplodiscidae

C = Capsalidae

Ch = Chimaericolidae

Cs = Calceostomatidae

D = Dactylogyridae

Di = Diclidophoridae

G = Gyrodactylidae

Go = Gotocotylidae

H = Hexabothriidae

He = Heteraxinidae

M = Microbothriidae

Mi = Microcotylidae

Mo = Monocotylidae

U = Udonellidae

Collectors

E.M.P. - E.M. Perkins

I.D.W. - I.D. Whittington

L.A.C. - L.A. Chisholm

Site Parasitised

Bm = Branchiostegal membranes

Bpf = Base of pectoral fin

Bw = Body wash
C = Cloaca
Ccs = Copepod carapace ex skin
Cf = Caudal fin
Ds = Dorsal skin
F = Fins
G = Gills
Gr = Gill raker
H = Head
N = Nares
Oc = Oral cavity
Pf = Pectoral fins
Pt = Pharyngeal teeth
S = Skin
Sd = Skin denticles
Vs = Ventral skin

*This species was listed as Trochopodinae sp. 2 in Perkins et al. (2009) and Chapter II
Unless mentioned otherwise taxa cited in Perkins et al. (2009) retain the same nomenclature

¹Nuclear dataset

²Combined dataset (nuclear and mitochondrial)

³EF1 α used in molecular dating

APPENDIX VI

Additional Materials and Methods, Results and Discussion for analyses in Chapter IV.

Table 1. Primers developed in this study for PCR amplification of two mitochondrial genes.

Gene	Primer ID	Sequence (5'-3')	Forward/Reverse
<i>COX3</i>	M1129	CAYGCTAGWTGTTTTTGTAC	F
<i>COX3</i>	M1155	ATGACWTGRYTWCCWRTWTATAAWGC	F
<i>COX3</i>	M1182	TGRTAYTGRCAYTTYGTDG	F
<i>CYTB</i>	M1156	GACATTTGRTGYCAHGGYARDATRTAMC	R
<i>CYTB</i>	M1166	GGWAYHCTYTGWACWACMGAAG	R
<i>CYTB</i>	M1167	GGHARDATRTAVCCYARRAACGCTTCHA CCATCA	R
<i>CYTB</i>	M1189	CTNGAATARTAHARNGCNCGDC	R
<i>CYTB</i>	M1190	TGAYGYCAAGGYAAWATRTAAC	R

MATERIALS AND METHODS

Voucher specimens (most mounted on slides but some are specimens or part specimens stored in 95% AR grade ethanol) of all but five monogenean species are deposited in the Australian Helminthological Collection (AHC) of the South Australian Museum (SAMA), Parasitology Section, North Terrace, Adelaide, 5000, Australia or in the Muséum National d'Histoire Naturelle (MNHN), Paris, France (see Appendix V).

RESULTS

(a) *DNA sequence characteristics*

Sequences from 77 ingroup and 30 outgroup taxa were available. There were no premature stop codons within the protein encoding regions of the sequences. A total alignment length of 1537 characters of sequence was produced from the concatenation of the 3 genes which included: 430 characters *28S rDNA*, 291 characters *H3* and 816 characters *EF1 α* . The five genes, for 78 ingroup taxa and 29 outgroup taxa, were concatenated for a total alignment of 2410 characters of sequence including: 430 characters *28S rDNA*, 291 characters *H3*, 816 characters *EF1 α* , 573 characters *COX3* and 300 characters *CYTb*. This included 104 parsimony informative sites for *28S rDNA*, 141 parsimony informative sites for *H3*, 348 parsimony informative sites for *EF1 α* , 414 parsimony informative sites for *COX3* and 236 parsimony informative sites for *CYTb*. We were unable to obtain sequence data for some taxa for certain genes (see Appendix V) and these taxa were included in analyses as missing data for those genes. The *EF1 α* sequence spanned an intron of variable length (50 to 100 bp), which we excluded from our analyses because it could not be aligned unambiguously due to high variability. Some primer pairs for *28S rDNA* generated larger sequence fragments (approximately 800 bp) but because alignment at the 3' end of this sequence was ambiguous, only approximately 400 bp were included in analyses. Other areas of *28S rDNA* and *EF1 α* sequence, where alignment was also ambiguous, were excluded from analyses reducing the final number of characters used in our analyses to 2153 bp. Indels occurred at 29 sites in the *28S rDNA* sequence data (20 of which occurred only in *Udonella*), 14 sites in the *EF1 α* sequence data and 15 sites in the *COX3* sequence data. Sequencing of some *28S rDNA*, *H3* and *EF1 α* sequences revealed heterozygotes, indicated by

overlapping signals for two kinds of bases in the sequence chromatograms data. These sites were scored with the IUPAC ambiguity codes for dimorphic sites.

(b) Phylogenetic analyses

The preliminary Bayesian analyses and AIC showed that four partitions for nuclear genes (*28S rDNA*, *H3* and *EF1 α* 1st codon position, *H3* and *EF1 α* 2nd codon position, and *H3* and *EF1 α* 3rd codon position) and seven partitions for all five genes (*28S rDNA*, *H3* and *EF1 α* 1st codon position, *H3* and *EF1 α* 2nd codon position, *H3* and *EF1 α* 3rd codon position, *COX3* and *CYTB* 1st codon position, *COX3* and *CYTB* 2nd codon position, *COX3* and *CYTB* 3rd codon position) were optimal for the data.

DISCUSSION

(a) Monophyly of the Capsalidae and sister group

Given the comprehensive taxon sampling (78 capsalid taxa in 28 genera) and the inclusion of taxa from all subfamilies, monophyly for the family has been tested. In analyses by Perkins *et al.* (2009) which included 47 capsalid taxa in 20 genera, a representative from the Dioncinae was unavailable and as this is the subfamily most recently united into the Capsalidae (see Whittington 2004), monophyly could not be tested. One species from the Dioncinae, *Dioncus remorae*, was included in present analyses and the family is monophyletic. The presence of accessory sclerites on the haptor, the perforated bead shape of the spermatid mitochondrion and the progressive disappearance of the microtubules of the zone of differentiation are morphological synapomorphies for the family (Justine & Mattei 1987; Whittington 2004). The absence of accessory sclerites appears to be an independent secondary loss in adults of two taxa, *Pseudonitzschia uku* (Appendix VIII, clade 1D) and *Calicobenedenia polyprioni* (Appendix VIII, clade 2C).

Two groupings have been proposed as the sister group to the Capsalidae and our analyses have been unable to resolve the sister to the family. Morphological phylogenetic analyses of the Monogenea have supported the Loimoidae and Monocotylidae as sister (Boeger & Kritsky 2001) but molecular phylogenetic analyses have typically supported the grouping of Udonellidae and Gyrodactylidae (see Olson & Littlewood 2002). The present analyses have included more monopithocotylean outgroups but the sister group remains unresolved. There are two

groupings that are the most likely sister groups: Gyrodactylidae and Udonellidae or Microbothriidae and Monocotylidae (Appendix VIII). To finally resolve relationships between the Capsalidae and other monogenean families, comprehensive phylogenetic analyses using multiple unlinked nuclear genes and representatives from all monopisthocotylean families are required.

(b) *The subfamily and generic classifications*

Perkins *et al.* (2009) found the Benedeniinae, Entobdellinae and Trochopodinae to be polyphyletic and this is supported further in our extended analyses. Taxa from the Benedeniinae and Trochopodinae are spread more widely throughout the phylogeny with representatives occurring in clades 1 and 2 (Appendix VIII). These large and diverse subfamilies need extensive morphological re-examination, appraisal and taxonomic revision in light of our results. The Entobdellinae remains polyphyletic with the inclusion of more taxa from other subfamilies. *Calicobenedenia polyprioni* is considered a member of the Benedeniinae but in our analyses grouped with entobdellines (Appendix VIII, clade 2C). While *C. polyprioni* (from the perciform *Polyprion americanus*) does not share a similar host association to the other entobdellines (i.e. Chondrichthyes and flatfish teleosts), it does have similar anterior attachment organ morphology (Kritsky & Fennessy 1999; Deveney 2002). With additional taxon representation to Perkins *et al.* (2009), monophyly was tested for the Encotyllabinae and was strongly supported (Appendix VIII, PP 100%, BS 100%). Many relationships from the analyses by Perkins *et al.* (2009) have changed with the addition of taxa but some have remained stable. Relationships between the capsalines remain largely unchanged and together with the Encotyllabinae are strongly monophyletic. Capsaline species have a restricted host range (exclusively pelagic fishes, predominantly Istiophoridae and Scombridae) and encotyllabine species have a restricted site range (predominately parasitise the pharyngeal tooth pads). In contrast species in Benedeniinae and Trochopodinae are polyphyletic and exhibit broad host and site ranges. Correlations between site specificity, host specificity, host associations and morphology warrant further examination as possible constraints on, or drivers of, radiation.

Members of the Dioncinae predominantly parasitise remora hosts, which attach to and are carried by larger fish such as sharks and rays. It has been proposed that, due to this behaviour, remoras may have acted as a vector for transmission of a

parasite from one host species to another (Whittington 2004). For example Whittington (2004) proposed that capsalids from elasmobranch hosts could have been transferred to a diversity of teleost hosts by remoras facilitating the radiation of the family (Whittington 2004) but this hypothesis is not supported by our analyses. Our present phylogenetic hypotheses provide the most detailed examination of the Capsalidae to date and frameworks for future studies to take into account the phylogenetic relationships of the family.

APPENDIX VII

Additional taxa used in molecular clock dating analyses in Chapter IV.

Table 1. Additional taxa used in molecular dating analyses in Chapter IV.

GenBank No.	Species	Taxonomy
AF360167	<i>Arabidopsis thaliana</i>	Viridiplantae
AY582824	<i>Monosiga ovata</i>	Choanoflagellida
AY580191	<i>Ephydatia cooperensis</i>	Demospongiae
DQ087497	<i>Trochospongilla pennsylvanica</i>	Demospongiae
AY580246	<i>Metridium senile</i>	Cnidaria
AY580239	<i>Obelia</i> sp.	Cnidaria
AY580170	<i>Antedon mediterranea</i>	Echinodermata
AY580177	<i>Asterina miniata</i>	Echinodermata
AY580197	<i>Dendraster excentricus</i>	Echinodermata
AY580204	<i>Encope michelini</i>	Echinodermata
AY580218	<i>Eucidaris tribuloides</i>	Echinodermata
AY580285	<i>Strongylocentrotus purpuratus</i>	Echinodermata
AY580280	<i>Saccoglossus kowalevskii</i>	Hemichordata
AY580292	<i>Ptychodera flava</i>	Hemichordata
NM_206593	<i>Drosophila melanogaster</i>	Insecta
AY580211	<i>Enallagma aspersum</i>	Arthropoda
AY580225	<i>Lestes congener</i>	Arthropoda
AY580299	<i>Priapulid caudatus</i>	Priapulida
AY580184	<i>Chaetopterus</i> sp.	Polychaeta
AY580265	<i>Modiolus americanus</i>	Bivalvia
AY580262	<i>Mytilus californianus</i>	Bivalvia
AY580270	<i>Mytilus edulis</i>	Bivalvia
AY580232	<i>Nucula proxima</i>	Bivalvia
AJ250908	<i>Leptoplana tremellaris</i>	„Turbellaria“
AF288068	<i>Microstomum lineare</i>	„Turbellaria“
AY580253	<i>Stylochus</i> sp.	„Turbellaria“
AY519355	<i>Clonorchis sinensis</i>	Trematoda
Y08487	<i>Schistosoma mansoni</i>	Trematoda
AB306938	<i>Echinococcus vogeli</i>	Cestoda
AF288066	<i>Grillotia erinaceus</i>	Cestoda

APPENDIX VIII

A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear and mitochondrial sequence data for 78 capsalid taxa and 29 outgroup taxa in Chapter IV.

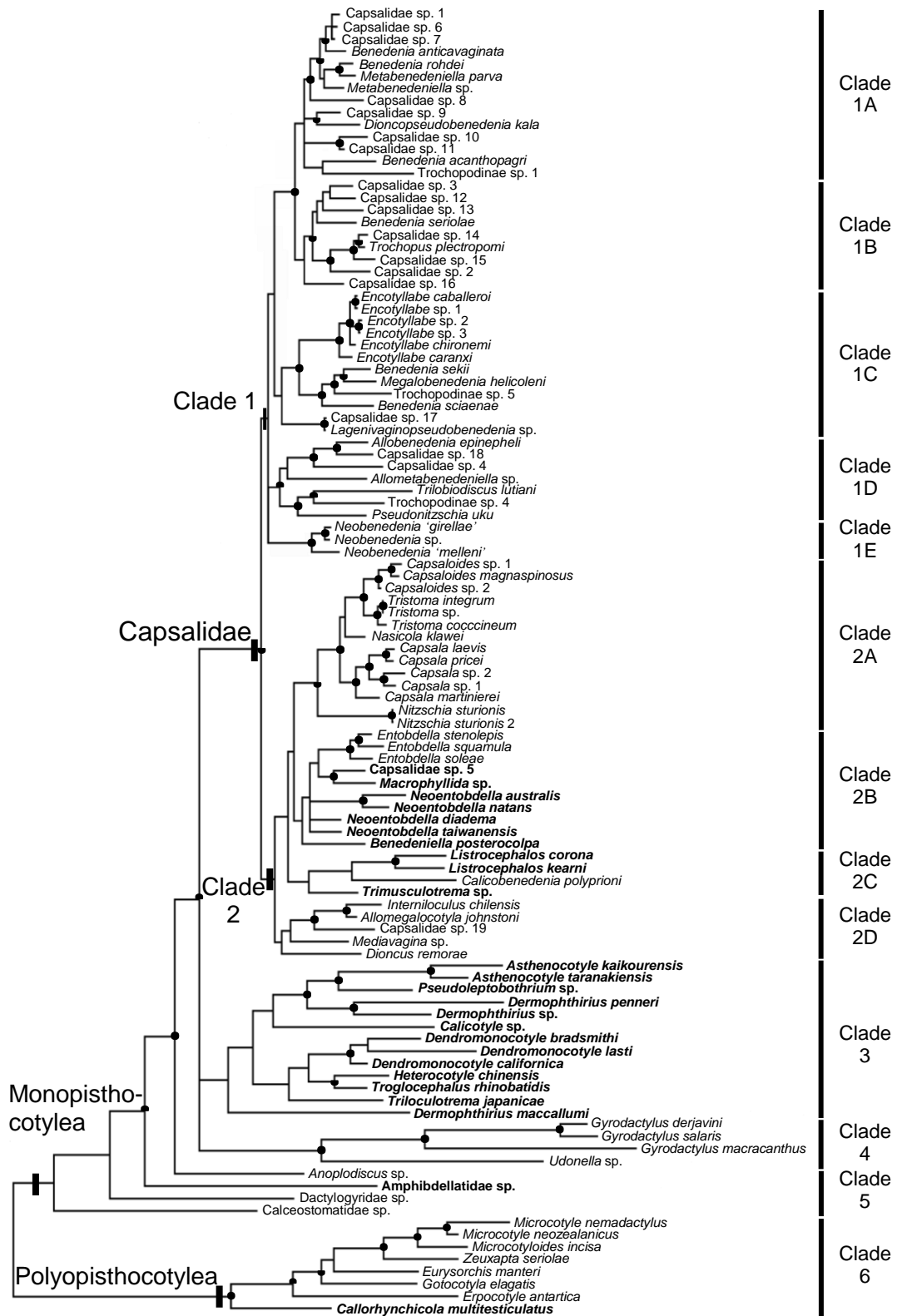


Figure 1. A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear and mitochondrial sequence data for 78 capsalid taxa and 29 outgroup taxa representing 14 monogenean families and 2 subclasses. Half circles above nodes indicate posterior probabilities above 90% and half circles below nodes indicate Maximum Likelihood bootstrap proportions above 70%. Complete circles indicate nodes with posterior probabilities above 90% and Maximum Likelihood bootstrap proportions above 70%. Taxa in bold parasitise elasmobranch hosts. See Appendix V for outgroup families.

APPENDIX IX

A 50% majority rule consensus tree produced from Bayesian inferences analyses of the combined nuclear sequence data for 77 capsalid taxa and 30 outgroup taxa in Chapter IV.

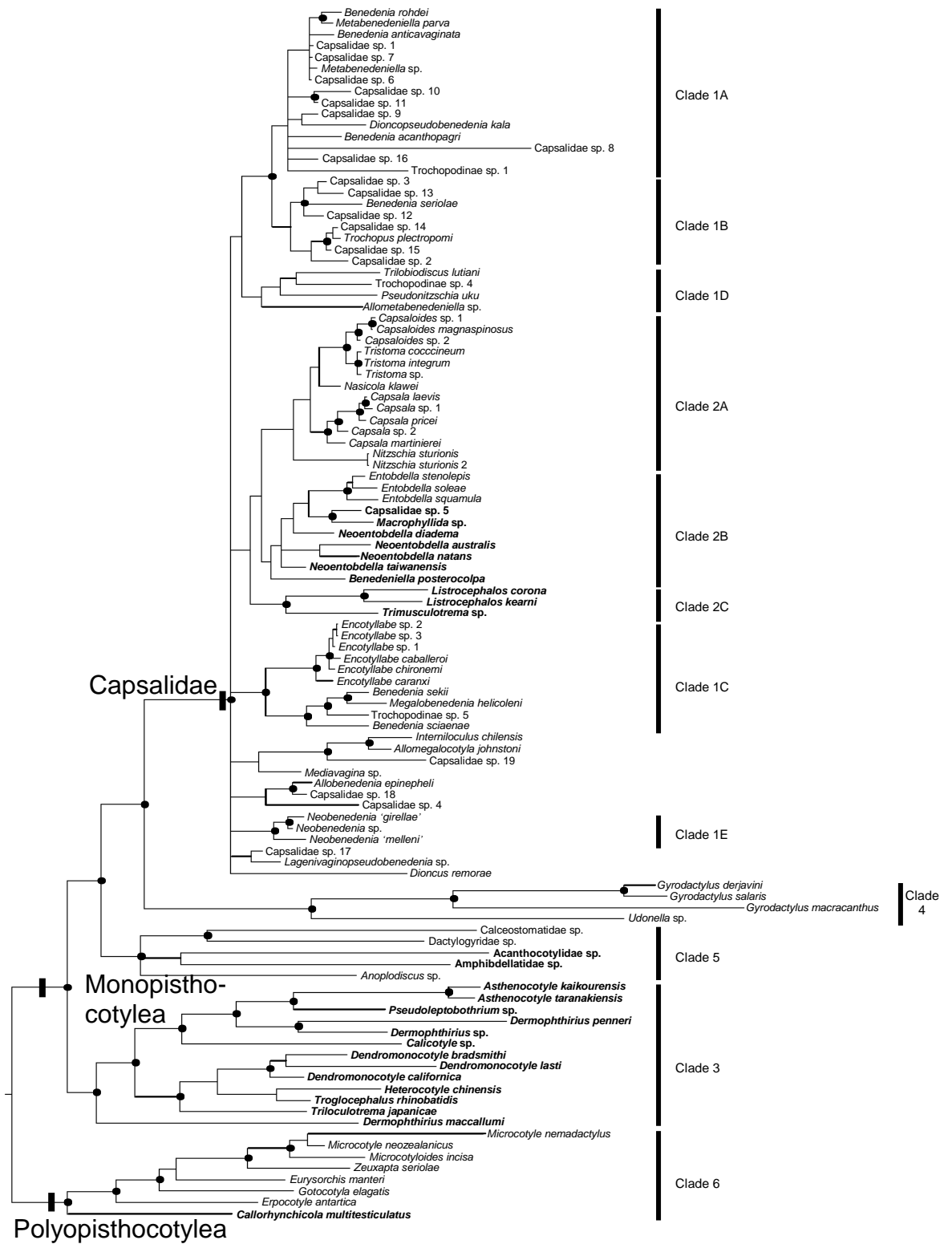


Figure 1. A 50% majority rule consensus tree produced from Bayesian inference analyses of the combined nuclear sequence data for 77 capsalid taxa and 30 outgroup taxa representing 14 monogenean families and 2 subclasses. Posterior probabilities above 90% are indicated by circles at those nodes. Taxa in bold parasitise elasmobranch hosts. See Appendix V for outgroup families.

APPENDIX X

The maximum likelihood tree derived from analyses of the combined nuclear and mitochondrial sequence data for the Capsalidae in Chapter IV.

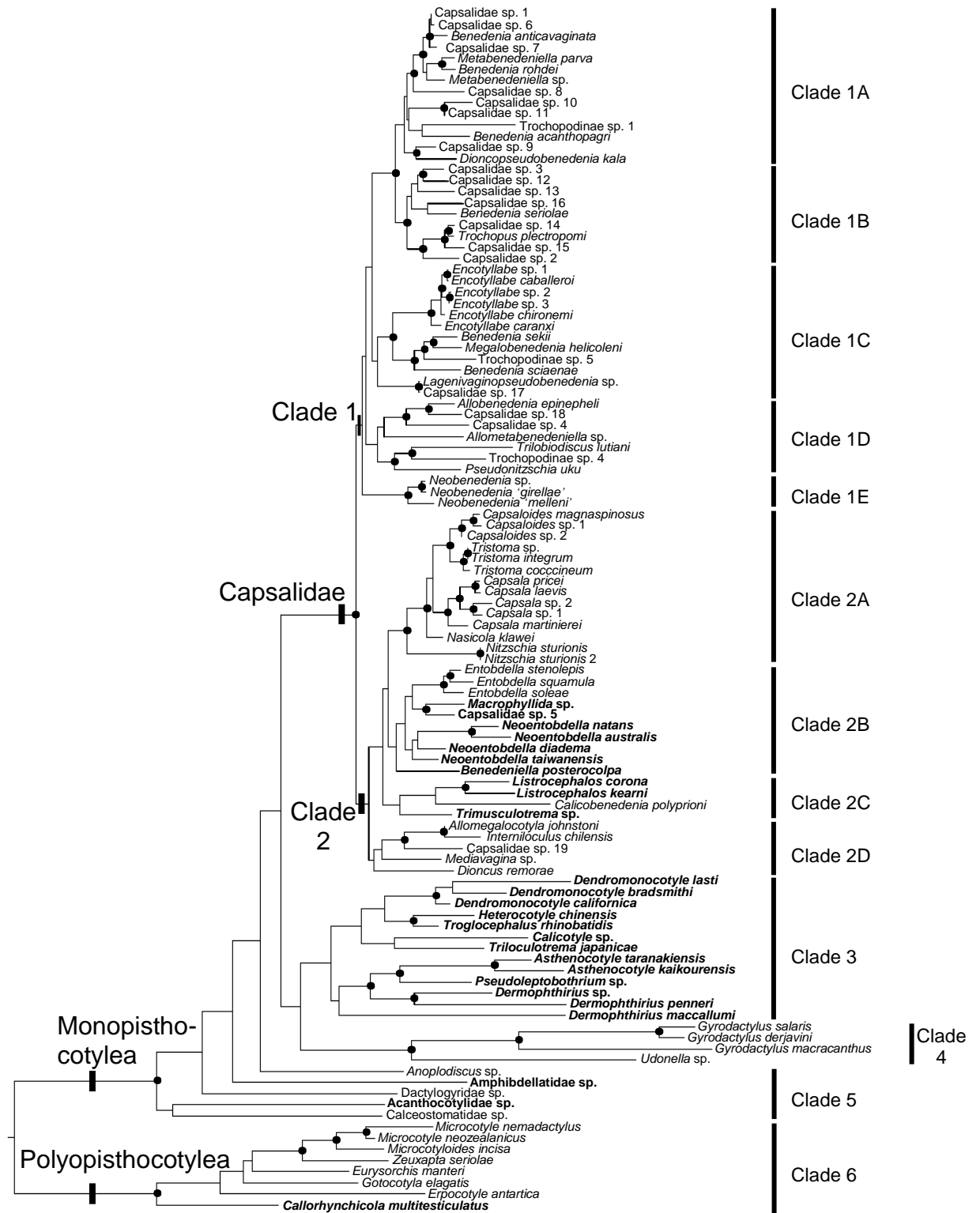


Figure 1. The maximum likelihood tree derived from analysis of the combined nuclear and mitochondrial sequence data for the Capsalidae. Non-parametric bootstrap support proportions above 70% are indicated by circles at those nodes. Log likelihood score -55078.47. Taxa in bold parasitise elasmobranch hosts. See Appendix V for outgroup families.

APPENDIX XI

GenBank numbers for sequences of fish taxa used in analyses in Chapter V.

Appendix XI. GenBank numbers for sequences used in analyses in Chapter V

	<i>COX1</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
<i>Acanthopagrus australis</i> ¹	DQ107856	AM265581	DQ533360	EU638155		XXXXXX ²			EU090914	EU638265
<i>Acipenser stellatus</i> ¹	NC_005795	NC_005795				NC_005795	AF369056	AF369073	AF137206	
Adrianchthyidae	NC_004387	NC_004387	AY655594	DQ168094		NC_004387	EF095641		AB001605	
Albulidae	NC_005800	NC_005796				NC_005800	AY430202			
Alepocephalidae	NC_011012	NC_011012				NC_011012			EU637933	
Alestiidae	NC_004699	NC_004699				NC_004699	DQ912097	AY804099		
Alopiidae	EU398646	L08031				XXXXXX ²	AF135482			
Amiiformes	NC_004742	NC_004742				NC_004742	AY430199	AF369079	AF137208	
Anguillidae	NC_006531	NC_006531				NC_006531			AJ249202	
Anomalopidae	NC_008128	NC_008128	DQ533450	DQ168101	AY362268	NC_008128	AY430223		AY141264	EU638242
Anura	NC_006839	NC_006839	X03104	NM_001089168		NC_006839	EF535914	NM_00109790 0	NM_00108704 8	
Aphredoderidae	NC_004372	NC_004372	DQ028082	DQ168038		NC_004372			DQ021403	
Aplocheilidae	NC_011176	NC_011176	DQ533424			NC_011176				
Apteronotidae	NC_004692	NC_004692				NC_004692	DQ492427	DQ492315		
<i>Aptychotrema rostrata</i> ¹						EU795620				
<i>Argyrosomus japonicus</i> ¹	EU523923	AY225662	DQ533357	EU638107	EU638030	DQ357206	EF095660	DQ874789	EU637942	EU638172
Ateleopodiformes	NC_003179	NC_003179				NC_003179	EU366725			
Atherinidae	NC_004386	NC_004386	AY655579	EU638137	EU638067	NC_004386	AY430225		EU637977	
Aulopidae	NC_002674	NC_002674	DQ028077	DQ168042		NC_002674	EU366705	DQ874791	EF439259	
Balitoridae	NC_001727	NC_001727	BC165500	X80802		NC_001727	EU711107	DQ366997	EU409636	
Batrachoidiformes	NC_006920	NC_006920	DQ533332	DQ168069	AY362246	NC_006920			DQ874822	EU638205
Belonidae	NC_011180	NC_011180	DQ533351	DQ168044	AY362273	NC_011180	AY308771	AY693520	EU036529	EU638184
Berycidae	NC_003188	NC_003188	DQ028088	DQ168045	AY362238	NC_003188	EF095636		DQ197831	EU638174
Blenniidae	NC_004412	NC_004412	AY539279	DQ168097	AY362255	NC_004412			AY141271	EU638237
Bythitidae	NC_004375	NC_004375		EU638130	EU638035	NC_004375			EU637947	
<i>Callorhynchus capensis</i> ¹	DQ108141					XXXXXX ²			EF565167	
Caproidae	NC_003191	NC_003191	DQ533343	DQ168048	AY362233	NC_003191	AY308785		AY141262	EU638178
Carapidae	NC_004373	NC_004373	AY539176		EU638058	XXXXXX ²	AY308782		DQ021402	
<i>Carcharhinus leucas</i> ¹	EF609311	L08032				XXXXXX ²	U62645		Y17586	
<i>Carcharhinus limbatus</i> ¹	DQ884979					XXXXXX ²	AY462152			
<i>Cephalopholis miniata</i> ¹	DQ107925	EF213724				XXXXXX ²			EF456040	

	<i>COX1</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
<i>Cephalopholis urodeta</i> ¹		AY786426			EU638036	XXXXXX ²				
Ceratodontiformes	NC_003127	NC_003127				NC_003127		AY442929	EF526298	
Cetomimidae	NC_003185	NC_003185				NC_003185				
Chanidae	NC_004693	NC_004693				NC_004693	AY430207		AF148142	
Characidae	NC_004700	NC_004700				NC_004700	AY430212	AY804053	EU409633	
Chaunacidae	NC_004381	NC_004381	DQ533350			NC_004381				
<i>Chelidonichthys capensis</i> ¹	DQ108041	AB326975	AY539210	DQ168053		XXXXXX ²			EF439099	EU638186
Chimaeridae	NC_003136	NC_003136				NC_003136				
Chirocentridae	NC_006913	NC_006913				NC_006913	DQ912127	DQ912153	AY158051	
Chlorophthalmidae	NC_003160	NC_003160	DQ028078		AY362219	NC_003160				
<i>Chrysoblephus gibbiceps</i> ¹		AF240719								
<i>Chrysophrys auratus</i> ¹		EF439217				AB124801	EU182627		DQ197878	
Clupeidae	NC_009575	NC_009575				NC_009575	DQ912117	DQ912151	EU224142	
Coelacanthiformes	NC_006921	NC_006921				NC_006921	AF369069	AF369087	AF131256	
Cottidae	NC_004404	NC_004404	AY539227	DQ168121	AY362217	NC_004404			AJ430489	EU638167
Cyclopteridae	NC_008129	NC_008129	AY539251	EU638116	AY362218	NC_008129			EU492176	
Dactylopteridae	NC_003194	NC_003194	DQ533358	DQ168059	AY362243	NC_003194			AY141282	
<i>Dasyatis brevis</i> ¹						XXXXXX ²	ABTC89931			
<i>Dasyatis fluviorum</i> ¹						XXXXXX ²	XXXXXX ²			
Diplophidae	NC_002647	NC_002647				NC_002647	EU366724			
<i>Echeneis naucrates</i> ¹		AY050763	DQ533366	DQ168062	AY362245	XXXXXX ²			AY141315	EU638197
<i>Elagatis bipinnulata</i> ¹	EF609345	AY050734			AY362263	XXXXXX ²	XXXXXX ²			EU638187
Eleotridae	NC_004415	NC_004415	DQ533447			NC_004415				
Elopidae	NC_005803	NC005803				NC005803				
Emmelichthyidae	EF609348	EF456004	DQ533371			NC_004407	EU182623		EF456054	
<i>Epinephelus coioides</i> ¹	DQ107873	DQ354156	AY539258	DQ168064	EU638049	EU043376		AY279869	FJ426122	EU638201
<i>Epinephelus fuscoguttatus</i> ¹	EU600140	DQ372725				EU043377				
<i>Epinephelus maculatus</i> ¹						FJ472837				
Esocidae	NC_004593	NC_004593				NC_004593	AY380541		AY158044	
<i>Etelis coruscans</i> ¹		U26961				XXXXXX ²	XXXXXX ²			
<i>Eugerres axillaris</i> ¹	AY541643		DQ533372			XXXXXX ²	EF095665		EF095623	
Eurypharyngidae	NC_005299	NC_005299				NC_005299				
Gadidae	NC_002081	NC_002081	DQ028083	DQ168066	EU638050	NC_002081	AF369064		EU492245	
Galaxiidae	NC_004594	NC_004594				NC_004594	AY430218		AY158043	

	<i>COX1</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
Gasterosteidae	NC_003174	NC_003174	DQ028089		EU638052	NC_003174	EF033039		EU637962	EU638264
Gobiesocidae	NC_008130	NC_008130	DQ533377		EU638031	NC_008130	XXXXXX ²		EU637943	
Gonorynchidae	NC_011018	NC_011018				NC_011018	EU409606		EU409632	
Gonostomatidae	NC_002574	NC_002574				NC_002574	AY438703		AY141256	
<i>Gymnocranius euanus</i> ¹						XXXXXX ²				
<i>Gymnocranius grandoculis</i> ¹		AF381275				XXXXXX ²				
<i>Helicolenus percoides</i> ¹	EF609370	NC_003195	AY539184	EU638146	EU638081	NC_003195	XXXXXX ²	DQ874787	DQ197858	EU638257
<i>Hemigaleus microstoma</i> ¹	EF609372	DQ422075				DQ422044				
Heterodontiformes	NC_003137	NC_003137				NC_003137	AY949030			
Hexanchidae	EU398837	M91186				XXXXXX ²				
<i>Himantura</i> sp. ¹	EU398859	AB021498				XXXXXX ²	ABTC79232			
Hiodontiformes	NC_005145	NC_005145				NC_005145	AY430200	AY504841		
<i>Hippoglossus stenolepis</i> ¹	NC_009710	NC_009710	DQ533442			NC_009710	AF369067	FJ870481	AF156264	
<i>Huso huso</i> ¹	NC_005252	NC_005252				NC_005252				
<i>Hyperoglyphe antartica</i> ¹	DQ107611	AB205458	DQ533381	EU638151	AY362269	XXXXXX ²	EF095677		EF439348	EU638248
Hypoptychidae	NC_004400	NC_004400	AY539179			NC_004400	AB445176			
Indostomidae	NC_004401	NC_004401	DQ533383		EU638057	NC_004401			EU637967	EU638209
Labridae	AP006018	AP006018	AY662886	EU638164	AY362222	AP006018	AY208617	AY279908	EF439121	EU638211
Lampridae	NC_003165	NC_003165		DQ168077		NC_003165	AY308764		DQ197861	
<i>Latris lineata</i> ¹	AF156246	AF156235				XXXXXX ²	XXXXXX ²			
Lepidosirenidae	NC_003342	NC_003342				NC_003342	AF369058	AY442930	AF137207	
Lepisosteiformes_1	NC_008104	NC_008104				NC_008104		AF369077		
Lepisosteiformes_2	NC_004744	NC_004744				NC_004744				
<i>Lethrinus miniatus</i> ¹	EF609388	AF381266								
<i>Lethrinus rubrioperculatus</i> ¹		AF240752	DQ533393							
Lophiidae	NC_004383	NC_004383	DQ028085	DQ168037	AY362215	NC_004383	AY308786	FJ219616	AY368324	EU638217
<i>Lutjanus argentimaculatus</i> ¹	DQ885025	DQ900672	DQ533398	EU638134	EU638064	AY600305	EU627659	EU627658	EF095620	EU638219
<i>Lutjanus argentiventris</i> ¹						XXXXXX ²		EU627674		
<i>Lutjanus carponotatus</i> ¹	EF609395	DQ784754				XXXXXX ²				
<i>Lutjanus russellii</i> ¹	EF609400	DQ784761				EF514208	EU627667			
<i>Lutjanus vitta</i> ¹	EF609402	DQ900677					EU627669	EU556712		
<i>Makaira nigricans</i> ¹		EF392595				DQ872418		DQ874778	DQ874810	
Mammalia	FJ979865	FJ979865	X57128	M22453	NM_0045	FJ979865	NM_000448	NM_000536	NM_000539	NM020914

	<i>COX1</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
Mastacembelidae	NC_003193	NC_003193	DQ533401	DQ168084	AY362249	NC_003193			AY141275	
Megalopidae	NC_005804	NC_005804				NC_005804	AY430204		AY158050	
Melanotaeniidae	NC_004385	NC_004385	AY655547		AY362271	NC_004385	EF095640		AY141267	
Merlucciidae	EF609408	DQ197962	DQ533406	DQ168124	EU638068	NC_004377	AY308787		EF427485	EU638227
<i>Misgurnus anguillicaudatus</i> ¹	NC_011209	NC_011209				NC_011209	EU711122	AY804103	EU409642	
Mitsukurinidae	NC_011825	NC_011825				NC_011825	AF135477			
<i>Mola mola</i> ¹	NC_005836	NC_005836	DQ533408	DQ168087	AY362251	NC_005836	AY700329		AF137215	EU638225
Moronidae	AY662754	AF240746	AY539255	EU638140	EU638072		AY308767		EU637981	EU638228
Mugiliformes	NC_003182	NC_003182	AY655593	DQ168082	AY362248	NC_003182	EF095639		Y18668	
Muraenidae	NC_004417	NC_004417				NC_004417	AY430203		AY862118	
<i>Mustelus antarcticus</i> ¹	DQ108315	NC_000890				NC_000890	AY462188			
Myctophidae	NC_003164	NC_003164	DQ533395		AY362201	NC_003163	AY430221		AY141258	
<i>Myliobatis australis</i> ¹						XXXXXX ²	ABTC82333			
<i>Myliobatis californica</i> ¹						XXXXXX ²	FJ235624			
Myxiniiformes_1	NC_002807	NC_002807				NC_002807				
Myxiniiformes_2	NC_002639	NC_002639				NC_002639				
<i>Narcine tasmaniensis</i> ¹	EU398929							AF369084		
<i>Naso unicornis</i> ¹	NC_009853	AY264660	DQ533412	EU638141	EU638074	NC_009853	EF530093		EU637984	EU638190
<i>Nemadactylus macropterus</i> ¹	AF156243	AF067091	AY539269	EU638142	EU638075	XXXXXX ²			EU637985	EU638231
<i>Nemadactylus valenciennesi</i> ¹		AF067089				XXXXXX ²				
<i>Nemipterus furcosus</i> ¹	EF609413	AB264298	DQ533430			XXXXXX ²				
Neoscopelidae	NC_003180	NC_003180	DQ533413			NC_003180	EU366727			
<i>Neotrygon kuhlii</i> ¹	EU398745	EU870496				XXXXXX ²	ABTC79231			
Notacanthoidei	NC_005144	NC_005144				NC_005144				
Notopteroidei	EF609328	AF201602				XXXXXX ²	AF369063	AF201653		
<i>Oncorhynchus mykiss</i> ¹	NC_001717	NC_001717	X01064			NC_001717	U15663	U31670	AF425074	
Orectolobiformes	EU398934	EU363745				XXXXXX ²	AY949032			
Osteoglossidae	NC_010570	NC_010570				NC_010570	AY430201	AY504838	AF137210	
<i>Paralabrax maculatofasciatus</i> ¹			DQ533427							
<i>Paralichthys californicus</i> ¹	NC_002386	NC_002386	DQ533428	DQ168119	AY362262			DQ874762	DQ874794	EU638266
<i>Pastinachus sephen</i> ¹	DQ108159	AB021505				XXXXXX ²	ABTC82862			
Percopsidae	NC_003168	NC_003168				NC_003168	AY308766			
Petromyzontiformes	NC_001626	NC_001626				NC_001626			AB116382	
Pholidae	NC_004410	NC_004410		DQ168100	AY362285	NC_004410			AY141298	EU638241

	<i>COX1</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
<i>Platax teira</i> ¹	DQ107756		DQ533348				EF530083	AY279871		
<i>Plectorhinchus chaetodonoides</i> ¹		DQ197979	AY539266		AY362230		EF095661	AY279872	DQ197881	EU638246
<i>Plectropomus leopardus</i> ¹	DQ107921	AY963556			EU638078	DQ101270	XXXXXX ²			
Poeciliidae	NC_009125	NC_009125	DQ533385	DQ168102	AY362203	NC_009125	EF017411		AY296735	EU638243
Polymixiiformes_1	NC_002648	NC_002648	DQ533436	DQ168104	AY362208	NC_002648	AY308765		AY368320	
Polymixiiformes_2	NC_003181	NC_003181	AY539175			NC_003181				
Polyodontidae							AF369057	AF369075	AF369050	
<i>Polyprion americanus</i> ¹	DQ107914	EF392605	DQ533437			XXXXXX ²	XXXXXX ²		EF427493	
Polypteriformes	NC_001778	NC_001778				NC_001778	AF369055	AF369072		
Pristidae	EU398986	D50024								
Pristiophoriformes	DQ108204	D50025				XXXXXX ²				
<i>Pristipomoides argyrogrammicus</i> ¹	DQ885044		DQ533440			XXXXXX ²	XXXXXX ²			
<i>Proscymnodon plunketi</i> ¹	DQ108219	EF090945				XXXXXX ²				
<i>Pseudobalistes fuscus</i> ¹	NC_011939	NC_011939	DQ533342	DQ168095		NC_011939	AY700314	DQ874786	EU108856	EU638233
<i>Pseudocaranx dentex</i> ¹	EF609442	EF392607	DQ533344			XXXXXX ²	EU477492		DQ197887	
<i>Pteroplatytrygon violacea</i> ¹						XXXXXX ²	XXXXXX ²			
Ptotopteridae	NC_001708	NC_001708				NC_001708	AF369070	AF369086	AF369054	
Rajidae	NC_00893	NC_000893				NC_000893			U81514	
Regalecidae	NC_009948	NC_009948	DQ533448	DQ168109	AY362266	NC_009948	EF107625		AY368328	EU638252
<i>Rhinobatos typus</i> ¹	EU398997	D50023				EU282427	ABTC79227			
<i>Rhinoptera bonasus</i> ¹	DQ108133					XXXXXX ²	AY949029			
Rhyacichthyidae	NC_004414	AB021257				NC_004414				
Saccopharyngidae	NC_005298	NC_005298				NC_005298				
<i>Salmo salar</i> ¹	NC_001960	NC_001960	BT043630			NC_001960	XXXXXX ²		AY214132	
<i>Scarus ghobban</i> ¹	NC_011599	NC_011599	AY662883	DQ168112	AY362212	NC_011599	EF095675	AY279952	EF095633	EU638254
<i>Sebastes capensis</i> ¹	NC_005450	DQ678420	AY539185	DQ168114		NC_005450	EF095642	DQ678722	EF212410	EU638258
<i>Seriola hippos</i> ¹	EF609459	EF439585				XXXXXX ²				
<i>Seriola lalandi</i> ¹	EF609460	AB269267				XXXXXX ²			EF439319	
<i>Seriola rivoliana</i> ¹		EF439235				XXXXXX ²			EF427513	
Siluriformes	NC_004697	NC_004697				NC_004697	DQ492458	DQ492389	AF028016	
<i>Solea solea</i> ¹	NC_008327	DQ198003		DQ168117	AY362275		EF095644		EU638009	EU638223
<i>Sparidentex hasta</i> ¹		AF240734								

	<i>COXI</i>	<i>CYTB</i>	<i>H3</i>	<i>IRBP</i>	<i>MLL</i>	<i>ND4</i>	<i>RAG1</i>	<i>RAG2</i>	<i>Rhod</i>	<i>RNF213</i>
<i>Sphoeroides annulatus</i> ¹	NC_010960	NC_010960		DQ168076	AY362221	NC_010960	AY308795		EF427517	EU638212
Squalidae	NC_002012	NC_002012	AF112370			NC_002012				
Squatiniformes	DQ108193	D50020				XXXXXX ²				
Stephanoberyciformes_2	NC_012046	NC_012046	DQ028087	DQ168041	EU638087	NC_012046			AY368327	
Sternopygidae	NC_004701	NC_004701				NC_004701	DQ492426	DQ492314		
Synbranchidae	NC_003192	NC_003192	DQ533410	DQ168088	AY362252	NC_003192			AY141276	EU638226
Syngnathidae	NC_010272	NC_010272		EU638126	AY362216	NC_010272	FJ905776		AY368330	EU638232
<i>Taeniura meyeni</i> ¹		AF110630				XXXXXX ²	XXXXXX ²			
<i>Tetrapterus audax</i> ¹	DQ107617	DQ882017				DQ872421			DQ080346	
<i>Tetrapterus belone</i> ¹		DQ882010				DQ872422				
<i>Thunnus albacares</i> ¹	DQ835945	NC_005317		DQ168113		NC_005317		DQ874771	DQ874803	
<i>Triakis megalopterus</i> ¹		DQ422089				DQ422058	AY462191			
<i>Trygonoptera mucosa</i> ¹	EU399061					EU795632				
Umbridae	NC_004592	NC_004592				NC_004592	AY380549		AY158047	
<i>Urobatis halleri</i> ¹		AF110624				XXXXXX ²				
<i>Urolophus cruciatus</i> ¹	DQ108142	U27265				XXXXXX ²	ABTC69572			
<i>Verasper variegatus</i> ¹	NC_007939	NC_007939				NC_007939				
<i>Xiphias gladius</i> ¹	DQ107623	EU224088	DQ533480	EU638163	EU638098		XXXXXX ²	DQ874779	DQ874811	EU638276
Zeidae	NC_003190	NC_003190	DQ028086	DQ168128	AY362287	NC_003190	AY308778		Y14484	EU638279
Zeniontidae	NC_004397	NC_004397		EU638124	EU638054	NC_004397				
Zoarcidae	NC_004409	NC_004409	AY539271	EU638136	EU638066	NC_004409			EU491979	

¹Fish species that host capsalid parasites studied

²Sequenced in this study

COXI – Cytochrome Oxidase 1

CYTB – Cytochrome Oxidase B

H3 – Histone 3

IRBP – Interphotoreceptor retinoid-binding Protein

MLL – Mixed lineage leukemia

ND4 – NADH dehydrogenase subunit 4

RAG1 – Recombination Activating Gene 1

RAG2 – Recombination Activating Gene 2

Rhod – Rhodopsin

RNF213 – anonymous nuclear protein coding gene

APPENDIX XII

Table of fossil calibrations, cladogram of phylogenetic relationships presented in Nelson (2006) and justification for constraints used in molecular phylogenetic analyses of fish data in Chapter V.

Table 1. Fossil Calibrations dates for molecular dating analyses of *RAG-1* fish data

Node	Constraint (mya)	
	Upper	Lower
1		L418 ²
2		L183 ²
3		L167 ²
4		L155 ²
5		L151 ²
6		L125 ²
7	U350	L330 ²
8	U392	L345 ¹
9		L284 ¹
10		L151 ¹
11		L130 ¹
12		L136 ¹
13		L146 ¹
14		L112 ¹
15		L90 ¹
16		L57 ¹
17		L50 ¹
18		L74 ¹
19		L94 ¹
20		L98 ¹

¹See Table 2 of Azuma et al. 2008.

²See Appendix of Underwood et al. 2006.

Justification for constraints used in molecular phylogenetic analyses of the fish data:

Cyclostome is the term used for the living jawless fishes (hagfishes and lampreys) however many paleontologists and morphologists consider this to be a paraphyletic group (Lovtrup 1977; Janvier 1996b; Gursoy et al. 2000). However, molecular evidence has supported monophyly (Kuraku et al. 1999; Mallatt et al. 2001; Delarbre et al. 2002; Takezaki et al. 2003). The purpose of this study was not to explore this issue and so our constraint supports parphyly as presented in Nelson (2006). Further information on this debate can be found in Janvier (2008).

Characters supporting monophyly of the Chondrichthyes are numerous (Jamieson 1991; Didier 1995; Janvier 1996b; Maisey 2001) with two key synapomorphies being the prismatic endoskeletal calcification and pelvic claspers (Grogan and Lund 2004). The Batoidea has traditionally been considered a derived

crown group of the Selachii (de Carvalho 1996; McEachran et al. 1996; Shirai 1996). However, recent molecular phylogenies differ placing the origins of the Batoidea from stem neoselachians and having no close relationship with the Selachii (Douady et al. 2003; Maisey et al. 2004; McEachran and Aschliman 2004; Winchell et al. 2004) and it has been concluded that this fits more closely the known neoselachian fossil record than do morphological phylogenies. Monophyly of the two subdivisions is also considered well established (Nelson 2006). Therefore, we constrain the Batoidea and Selachii to be sister groups and each monophyletic.

We have used the term Sarcopterygii to describe a monophyletic group that includes the tetrapods and have constrained it to be monophyletic and sister to the Actinopterygii following Wiley (1979), Rosen et al. (1981), Nelson (1994), Cloutier and Ahlberg (2001) and Nelson (2006). There has been extensive examination of this group in Long (1995), Janvier (1996b), Maisey (1996), Schultze and Cloutier (1996), Ahlberg (2001) and Clack (2002). While not diagnosed by a strong set of derived characters, the Actinopterygii is still considered to be monophyletic. Diversification of this group has been reviewed by Lauder and Liem (1983) and also revised by Cloutier and Arratia (2004). A monograph with anatomical drawings with an emphasis on the Actinopterygii is provided by Springer and Johnson (2004). As such, we constrain the Actinopterygii to be monophyletic. The Acipenseriformes and Polypteriformes are constrained as sister to the neopterygii based on work by Venkatesh et al. (2001) and recent support from Britz and Bartsch (2003) and Nelson (2006). There is general agreement that the Neopterygian fishes are monophyletic but much uncertainty remains on relationships with the group (Grande and Bemis 1998; Arratia 2004; Nelson 2006) and so we have constrained monophyly in our analyses. The Teleostei is also considered to be a monophyletic group but there is disagreement over its boundaries. de Pinna (1996) described 27 anatomical synapomorphies to support monophyly of the group to the exclusion of the Amiiiformes and Lepisosteiformes. Summaries of recent works are available in Arratia (1997, 1999, 2004). We have therefore constrained the Teleostei to be monophyletic to the exclusion of the Amiiiformes and Lepisosteiformes.

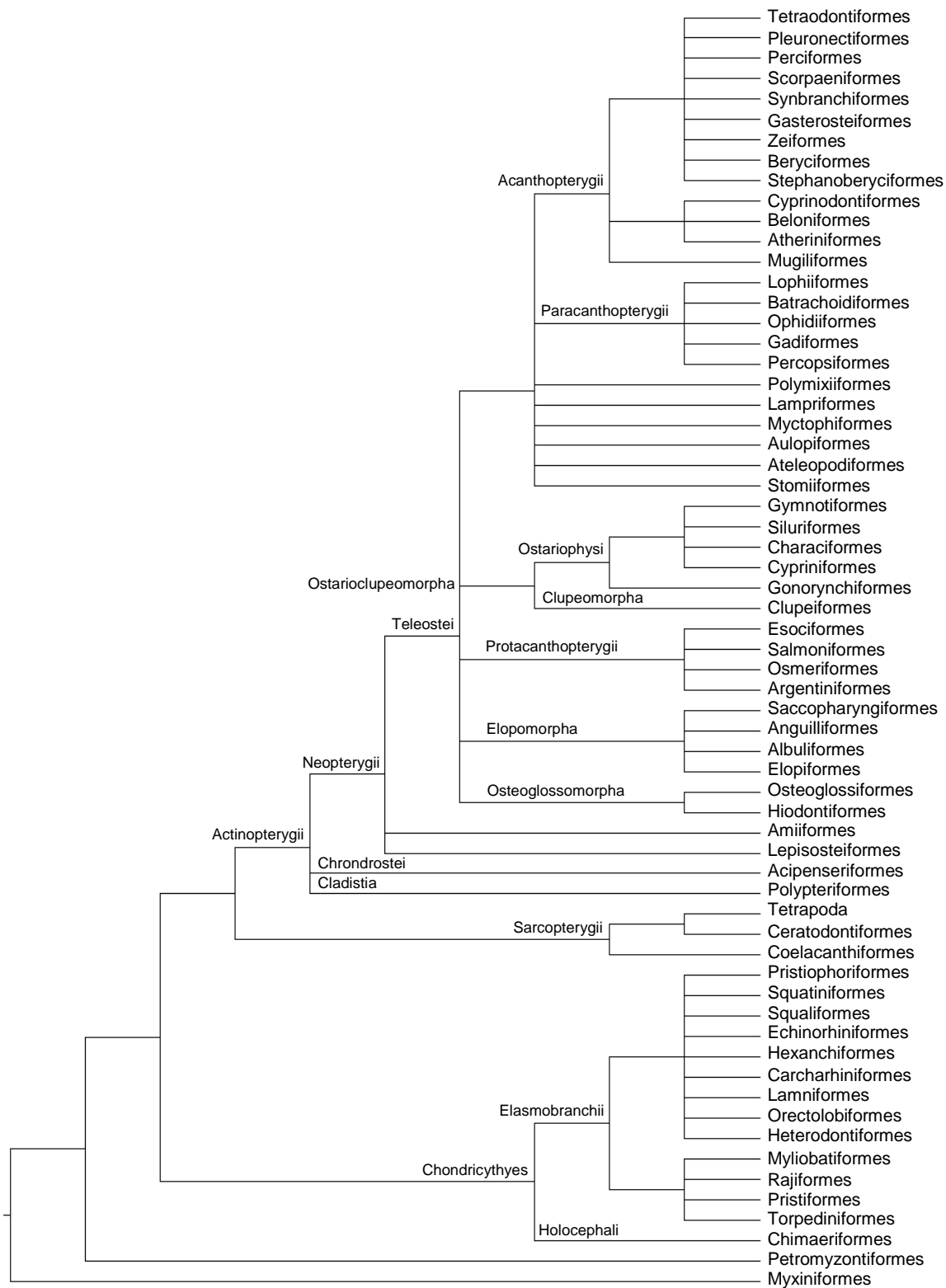


Figure 1. Cladogram representing relationships of the 62 orders of fishes and including the Tetrapoda as presented in Nelson (2006).