# The taxonomy, systematics and evolutionary biology of the Gyliauchenidae Fukui, 1929 (1918) (Platyhelminthes: Digenea)

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# The taxonomy, systematics and evolutionary biology of the Gyliauchenidae Fukui, 1929 (1918) (Platyhelminthes: Digenea)

## VOLUME I

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Thesis submitted as fulfillment of the requirements of a Doctor of Philosophy by research
5th September, 2003

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# Declaration

I, **Kathryn Ann Hall**, hereby declare that this thesis completes, in full, the requirements for a Doctor of Philosophy by research. All work contained herein is my own, unless stated otherwise by reference or other acknowledgement, and was completed while I was enrolled in a higher degree programme at The University of Queensland. This work has not been previously presented in partial or total fulfillment of any other degree at any other university. Research leading to the completion of this thesis was undertaken largely at the St. Lucia campus of The University of Queensland within the Department of Microbiology and Parasitology. Additional research was completed at the following facilities: The University of Queensland Veterinary Farm at Pinjarra Hills; Heron Island Research Station (The University of Queensland); Lizard Island Research Station (Australian Museum Trust); and the Natural History Museum, London, United Kingdom.

Many people were involved in the collecting of specimens used in the course of this study; they collected fish and helminths and are acknowledged in full in the collection records which form Appendix II. I am grateful for their tremendous contribution for, without them, this research would not be comprehensive or able to incorporate material from so many hosts across such a wide geographical area. Their efforts have truly facilitated this study.

I hereby give my permission for this thesis to be reproduced in part or full for the purposes of lending, teaching or other educational purposes according to the copyright legislation of the Commonwealth of Australia.

I declare that these are my own words,

Kathryn Hall.

Dated this 22$^{nd}$ day of July, in the year 2004.

# Abstract

This thesis presents a review of the taxonomy, systematics and evolutionary biology of the Gyliauchenidae Fukui, 1929 (1918). A review of the literature, including taxonomic descriptions and host records, the systematic history and biological observations has been conducted. The most recent review of the family, by Nahhas & Wetzel (1995), recognised 25 species in six genera; *Telotrema* Ozaki, 1933 was included as a junior synonym of *Gyliauchen*. The genera *Petalocotyle* Ozaki, 1934 and *Robphildollfusium* Paggi & Orecchia, 1963 were not attributed to the Gyliauchenidae in the review of Nahhas & Wetzel. This review recognises all genera covered by Nahhas & Wetzel in addition to *Telotrema*, *Petalocotyle* and *Robphildollfusium*. *Progyliauchen* Shalaby & Hassanine, 1997 is recognised as valid and six new genera are proposed. I have expanded the group to comprise 68 species within 16 genera classified in three subfamilies.

The Gyliauchenidae is a morphologically distinctive family of trematodes which is generally restricted to reef-endemic herbivorous fishes of the Indo-West Pacific; over 4 300 individual host fishes from Indo-Pacific waters have been examined during the period of this study. The recovered gyliauchenids have been examined using a combination of morphological and molecular techniques to examine the taxonomy, systematics and biology of the group. I present a new method for the preparation of gyliauchenid specimens for microscopy. The ultrastructure of the digestive system and morphology of the male terminal genitalia has been examined by histological sectioning. Genetic characterisation of gyliauchenid species, using a combination of four genetic loci (internal transcribed spacer (ITS2) and partial large subunit (28S D1-D3) rDNA and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) and large subunit (16S) mtDNA genes), has been performed for use in the molecular discrimination of species and for the phylogenetic inference of the interrelationships of the group. A morphological matrix, using morphometric data, has also been used for the cladistic analysis of gyliauchenid interrelationships. The results from the phylogenetic studies have been used to propose a systematic classification for the Gyliauchenidae and to explore the observed patterns of host and geographical distribution and morphological evolution of the group.

The taxonomy presented here incorporates a new diagnosis of the family, which recognises two synonyms, Dissotrematidae Goto & Matsudaira, 1918 and Robphildollfusiidae Paggi & Orecchia, 1963. The recognition of the family-group name Gyliauchenidae Fukui, 1929 (1918) is justified within the recommendations of the International Code for Zoological Nomenclature (ICZN). The Gyliauchenidae is united by the synapomorphic morphology of the male terminal genitalia, in which the prostate gland cell bodies are external to the cirrus-sac. Three subfamilies, Gyliaucheninae Fukui, 1929 (1919), Petalocotylinae Ozaki, 1937 and Robphildollfusiinae Paggi & Orecchia, 1963 are diagnosed. Ichthyotreminae Caballero & Bravo-Hollis, 1952 and Apharyngogyliaucheninae Yamaguti, 1958 are recognised as junior synonyms of Gyliaucheninae. The Gyliaucheninae is united by the posterior position of the ventral sucker and is proposed to comprise 14 genera; a dichotomous key to genera is presented. The composition of each of these genera is comprehensively reviewed and keys to species are provided.

*Gyliauchen* Nicoll, 1915 comprises eight species, of which six are new, from siganid fishes; the type species, *G. tarachodes* Nicoll, 1915 was described from an undescribed species of *Tachysurus* (Ariidae). A new generic diagnosis is provided and *Dissotrema* Goto & Matsudaira, 1918 is recognised as a junior synonym of *Gyliauchen*. The species of *Gyliauchen* are united by the ventroterminal position of the ventral sucker and the presence of a postovarian uterus. *G. tarachodes* and *G. papillatus* (Goto & Matsudaira, 1918) are redescribed. *G. bylgia* n. sp. is described from species of *Siganus* (Siganidae) from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *G. castor* n. sp. is described from species of *Siganus* from Noumea, New Caledonia. *G. cymodoce* n. sp. is described from species of *Siganus* collected at Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *G. doris* n. sp. is described from *Siganus corallinus* (Valenciennes, 1835) from Heron Island, Great Barrier Reef, Queensland, Australia. *G. galatea* n. sp. is described from species of *Siganus* from Heron Island, Great Barrier Reef, Queensland, Australia; one specimen is also recorded from *Thalassoma lunare* (Linnaeus, 1758) (Labridae) from Heron Island. *G. pollux* n. sp. is described from *S. fuscescens* (Houttuyn, 1782) from Ningaloo Reef, Ningaloo, Western Australia, Australia. *G. castor* and *G. pollux* are hypothesised as bigemminate species. The species *G. indicum* Gupta & Tandon, 1983, *G. oligoglandulosus* Gu & Shen, 1979, *G. ozakii* Srivastava, 1938 and *Gyliauchen* sp. of Nahhas & Wetzel (1995)

are included as *species inquirenda*; *Gyliauchen* sp. of Toman & Kamegi (1974) is similarly not recognised and is included here as a *nomen nudem*.

       *Affecauda* n. g. is proposed for three morphologically unusual species from acanthurid and siganid fishes from the Great Barrier Reef, Australia, and is diagnosed by the combination of an elongate, slender body shape, annulation of the tegument, and a sigmoid oesophagus (or with a single loop). *A. annulata* n. sp. is designated as the type-species and is described from species of *Naso* (Acanthuridae) from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *A. rugosa* n. sp. is described from *Zebrasoma veliferum* (Bloch, 1797) (Acanthuridae) from Noumea, New Caledonia. *A. salacia* n. sp. is described from *Siganus corallinus* (Valenciennes, 1835) from Lizard Island, Great Barrier Reef, Queensland, Australia.

       *Apharyngogyliauchen* Yamaguti, 1942 is reviewed and diagnosed chiefly by the absence of the oesophageal bulb, in addition to the pyriform body shape. Species of *Apharyngogyliauchen* are known only from labroid hosts and have a wide distribution in the Indo-West Pacific, ranging from the Red Sea to French Polynesia. The type-species, *A. callyodontis* Yamaguti, 1942, is redescribed and *A. scarustis* Gu & Shen, 1983 is recognised as a junior synonym. *A. asymbletis* n. sp. is proposed for specimens attributed to *A. callyodontis* by previous workers; *A. callyodontis* of Nagaty (1956) (in part), *A. callyodontis* of Ramadan (1986) and *A. callyodontis* of Rabie & Ahmed (2000), all from labroid fishes of the Red Sea, are here attributed to *A. asymbletis*. *A. catomus* n. sp. is described from scarid fishes from Heron Island, Great Barrier Reef, Queensland, Australia and Moorea, French Pacific. *A. hryga* n. sp. is described from *Chlorurus microrhines* (Bleeker, 1854) (Scaridae) from Noumea, New Caledonia. *A. opisthovarius* Gu & Shen, 1983, from *Cirrhilabrus* sp. (Labridae) from China is redescribed. The new combination of *A. thalassamae* (Wang, 1977) is proposed; this species was formerly attributed to *Gyliauchen* and is known from *Thalassoma hardwicke* (Bennett, 1830) (Labridae) from China. *Apharyngogyliauchen* sp. of Nahhas & Wetzel (1995), recorded from *Scarus ghobban* Forsskål, 1775 (Scaridae) collected from Laucala Bay, Suva, Fiji, is redescribed based on examination of deposited type material. *Apharyngogyliauchen* sp. A n. sp. is described from species of scarids from Heron Island, Great Barrier Reef, Queensland, Australia.

       *Cincinnogyliauchen* n. g. is proposed for eight new species from acanthuroid fishes from the Indo-West Pacific and is diagnosed by the combination of an elongate, dorso-

ventrally curved body shape, presence of at least a small excretory papilla, and an oesophagus which always executes a single dextral loop crossing the midline. The monophyly of this genus is supported by genetic data. *C. bellus* n. sp. is designated as the type-species and is described from *Zebrasoma scopas* (Cuvier, 1829) (Acanthuridae) from Lizard Island, Great Barrier Reef, Queensland, Australia and Noumea, New Caledonia. *C. chasmemus* n. sp. is described from *Z. scopas* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *C. geminus* n. sp. is described from *Z. scopas* from Lizard Island, Great Barrier Reef, Queensland and Ningaloo Reef, Ningaloo, Western Australia, Australia. *C. peratus* n. sp. is described from *Z. scopas* from Moorea, French Pacific. *C. procerus* n. sp. is described from *Z. veliferum* (Bloch, 1797) from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *C. raritas* n. sp. is described from *Naso lituratus* (Forster, 1801) (Acanthuridae) from Moorea, French Pacific. *C. surreptivus* n. sp. is described from *N. fageni* Morrow, 1954 from Ningaloo Reef, Ningaloo, Western Australia, Australia. *Cincinnogyliauchen* sp. A n. sp. is described from *N. annulatus* (Quoy & Gaimard, 1852) from Lizard Island, Great Barrier Reef, Queensland, Australia. Two distinct morphological groups are recognised within *Cincinnogyliauchen*. The *C. bellus*-group comprises *C. bellus*, *C. chasmemus*, *C. geminus* and *C. peratus* and is characterised by a restriction of the vitelline follicles to X-shaped distributions centred at the intestinal bifurcation. The *C. procerus*-group comprises the remaining species and is marked by an elongate body shape and no restriction of the vitelline follicles into an X-shaped distribution. Within the *C. bellus*-group, *C. chasmemus* and *C. geminus* are hypothesised as bigemminate taxa.

   *Flagellotrema* Ozaki, 1936 is recognised here principally by the possession of a looped oesophagus and the position of the genital pore posterior to the termination of the caeca. The type-species, *F. convolutum* Ozaki, 1936 is redescribed. *F. amphitrite* n. sp. is described from *Prionurus maculatus* Ogilby, 1887 (Acanthuridae) and species of *Siganus* from Heron Island, Great Barrier Reef, Queensland, Australia.

   *Hadrobolbus* n. g. is proposed for three species previously attributed to *Flagellotrema*. These species are distinguished from *Flagellotrema* and united by the massive oesophageal bulb, short caeca and the symmetrical testes, which lie entirely anterior to the ventral sucker. All of the species of *Hadrobolbus* are parasites of chaetodontid and pomacanthid fishes of Hawaii. *H. chaetodontis* (Manter & Pritchard, 1962) n. comb. is designated as the type-

species and is redescribed.  The new combinations of *H. centropygis* (Yamaguti, 1970) and
*H. potteri* (Yamaguti, 1970) are proposed and redescribed from examinations of type material.

The monotypic *Ichthyotrema* Bravo-Hollis, 1952 is recognised.  *I. vogelsangi* Caballero & Bravo-Hollis, 1952 is redescribed from voucher material from *Prionurus punctatus* Gill, 1862 (Acanthuridae), collected from Puerto Vallarta, Mexico.

*Leptobulbus* Manter & Pritchard, 1962 is recognised as a monotypic genus. *L. magnacirratus* Manter & Pritchard, 1962 is redescribed from voucher material collected from scarid fishes of Hawaii.

*Medousogyliauchen* n. g. is proposed for three new species from siganid fishes of the Indo-West Pacific; I also propose the new combinations of three species of *Gyliauchen*.  This genus is defined principally by the unique double U-shaped conformation of the oesophagus. The type-species, *M. nahaensis* (Ozaki, 1937) n. comb.,  is redescribed based on published accounts.  *M. cydippe* n. sp. is described from species of *Siganus* from Heron Island, Great Barrier Reef, Queensland, Australia.  *M. panope* n. sp. is described from species of *Siganus* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia.  The new combination of *M. parapapillatus* (Nahhas & Wetzel, 1995) is proposed; *G. parapapillatus* Nahhas & Wetzel, 1995 and *G. papillatus* of Durio & Manter (1969) are synonymised.  This species is known from siganid fishes of the Great Barrier Reef, Queensland, Australia and Fiji. *M. spio* n. sp. is described from species of *Siganus* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia, Noumea, New Caledonia and Fiji.  This species is recorded also from *Zanclus cornutus* (Zanclidae) from Heron Island.  *G. nahaensis* of Nahhas & Wetzel (1995) (in part), *G. nahaensis* of Hughes-Stamm *et al.* (1999) and *G. nahaensis* of Jones *et al.* (2000) are recognised as synonyms of *M. spio*.  *M. volubilis* (Nagaty, 1956) from siganid and scarid fishes from the Red Sea is proposed as a new combination.

*Paragyliauchen* Yamaguti, 1934 is recognised as comprising two previously described species; a further five species are described from pomacanthid fishes from the Indo-West Pacific.  Two morphological groups are recognised within *Paragyliauchen*: the *P. chaetodontis*-group, containing only two species, *P. chaetodontis* Yamaguti, 1934 and *P. scaeodicliditus* n. sp., and the *P. arusettae*-group.  The type-species, *P. chaetodontis*, is redescribed from type material.  *P. scaeodicliditus* is described from species of *Centropyge* (Pomacanthidae) from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia and Moorea, French Pacific.  The following species belong to the *P. arusettae*-group.

*P. arusettae* Machida, 1984 is redescribed from review of the literature.  *P. atractus* n. sp. is described from species of *Centropyge* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia.  *P. fractoporus* n. sp. is described from species of pomacanthids from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia.  A single specimen previously attributed to *P. arusettae* by Dyer *et al.* (1988) is here considered as a distinct species, *Paragyliauchen* sp. A n. sp.  *Paragyliauchen* sp. B n. sp. is described from *Pomacanthus semicirculatus* (Pomacanthidae) from Ningaloo Reef, Ningaloo, Western Australia, Australia.

The monotypic *Progyliauchen* Shalaby & Hassanine, 1997 is recognised by the presence of a unique conformation of the oesophagus and a four-lobed ovary.  *P. sigani* Shalaby & Hassanine, 1997 is redescribed.

*Ptychogyliauchen* n. g. is proposed for four new species from siganid fishes of the Indo-West Pacific and is distinguished from the remainder of the Gyliauchenidae principally by the possession of a uniquely double-looped oesophagus and the distinctive lining of the ejaculatory duct.  *P. thetidis* n. sp. is designated as the type-species and is described from species of *Siganus* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *P. himinglaeva* n. sp. is described from *S. corallinus* (Valenciennes, 1835) from Lizard Island, Great Barrier Reef, Queensland, Australia.  *P. leucothea* n. sp. is described from species of *Siganus* from Ningaloo, Western Australia, Australia.  *P. thistilbardi* n. sp. is described from species of *Siganus* from Noumea, New Caledonia.  This species is recorded also from *Chaetodon citrinellus* Cuvier, 1831 (Chaetodontidae) from Moorea, French Pacific.

*Telotrema* Ozaki, 1933 is recognised by the straight structure of the oesophagus and the presence of a conspicuous and wide excretory papilla.  The type-species, *T. caudatum* Ozaki, 1933 is reviewed.  *T. brevicaudatum* n. sp. is described from *Acanthurus xanthopterus* Valenciennes, 1835 (Acanthuridae) from Lizard Island, Great Barrier Reef, Queensland, Australia.  The new combination of *T. pomacentri* (Nahhas & Wetzel, 1995) is proposed from the examination of the type-specimen.

*Xenogyliauchen* n. g. is proposed for two species of gyliauchenids from siganid and zanclid fishes.  The type-species, *X. zancli* (Nahhas & Wetzel, 1995) n. comb. is redescribed and *G. nahaensis* of Nahhas & Wetzel (1995) (in part) is recognised as a synonym.  *X. buseyra* n. sp. is described from species of *Siganus* from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia.

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The Petalocotylinae Ozaki, 1937 is distinguished from other subfamilies of the Gyliauchenidae by the combination of the position of the ventral sucker in the anterior half of the body and the possession of a common genital pore.  The Petalocotylinae contains only one genus, *Petalocotyle* Ozaki, 1934, of which there are four species.  The type-species, *P. nipponica* Ozaki, 1934, is redescribed.  *P. adenometra* n. sp. is described from *Prionurus microlepidotus* Lacepède, 1804 (Acanthuridae), from Amity Point, Stradbroke Island, Queensland, Australia.  *P. diverticulata* n. sp. is described from species of *Acanthurus* (Acanthuridae) from Heron and Lizard islands, Great Barrier Reef, Queensland, Australia. *Petalocotyle* sp. A n. sp. is described from *Acanthurus blochii* Valenciennes, 1835 (Acanthuridae), Noumea, New Caledonia.

The Robphildollfusiinae Paggi & Orecchia, 1963 is diagnosed principally by the presence of separate male and female genital pores.  *Robphildollfusium* Paggi & Orecchia, 1963 is the only genus in the subfamily.  *R. fractum* (Rudolphi, 1819) and *R. martinezgomezi* López-Román, Gijón-Botella, Kim & Vilca-Chocque, 1992 are redescribed from the literature and examination of type material.

The phylogenetic interrelationships of the Gyliauchenidae have been inferred using molecular data from four genetic loci (ITS2 and 28S rDNA and ND1 and 16S mtDNA) analysed as independent datasets and using a total evidence approach.  Each dataset was analysed using the criteria of Maximum Parsimony, minimum evolution and maximum likelihood.  Three different *a priori* weight sets were assumed for analysis of the ND1 mtDNA gene alignment.  Phylogenetic relationships were also inferred by parsimony for the translation of this gene into amino acids.  A morphological dataset, which combined morphometric and morphological characters, was devised and analysed by parsimony.  The results of the phylogenetic analysis were used to hypothesise a classification of the Gyliauchenidae.  This classification was consistent with the recognition of the three subfamilies.  Within the Gyliaucheninae, two tribes were recognised: Gyliauchini and Paragyliauchini.  Paragyliauchini comprises the species of the genera *Paragyliauchen*, *Hadrobolbus* and *Leptobulbus*.  The Gyliauchini is further classified into three clades identified in the phylogenetic analysis.  The *Gyliauchen*-group contains *Gyliauchen*, *Apharyngogyliauchen* and *Xenogyliauchen*.  The *Affecauda*-clade contains *Affecauda*, *Cincinnogyliauchen*, *Flagellotrema*, *Ichthyotrema*, *Ptychogyliauchen* and *Telotrema*.  The

species of *Medousogyliauchen* are classified within a monogeneric clade. *Progyliauchen* is classified within the Gyliauchini as *incertae sedis*.

The proposed systematic classification of the Gyliauchenidae has been used to explore macroevolutionary patterns in the observed biogeographical and host distribution of the family. The origins of the unique morphological adaptations of the group have also been investigated in an evolutionary context. Molecular data have been used to hypothesise the time of divergence of the Gyliauchenidae from a lepocreadoid ancestor. I propose that the current observed morphology and host and geographical distribution of the Gyliauchenidae reflects a rapid, but intense, history of association with acanthuroid fishes; the distinctive morphology of the group is a direct consequence of their association with the dominant herbivorous fishes of the Indo-West Pacific.

# Acknowledgements

It was a typically hot, stickily stifling, listless day. Mid-March, with the sun already packing blistering heat by 10am. I walked up the spiralling, cool, concreted stairway from the road to the ground floor of the North wing, Seddon Building, Building 82. Up to the grassy green laminex benches of NP19, the room that four months previously, I had thought I would never see again. There was no air-conditioning in NP19, no relief from the oppressive summer outside; only the whirr of the powerful 486s cut the thick and syrupy air. The tinkling of spoons in tea-cups, the shuffling of feet as bodies edged around the urn, the definitive, magnetic snapping closed of the fridge door, voices raised in spirited, competitive conversation filtering down the lino hall. Morning Tea. Tom had told me it was important to always make sure that I arrived for Morning Tea. As the years passed, the laminex changed, air-conditioning arrived, 486s have assumed their place as historical treasures, departments merged and morphed and I hardly ever made it in for Morning Tea. But I haven't forgotten that important day, a day on which I made a commitment to Parasitology and to the Gyliauchenidae. If this sounds like a solemn vow, it is not so much for poetic effect as to tell it plainly. It hasn't always been a smooth road, it hasn't always been a pleasant road, but it has always been a road that I have been following and every day has taught me something new. I have been so very lucky to have met some wonderful people who have helped me and taught me more than just about science. I am grateful for the opportunity to thank them here for their assistance and for the richness they have added to this body of work, be that this thesis, or this person.

There are many people deserving of acknowledgement for their contributions to this thesis and research. The list is long:

Here, for the benefit of those who would otherwise deem this thesis a failure, I include a musical reference:

<div style="text-align:center">

"*Cigarettes will kill you*" Ben Lee, 1998.

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# List of Publications

The following is a list of publications arising from research contributing to this thesis. They are to be found in full (peer-reviewed papers only) in Appendix I.

## Book Chapters

**K. A. Hall** & T. H. Cribb. (in press; accepted December, 2002). The Gyliauchenidae Fukui, 1929 (1918). In: *Classification of the Trematoda. Vol III*. CABI Publishing, London.

## Peer-Reviewed Papers

**K. A. Hall** & T. H. Cribb. (in press; accepted June, 2004). Revision of *Affecauda* Hall & Chambers, 1999 (Digenea, Gyliauchenidae Fukui, 1929), including the description of two new species from fishes of the Indo-West Pacific. *Zootaxa*.

**K. A. Hall** & T. H. Cribb. 2000. The status of *Petalocotyle* Ozaki, 1934 (Digenea: Gyliauchenidae), including the description of two new species from acanthurid fishes in Queensland, Australia. *Systematic Parasitology*, **47**, 145-156.

**K. A. Hall** & C. B. Chambers. 1999. A new genus of the Gyliauchenidae Goto & Matsudaira, 1918 (Digenea) from *Naso tuberosus* (Percomorpha: Acanthuridae) on the Great Barrier Reef, Queensland, Australia. *Acta Parasitologica* **44**, 229-232.

**K. A. Hall** & T. H. Cribb. (submitted). The proposal of *Ptychogyliauchen*, a new genus of Gyliauchenidae Fukui, 1929 (Platyhelminthes, Digenea), including the description of four new species from siganid fishes of the Indo-West Pacific.

**K. A. Hall** & T. H. Cribb. (submitted). Revision of *Telotrema* Ozaki, 1933 (Digenea, Gyliauchenidae Fukui, 1929), including the description of a new species from an acanthurid fish of the Great Barrier Reef, Queensland, Australia.

## Conference Papers:

**K. A. Hall**, T. H. Cribb & R. A. Bray. 2000. Gyliauchenid trematodes and morphometrics: is size phylogenetically informative? Joint meeting of the New Zealand Society for Parasitology and Australian Society for Parasitology, September, 2000.

T. H. Cribb, R. A. Bray, C. B. Chambers, A. D. Dove, **K. Hall**, S. Pichelin & T. Wright. 2000. The futility of life-cycle studies in the face of megadiversity: trematodes, fishes and molluscs on the Great Barrier Reef. Joint meeting of the New Zealand Society for Parasitology and Australian Society for Parasitology, September, 2000.

**K. A. Hall**. 2000. Worms and fish: more than just bait. Departmental seminar, Department of Microbiology & Parasitology, UQ, post-graduate seminar series.

**K. A. Hall**, T. H. Cribb, R. A. Bray & D. T. J. Littlewood 1999. An evolutionary hypothesis for the radiation of the Gyliauchenidae Goto & Matsudaira, 1918 (Platyhelminthes: Digenea). The Australian Society for Parasitology Conference, September, 1999.

**K. A. Hall**, T. H. Cribb & R. A. Bray. 1999. Gyliauchenidae Goto & Matsudaira, 1918 (Platyhelminthes: Digenea): co-evolution, parallelisms and diverging paths. The Systematics Association Biennial Conference, August 1999.

**K. A. Hall**, T. H. Cribb, R. A. Bray & D. T. J. Littlewood. 1999. Gene-tree gives insight into evolution of unusual group (poster presentation). Interrelationships of the Platyhelminthes, symposium held by The Linnean Society of London in association with The Systematics Association and The British Society for Parasitology, July 1999.

**K. A. Hall** & T. H. Cribb. 1998. Primitive *Petalocotyle* (Digenea: Gyliauchenidae) in primitive *Prionurus* (Acanthuridae). The Australian Society for Parasitology Conference, September 1998.

**K. A. Hall**, T. H. Cribb & R. A. Bray. 1998. Trematodes in Tangs: species identification and cluster observations. The American Society of Parasitology Conference, August 1998.

# Abbreviations and Symbols

Symbols and abbreviations have been explained in full in the text where appropriate. Standard scientific symbols have been used throughout this thesis. The following abbreviations have been used in taxonomic descriptions:

BMNH    Natural History Museum Parasitic Worms Collection, UK
HWML    H. W. Manter Laboratory Parasite Collection, USA
KH      Hall gyliauchenid specimen collection
MPM     Meguro Parasite Museum Collection, Japan
UNAM    Universidad Nacional Autonoma de Mexico, Institute of Biology collection, Mexico
USNPC   United States National Parasite Collection, USA
UQ      The University of Queensland, Australia
THC     Cribb host database collection

# Foreword

The Convention on Biological Diversity (Brazil, 1998) focussed the international attention of politicians, scientists and the community alike on the global lack of knowledge about biodiversity and environmental destabilisation.  Improvements in this knowledge crisis are hampered by "the taxonomic impediment"; there is a chronic shortage of expertise in taxonomy (*eg*: Brooks & Hoberg, 2000).  A Global Taxonomic Initiative (GTI) was ratified at the convention and aimed to establish a solid understanding of the identity of plant and animal species and their ecosystems.  Astute use of this knowledge is integral to the drafting of rational and flexible conservation policies which provide for biodiversity and environmental conservation and promote industry and development in a metered, sustainable and prosperous manner.  Fundamental to the success of the GTI is not only the accurate identification and classification of biota, but also comparative biological surveys which integrate taxa, their phylogenetic histories and their historical associations with other taxa and physical environments.

Taxonomy has undergone a resurgence in popularity in the time since the ratification of the GTI, and is now seen as a "fashionable", contemporary science (Mallet & Willmott, 2003).  The new-found enthusiasm for taxonomy is, in part, due to the creation of web-based taxonomic initiatives (Godfray, 2002; Mallet & Willmott, 2003), such as All-Species (http://www.allspecies.org/), GBIF (http://www.gbif.org/), and Species 2000 (http://www.sp2000.org/).  Further, there is strong advocacy for the adoption of DNA-based taxonomy, similar to the system currently used by microbiologists (Tautz *et al*., 2003).  Exploitation of the advances in genome sequencing, which mean that it is now less expensive, more rapid and more efficient than ever before, is seen as an opportunity to catalogue the biosphere and overcome the problem of instability in the existing Linnaean hierarchy (Tautz *et al*., 2003).  A claimed added benefit of a centralised move to DNA-based taxonomy is the revival of the image of taxonomy; it would no longer be fusty, museum-based pseudo-science, but modern, sophisticated, "real science" (Godfray, 2002; Tautz *et al*., 2003).  Critics (Lipscomb *et al*., 2003; Seberg *et al*., 2003) dispute that DNA-based taxonomy would accomplish these aims; Lipscomb *et al*. (2003) argue that *any* character, molecular or morphological, which is stable within a species and variable among taxa, will add rigour to

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taxonomy. Further, critics believe that the widespread adoption of DNA-based taxonomy could serve to ostracise workers from developing nations and would be harmful to the discipline:

> *"To relegate taxonomy, rich in theory and knowledge, to a high-tech service industry would be a decided step backward for science."* (Lipscomb *et al.*, 2003)

> *"We feel that a DNA-based taxonomy along the suggested lines* [of Tautz *et al.*, 2003] *would catastrophically retard taxonomic activity..."* (Seberg *et al.*, 2003)

There are additional, operational concerns associated with the use of DNA-based taxonomy. Tautz *et al.* (2003) propose a scheme, in which samples are taken for molecular characterisation, and the remainder of the specimen designated as a neotype. The authors accept that such a proposal is feasible for large animals, such as birds, where only a single feather would be required for the extraction of DNA. Tautz *et al.* (2003) suggest that for smaller organisms, where the whole specimen is needed for DNA extraction, that photographs be taken of specimens, prior to destruction. Seberg *et al.* (2003) believe this is a suboptimal compromise; future needs for type material can not reasonably be anticipated, and in such circumstances, type destruction is seen as perilously injudicious. I would suggest further that even photographic records of platyhelminth material would be virtually useless. Accurate identification of material depends critically on the good preparation of whole mounts for light microscopy; heat-fixed material is best for these purposes, and it is currently almost impossible to extract high quality DNA for high-fidelity amplification from material fixed appropriately for light microscopy.

Regardless of the criticism of proposals for DNA-based taxonomy, there is a well acknowledged need for reform in the discipline (*eg*: Godfray, 2002; Mallet & Willmott, 2003; Minelli, 2003; Wilson, 2003). Minelli (2003) calls for a centralised register for zoological taxonomic literature, united by publication, in English, using electronic forms of communication. Minelli believes that this register would make significant improvements to the availability of literature and ameliorate many of the problems associated with instability in the Linnaean hierarchy; a continually evolving register would provide easy, stream-lined access to changes in taxon status. The web-based "Encyclopedia of Life" proposed by Wilson (2003) also aims to reduce taxonomic confusion and make taxonomy more accessible. Mallet & Willmott (2003) are more cautionary; they believe that discussion and exploration of future

developments in taxonomy are useful, however, they assert that now is the time for action. Mallet & Willmott suggest that a greater emphasis on cohesive debate is needed to capitalise on the surge of interest in taxonomy; indecision and discord risk fragmenting the discipline.

The inherent importance of taxonomic studies in understanding biodiversity is well documented and understood (Richardson, 1984; Gibson, 1992; MacFadden & Parker, 1994; Renner & Ricklets, 1994; Brooke, 2000; Brooks & Hoberg, 2000; Erwin & Johnson, 2000), yet even some of these authors question the validity of, or scientific imperative behind, the current urgency for biodiversity documentation. MacFadden & Parker (1994) suggested that current political and community concern is merely a reflection of human values, and that given this concern, we must continue to act in accordance with our consciences. They suggested that humanity represents nothing more than a new ecological perturbation, similar in many ways to such natural phenomena as earthquakes, and as such, extinctions are an inevitable consequence. These authors cited the impressive statistic that 99% of all species are now extinct and contend that current imperatives in biodiversity research are borne purely out of hysteria. MacFadden & Parker concluded, rather dispassionately, that taxonomy is probably a "waste of time", but if it serves to salve the global conscience then it can't really be a bad thing. Renner & Ricklets (1994) were more scathing and dismissive of taxonomy, suggesting that the focus on inventory building is nothing more than a "fad" and that biologists would be better able to contribute to conservation by devising practical and rational preservation practices. The authors contempt is manifested in their comments that taxonomy serves as nothing more than a platform for the more "creative" and "intellectual" branches of systematics research. This contention, which, in my opinion, undervalues and belittles taxonomy, is one which is often perpetuated by workers in their rush to validate the contribution of taxonomy to other biological sciences (Gibson, 1992; Brooks & Hoberg, 2000). Even the well-respected and eminent Ernst Mayr (1976) suggested that taxonomists are selfless workers who dedicate their lives to the documentation of animal species, implying that the role of the taxonomist is no more significant than that of a philatelist. Simpson (1961) simply and readily removed taxonomy from the debate surrounding whether or not it constitutes science by defining it as "art". Further, Renner & Ricklets (1994) contended that the current focus on taxonomy (which is, they intimate, inherently dull) actively discourages good students from entering into systematics research!

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The derogatory remarks flung at taxonomists by other biologists reflect fundamental misconceptions and narrow-mindedness about the nature and philosophy of science. Karl Popper was the foremost exponent of the philosophy of science in the 20th century. There have been many works published which outline his philosophy of falsifiability (*eg*: 1934; 1957; 1972; 1974), however, the spirit of Popper's vision and conception of science is often lost. Popperian science is an activity that seeks the truth through documentation of observation and the devising of tests which will falsify explanation of that observation. The perception that taxonomy is somehow "not quite a science" is founded in the misconception that it is not falsifiable. It is easy to sympathise with this view; once a biological specimen is labelled with a name and taxonomists publish those descriptions, that name becomes fact, and in Popperian terms, if it is true today, then it is true tomorrow. Is it possible to falsify something that is fundamentally true? Philosophically then, it would seem that taxonomy can not, by definition, be "science". Settle (1979) convincingly argued that the inability to devise a test to falsify taxonomy does not invalidate it as science:

> "*...imagine a taxonomist has conjectured a hypothesis he was unable to submit to a satisfying rigorous test... Should we say he has failed as a scientist?... It is worth mentioning that no test was devised that could distinguish empirically between Copernicus's theory of the solar system and Ptolemy's until 1851... If I were a taxonomist, I should not worry overmuch about whether to call a hypothesis I did not know how to test, a piece of "science" or a piece of "metaphysics" so long as my style was right: so long as I was after the truth, and held my conjectures open to criticism.*" (pp. 528-529)

Settle's argument pertained to the spirit of Popperian science; Settle contended that the focus on falsifiability, rather than the search for truth, has overshadowed perceptions of taxonomy as a science. Taxonomy seeks to document observations of the biological world through the classification of organisms, it presents hypotheses in the form of classifications, and these classifications are able to be falsified; taxonomy fulfills the definition of a "science". Lipscomb *et al.* (2003) state that taxonomy is an hypothesis-driven science that draws on multiple data sources in order to explore fundamental questions in evolutionary patterns. That taxonomy is intimately enmeshed with other branches of systematic and ecological research only intensifies, rather than diminishes, its value; taxonomy is fundamentally important as a vital science in its own right, that it contributes further through tangible benefits to other scientific sectors is merely a bonus.

It is clear that taxonomy is, scientifically, both rigorous and valid, in addition to the valuable contribution it lends to other branches of biological science. Despite the importance of taxonomy, however, the comments of Gibson (1992) highlight the current status of the discipline with respect to the Platyhelminthes:

> *"Taxonomy is seen as an unpopular discipline in the developed world. Intractable difficulties with regard to funding and employment opportunities mean that few students are being attracted to the field. Furthermore, the enormous size of some of the groups and the length of time it takes to acquire enough experience to work with these groups are also great deterrents. There are, therefore, few modern replacements for the great helminth systematists of the past."* (p. 58)

Perhaps more alarming for Australians is the apparent dearth of knowledge of our own helminth fauna. Richardson (1984) suggested that there may be as many helminths as insects in Australia, however, deficiencies in the taxonomic research make it impossible to even accurately estimate the number of helminth species that remain to be described. At the time of the article of Richardson, working taxonomists in Australia were few, and their expertise was overwhelmingly concentrated on better-known fauna, such as the tetrapods (Richardson, 1984). Brooks & Hoberg (2000) provide a global perspective, which echoes concern about the shortage of working taxonomists and suggests that little has changed in the 15 years hence. Despite the myriad ways in which parasite systematics can contribute to wider understanding of the biosphere – parasites can act as models for studying macroevolutionary trends in character evolution, adaptive radiations, the mechanisms of speciation and the evolution of ecological communities – there remains an enormous volume of taxonomic studies to be undertaken. It would seem timely, therefore, to present this thesis: **The taxonomy, systematics and evolutionary biology of the Gyliauchenidae Fukui, 1929 (1918) (Platyhelminthes: Digenea)**.

# Chapter 1

# Literature Review

# 1.0 Introduction

The Gyliauchenidae Fukui, 1929 (1918) is a family of intestinal helminths which is almost entirely restricted to herbivorous teleost fishes of the Indo-West Pacific. This niche restriction is correlated with a suite of morphological adaptations which may be used to define the group. Gyliauchenids are characterised principally by specialisation of the digestive system: the oral sucker has been lost and functionally replaced by the pharynx; in some presumably derived groups the oesophagus is long and convoluted, forming many coils within the forebody; and the function of the pharynx has been accommodated by a *de novo* structure, the oesophageal bulb, which is derived from increasing muscularisation of the oesophageal wall. These trematodes are usually further adapted to the gut of herbivorous fishes by the possession of a posterior ventral sucker. This unusual morphology has seen gyliauchenids associated with a number of digenean groups, including the Paramphistomidae Fischoeder, 1901, with which they share superficial similarity. Further, the complexity of the structure of the oesophagus of gyliauchenids, coupled with the paucity of other morphological features, has confounded their taxonomy. Nahhas & Wetzel (1995) conducted the most recent review of the group, however, this work only superficially covered many species and did not recognise the phylogenetically important and morphologically uncharacteristic genus, *Petalocotyle* Ozaki, 1934.

The following sections provide an historical account of records of the Gyliauchenidae from the literature, including a review of the taxonomy, systematics and biology of the group. It is important that this review also explores the biological and physical environment of gyliauchenids. These trematodes exhibit restriction of both host fish and geographic range; consequently, I provide a review of the major host groups and the biogeography of the Indo-West Pacific region. These reviews become useful in the context of an examination of the macroevolutionary patterns for the Gyliauchenidae; a brief statement regarding current theory and best practice in the study of the evolutionary biology of historical biogeography and host-parasite associations is therefore warranted. This review is intended to highlight the deficiencies in, and the utility of, the large body of reference material pertaining to gyliauchenids, to consolidate the current status of the family and illuminate the biological and physical context of the Gyliauchenidae, thereby establishing a platform from which the systematics and macroevolutionary patterns can be meaningfully investigated.

## 1.1 Taxonomic History of the Gyliauchenidae Fukui, 1929 (1918)

The first record of a gyliauchenid trematode was the description of *Gyliauchen tarachodes* Nicoll, 1915 from the intestine of a pilot-fish, "*Tachysurus* n. sp." (Ariidae) from North Queensland, Australia. Nicoll established the new genus, *Gyliauchen*, which he assigned to the Paramphistomidae Fischoeder, 1901 but could not place within any of the subdivisions of the group. Nicoll acknowledged the aberrant morphology of *Gyliauchen*, with its unusual convoluted oesophagus and the absence of pharyngeal sacs, however, believed that it was sufficiently similar to the other members of the Paramphistomidae to classify this new genus within that family.

*Dissotrema* Goto & Matsudaira, 1918 was established to house a morphologically unusual amphistomatous trematode from the intestine of *Siganus fuscescens* from Japanese waters (Goto & Matsudaira, 1918). Although they acknowledged significant superficial similarity between *Dissotrema* and other amphistomes, most noticeably those of the Paramphistomidae, Goto & Matsudaira believed that consistent differences in the topography of the gonads and the nature of the vitellarium warranted the erection of a new family. They established the monotypic Dissotrematidae, which was diagnosed by a posterior ventral sucker, a long and convoluted oesophagus with a distal oesophageal bulb, a pre-testicular ovary and the absence of buccal pouches (pharyngeal sacs). The following year, Goto synonymised *Dissotrema* with *Gyliauchen* Nicoll, 1915. Goto (1919) was less convinced of the affinity of *Gyliauchen* with the paramphistomes than Nicoll, and maintained that the atypical morphology of *Gyliauchen* justified a separate and distinct family. At no point, however, did Goto specify a name for the new family. It was not until almost a decade and a half later, that the name Gyliauchenidae was to appear in the literature.

Ozaki (1933) described a new genus and species of the "Gyliauchenidae". Although Fukui (1929) had previously placed *Gyliauchen* within the Gyliaucheninae as a subfamily of the Opistholebetidae, Ozaki (1933) represents the first explicitly published use of the family-level name Gyliauchenidae (see Taxonomy, p. 80, for full discussion). Since that time, the Gyliauchenidae has consistently retained its family status. The Gyliauchenidae has been formally reviewed twice (Ozaki, 1937b; Nahhas & Wetzel, 1995) and, although composition of the family has changed through the addition and subtraction of various genera, the

diagnosis of the family has remained comparable to that initially proposed by Goto & Matsudaira (1918) for the Dissotrematidae.  Yamaguti (1971) primarily defined the Gyliauchenidae by: the posterior position of the ventral sucker; presence of a convoluted oesophagus with terminal oesophageal bulb; the unusual location of both the seminal vesicle and the *pars prostatica* free in the parenchyma, coupled with the presence of a true cirrus-sac; and the entirely pre-ovarian uterus.  Table I presents the species which I include within the Gyliauchenidae for the purposes of this review.  This list is based on the reviews of the family (Ozaki, 1937b; Nahhas & Wetzel, 1995), the most recent and comprehensive review of the Digenea (Yamaguti, 1971) and individual records of new species scattered throughout the literature subsequent to, or not included in, these reviews.  It should be noted that at no time has *Robphildollfusium* Paggi & Orecchia, 1963 ever been included within the Gyliauchenidae; affinity with some members of the family, however, has been suggested (Goto, 1919; Yamaguti, 1971).

**Table I. Described species included as members of the Gyliauchenidae Fukui, 1929 (1918) within this study.** Type species are indicated with an asterisk (*). Note that *Robphildollfusium* Paggi & Orecchia, 1963, which is classified by Lopez-Roman *et al.*, 1992 within the Robphildollfusiidae Paggi & Orecchia, 1963, and *Petalocotyle* Ozaki, 1934, classified within the Lepocreadiidae Nicoll, 1914 by Yamaguti (1971), are included here; the species of *Robphildollfusium* are considered in this study to be a sister group to *Petalocotyle* Ozaki, 1934, and both genera are classified within the Gyliauchenidae[1].

| Taxon | | Authority |
|---|---|---|
| *Gyliauchen* | *caudatum* | (Ozaki, 1933) |
| | *indicum* | Gupta & Tandon, 1983 |
| | *nahaensis* | Ozaki, 1937 |
| | *ozaki* | Srivastava, 1938 |
| | *oligoglandulosus* | Gu & Shen, 1979 |
| | *papillatus* | (Goto & Matsudaira, 1918) |
| | *parapapillatus* | Nahhas & Wetzel, 1995 |
| | *pomacentri* | Nahhas & Wetzel, 1995 |
| | *tarachodes** | Nicoll, 1915 |
| | *thalassamae* | Wang, 1977 |
| | *volubilis* | Nagaty, 1956 |
| | *zancli* | Nahhas & Wetzel, 1995 |
| | sp. | of Toman (1974) |
| | sp. | of Nahhas & Wetzel (1995) |
| *Apharyngogyliauchen* | *callyodontis** | Yamaguti, 1942 |
| | *opisthovarius* | Gu & Shen, 1983 |
| | *scarustis* | Gu & Shen, 1983 |
| | sp. | of Nahhas & Wetzel (1995) |
| *Flagellotrema* | *centropygis* | Yamaguti, 1970 |
| | *chaetodontis* | (Manter & Pritchard, 1962) |
| | *convolutum** | Ozaki, 1936 |
| | *potteri* | Yamaguti, 1970 |
| *Ichthyotrema* | *vogelsangi** | Caballero & Bravo-Hollis, 1952 |
| *Leptobulbus* | *magnacirratus** | Manter & Pritchard, 1962 |
| *Paragyliauchen* | *arusettae* | Machida, 1984 |
| | *chaetodontis** | Yamaguti, 1934 |
| *Progyliauchen* | *sigani** | Shalaby & Hassanine, 1997 |
| *Petalocotyle* | *nipponica** | Ozaki, 1934 |
| *Robphildollfusium* | *fractum** | (Rudolphi, 1819) |
| | *martinezgomezi* | Lopez-Roman, Gijon-Botella, Kim & Vilca-Choque, 1992 |

[1] The classification and internal systematic relationships of the Gyliauchenidae are discussed in full in Systematics (p. 439). Justification for the proposed classification, using molecular and morphological evidence, is provided in this section.

## 1.2   Systematic History of the Gyliauchenidae Fukui, 1929 (1918)

### Family level affinities

Early workers observed many similarities between the Gyliauchenidae and another amphistomatous parasite group, the Paramphistomidae Fischoeder, 1901.  Nicoll (1915) first proposed *Gyliauchen tarachodes* within the paramphistomes, but was unable to definitively place it within any of the known subfamilies.  A relationship within the Paramphistominae Fischoeder, 1901 was rejected by Nicoll because of the possession of a cirrus-sac by *G. tarachodes*; the absence of pharyngeal pouches prohibited an association with the Cladorchiinae.  Nicoll (1915) hypothesised that *G. tarachodes* might be most closely related to *Pseudocladorchis* Daday, 1906 from South American fishes, however, he qualified this relationship as remote.  The similarity between *Gyliauchen* and the paramphistomes was also observed by Goto (1919).  Goto (1919) concluded that the differences between these two groups were slight, such that the erection of a new family was more of a convenience than a necessity.  Despite these reservations, Goto (1919) retained recognition of the monogeneric Dissotrematidae and synonymised *Dissotrema* with *Gyliauchen*.  Goto speculated on further associations of the gyliauchenids, suggesting that the asymmetrical testes, pre-testicular ovary and posterior ventral sucker indicated similarity with *Balanorchis* Fischoeder, 1901 (Paramphistomidae), however, the presence of a cirrus-sac confounded this relationship.  Associations with both *Opistholebes* Nicoll, 1915 and *Maculifer* Nicoll, 1915 (Opistholebetidae Fukui, 1929) were also discounted by Goto (1919).  The shared presence of gland cells surrounding the long and winding oesophagus was considered suggestive of a relationship with *Distomum fractum* Rudolphi, 1819 (now *Robphildollfusium fractum* (Rudolphi, 1819) (Robphildollfusiidae Paggi & Orecchia, 1963)), however Goto (1919) did not favour this proposal, dismissing the possibility of any close phylogenetic relatedness.

The classification of Fukui (1929) asserted a relationship between *Gyliauchen* and *Opistholebes*, previously rejected by Goto (1919).  Fukui did not support association of *Gyliauchen* with the Paramphistomidae, on the basis of the pre-testicular ovary and presence of a seminal receptacle and cirrus-sac in species of *Gyliauchen*, and placed *Gyliauchen* within the Gyliaucheninae as a subfamily of the Opistholebetidae.  Although Fukui acknowledged the superficial similarities between *Gyliauchen* and paramphistomes, his classification recognised

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the Opistholebetidae as occupying a position intermediate between the distomes and amphistomes.  This classification identified two subfamilies: the Opistholebetinae Fukui, 1929 containing *Opistholebes amplicoelus* Nicoll, 1915; and the Gyliaucheninae Fukui, 1929 containing *G. tarachodes* and *G. papillatus*.  Yamaguti (1934) endorsed the classification of Fukui and placed the new genus *Paragyliauchen* Yamaguti, 1934 within the Gyliaucheninae, Opistholebetidae.  Ozaki (1933; 1936), however, did not recognise the classification of *Gyliauchen* within the Opistholebetidae and described *Telotrema* Ozaki, 1933 and *Flagellotrema* Ozaki, 1936 within the Gyliauchenidae.

A comparative study of the morphology of the Opistholebetidae and Gyliauchenidae was undertaken by Ozaki (1934a; 1937a; b) to investigate the relationships of these groups.  An initial study of the excretory canal structure of *Telotrema caudatum* suggested to Ozaki (1934a) that the gyliauchenids may be more closely related to the Angiodictyidae Looss, 1902 (= Microscaphidiidae Looss, 1900) than the paramphistomes.  Subsequent and more detailed examinations of species from the Opistholebetidae and Gyliauchenidae, however, led Ozaki (1937a; b) to provide more confident statements of relatedness.  The excretory vesicle of gyliauchenids opens via a pore terminal on a papilla, or at the posterior extremity of the body in species which lack distinct papillae.  The vesicle is generally a short, simple undivided sac, which lies dorsal to the testes; two main collecting ducts arise anteriorly (Ozaki, 1937b).  Ozaki found a positive correlation between the size of the vesicle and the length of the papilla.  This arrangement of the excretory bladder, in addition to flame cell patterns, differs from those of both the Paramphistomidae and Microscaphidiidae, however, a coincidental pattern is seen in the Dicrocoeliidae Odhner, 1910 and Allocreadiidae Stossich, 1904.  Ozaki (1937b) concluded that the shared morphology of the excretory system indicated phylogenetic relationship between the Gyliauchenidae and the Dicrocoelioidea Odhner, 1910.

Investigations of the lymph system contradicted the findings from the study of the excretory system.  Ozaki (1937b) suggested that the overall morphology of the lymph system of gyliauchenids was best paralleled in the Microscaphiidae and Paramphistomidae; in these groups, the system consists of several independent longitudinal canals, each with one large nucleus.  *Petalocotyle nipponica* Ozaki, 1934 was found to display the most basal condition; there are canals in the neck region only and much unfilled space near the gut, which is used to store nutrients.  Ozaki hypothesised that lengthening and anastomosing of these canals developed in the other groups, from the apparently basal condition exemplified by

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*P. nipponica*, as a nutrient transport system. Despite the differences observed between the excretory system of gyliauchenids and that of the paramphistomes and microscaphidiids, Ozaki (1937b) suggested that the amphistomes were basal to the distomes, rather than simplified distomes; evolution within the Digenea had proceeded from the amphistomatous condition to the distomes. Ozaki concurred with the systematic position of Nicoll (1915), Goto & Matsudaira (1918) and Goto (1919) that the Gyliauchenidae shares common ancestry with the Paramphistomidae, however, maintained that the Gyliauchenidae should be retained as a separate family. Ozaki proposed a classification of the Paramphistomoidea, containing three families, the Paramphistomidae, Microscaphidiidae and Gyliauchenidae. Under this scheme, the Gyliauchenidae was composed of two subfamilies: Gyliaucheninae Fukui, 1929 and Petalocotylinae Ozaki, 1937 (*Petalocotyle* was removed from the Allocreadiidae). The Paramphistomoidea was recognised as separate from another group of amphistomes comprising the Opistholebetidae and Cephaloporidae Trevassos, 1934.

Although there is no direct statement of affinity, the Gyliauchenidae is listed directly after the Paramphistomidae in the classification of the Digenea by Yamaguti (1971); the implication from this listing is that the two groups are sufficiently similar enough to be identified along side one another. Yamaguti (1971) based his classification scheme on a combination of adult morphology, life-cycle information and ecological data such as habitat within host, types of host infected and environment of host. The interpretation of the Gyliauchenidae by Yamaguti (1971) does not include *Petalocotyle* within the family. Yamaguti (1971) classified *Petalocotyle* with *Robphildollfusium* Paggi & Orecchia, 1963 as the Petalocotylinae within the Lepocreadiidae Nicoll, 1914 and suggested that the Robphildollfusiidae Paggi & Orecchia, 1963 should be suppressed in favour of the Lepocreadiidae.

The contention that distomatous digeneans arose from amphistomatous ancestors (Ozaki, 1937b) was strongly refuted by Cable (1954). Cable hypothesised that the amphistomatous condition had arisen independently from distomes in several lineages of digeneans and that gyliauchenids were in fact aberrant lepocreadioids. Ironically, Cable supported this suggestion with evidence from Ozaki. Cable suggested that the lymph channels possessed by gyliauchenids are similar to those of the Megasolenidae Manter, 1935, Megaperidae Manter, 1934 and the genera *Apocreadium* Manter, 1937 and *Choanodera* Manter, 1940 (Apocreadiidae Skrjabin, 1942). The posterior migration of the ventral sucker is

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common and widespread in the Digenea; examples can be seen in the families Hemiuridae Looss, 1899, Opistholebetidae, Cephaloporidae and Paramphistomidae. Cable asserted that in these instances, each group represents an independent derivation from distomatous ancestors. A previous survey of digeneans from fishes of the Galapagos Islands (Manter, 1940) identified the Megasolenidae within the Gyliauchenidae, in a classification comparable to that proposed by Cable (1954). The classification of the Digenea based on the structure of the excretory system proposed by La Rue (1957) paralleled that of Cable: the Gyliauchenidae was provisionally assigned to the Allocreadioidea Looss, 1902, an assemblage comprising the Allocreadiidae Looss, 1902, Acanthocolpidae Luhe, 1906, Lepocreadiidae, Megaperidae, Monorchiidae Odhner, 1911, Opecoelidae Ozaki, 1925 and Opistholebetidae. Three other families, the Gorgoderidae Looss, 1901, Troglotrematidae Odhner, 1914 and Zoogonidae Odhner, 1902, were also assigned to this group. Membership of the Gyliauchenidae within the Allocreadioidea was upheld by Srivastava & Ghosh (1972). These authors contended that the gyliauchenids were most closely related to the Lepocreadiidae.

The two established hypotheses of the affinities of the Gyliauchenidae, that they are related to paramphistomes or that they are aberrant lepocreadiids, were tested with molecular data (Blair & Barker, 1993; Barker *et al.*, 1993). Parsimony analysis of an alignment of partial small subunit (18S) rDNA genes lent strong support in favour of a sister-group relationship between the Gyliauchenidae and Lepocreadiidae. Blair & Barker (1993) found no support for any association between gyliauchenids and paramphistomes; the authors concluded that any morphological similarities are homoplastic. A second analysis, using partial large subunit (28S) rDNA genes (Barker *et al.*, 1993), supported the findings of Blair & Barker (1993) that gyliauchenids are more closely related to lepocreadiids than either is to paramphistomes, however, the results were less definitive. Bootstrap resampling of the data set generated only 52-56% support for monophyly of Lepocreadiidae + Gyliauchenidae. The results of this study may have been confounded by poor taxon sampling; one of the species of lepocreadiid, *Tetracerasta blepta* Watson, 1984, was suggested by Bray & Gibson (1991) to belong within the Enenteridae Yamaguti, 1958, although current studies (Cribb *et al.*, 2001; Olson *et al.*, 2003) place this species within lepocreadiids. Parsimony analyses of 18S (Olson *et al.*, 2003) and 28S (Cribb *et al.*, 2001) rDNA genes indicate good support for a monophyletic assemblage comprising the Lepocreadiidae, Enenteridae and Gyliauchenidae.

## Intra-familial affinities

There have been few attempts to investigate the inter- and intra-generic relationships of the Gyliauchenidae. Early classifications recognised that *Apharyngogyliauchen* Yamaguti, 1942 was unusual in lacking an oesophageal bulb and Yamaguti (1958) placed the genus within its own subfamily, the Apharyngogyliaucheninae Yamaguti, 1958, to the exclusion of the remainder of the family. Manter & Pritchard (1962) invalidated this subfamily; they contended that the monotypic *Leptobulbus* occupied a position intermediate between *Paragyliauchen* and *Apharyngogyliauchen*. Although an oesophageal bulb is present in *Leptobulbus*, its development is poor and, according to Manter & Pritchard, warranted exclusion of *L. magnacirratus* from *Paragyliauchen*. Manter & Pritchard also drew parallels between *Leptobulbus* and *Apharyngogyliauchen*; *L. magnacirratus* possesses an almost straight oesophagus and is found in scarid hosts. Yamaguti (1970) maintained the validity of *Leptobulbus*, citing the poor development of the oesophageal bulb and well developed male terminal genitalia as justification for the genus. The division of gyliauchenids into two subfamilies, the monogeneric Apharyngogyliaucheninae and the Gyliaucheninae, was retained by Yamaguti (1971), however, no proposal of the internal relationships of the Gyliaucheninae was suggested. A third subfamily within the Gyliauchenidae was recognised by Ozaki (1937b); the monotypic Petalocotylinae was later placed with *Robphildollfusium* within the Lepocreadiidae by Yamaguti (1971). Neither *Petalocotyle* nor *Robphildollfusium* were acknowledged as members of the Gyliauchenidae in the review by Nahhas & Wetzel (1995).

## 1.3  Biology of the Gyliauchenidae Fukui, 1929 (1918)

Very little is known of the biology of the Gyliauchenidae; the life-cycle is unknown and only recently has the functional anatomy of the digestive tract been described (Jones *et al*., 2000). Cannon (1978) described a cercaria from the marine gastropod, *Cerithium moniliferum* Kiener, from Heron Island, Queensland, Australia, which he suggested may belong within the Gyliauchenidae. This life-cycle has, however, never been completed, and the association of this cercaria with the Gyliauchenidae can not be confirmed. Many authors record that gyliauchenids are a pale yellow to bright orange-red colour in life (*eg*: Goto & Matsudaira, 1918; Ozaki, 1937b; Nagaty, 1956; Manter & Prichard, 1962; Wang, 1977; Machida, 1984; Nahhas & Wetzel, 1995). Goto & Matsudaira (1918) attributed this distinctive colouration to

the presence of fine pigment granules distributed throughout the parenchyma. Hughes-Stamm *et al*. (1999) examined the ultrastructure of the tegument of *M. spio* n. sp. (as *Gyliauchen nahaensis* Ozaki, 1937) using scanning Electron Microscopy. These authors found that the syncytial tegument supported a rich diversity of micro-organisms, which forms a non-invasive biofilm. The presence of mitochondria in the basal matrix suggests that the tegument functions in nutrient absorption (Hughes-Stamm *et al.*, 1999).

The Gyliauchenidae is distinguished by an unusual suite of adaptations to herbivory, the most striking of which occur in the gut (Jones *et al.*, 2000). Most digeneans have a simple, blind-ended gut. They are lumenal parasites, feeding on easily digestable mucosa, supplemented with host gut content. Gyliauchenids, however, apparently have efficient strategies to ferment and digest the high cellulose diet. The gut is an efficient digestive organ. In some cases, the oesophagus is grossly elongated and coiled, and may function as a fermentation chamber, whereas the caeca are reduced in size, but have a thickened gastrodermal lining which is dedicated to absorption (Jones *et al.*, 2000). There has been much debate surrounding the terminology of the organs of the digestive tract. Early workers regarded the anterior-most organ as an oral sucker, and the organ immediately anterior to the caeca as a pharynx. Ozaki (1937b) examined the histology of the "pharynx" and determined that it was derived from oesophageal cells and was not a separate, membrane-bound structure. This organ has strongly developed longitudinal and circular muscles and is referred to as the oesophageal bulb. The homology of the anterior-most organ is still unclear; Pearson (1992) presents histological evidence which suggests that the single structure seen in paramphistomes is a pharynx, rather than an oral sucker. Pearson (1992) concludes that the oesophageal bulb is of oesophageal origin and is not a pharynx. Histological sections have, however, been of little value in determining the homology of the anterior organ in gyliauchenids (Blair & Barker, 1993). The criterion of symmetry, delineated by Pearson (1992), suggests that this anterior-most structure is best interpreted as a pharynx.

The morphological specialisation of the Gyliauchenidae is coincident with a limited range in terms of both host taxa and geography. Gyliauchenids are almost entirely restricted to herbivorous host fishes, principally members of the Acanthuroidei (Acanthuridae, Zanclidae and Siganidae) and the Chaetodontidae, Pomacanthidae and Scaridae, although small numbers of specimens have been obtained from other groups of fishes including the Labridae and Pomacentridae. The majority of gyliauchenids, however, is restricted to the surgeon fishes

(Acanthuridae) and rabbit fishes (Siganidae) (Nahhas & Wetzel, 1995). Gyliauchenids are found only in fishes of the reefs of the Indo-Pacific; gyliauchenids have never been recorded from fishes of the Atlantic Ocean despite the comprehensive parasite surveys in these waters, and although some host taxa are represented in the Mediterranean and Caribbean seas.

## 1.4 Macroevolutionary patterns

Macroevolution may be loosely defined as "evolution on a grand-scale", a process which encompasses morphological change, driven by inheritance, which results in the formation of taxa above the specific level (Erwin, 2000; Ridley, 1997). Macroevolutionary studies aim to understand processes and patterns which influence the evolution of entire clades. Erwin (2000) suggests that there is no distinct process of "phylisation"; clades and higher taxa, and the morphological characters which define them, result from multiple speciation events distributed across space and time. Whether clade-wide patterns are the result of the accumulation of microevolution (speciation) or driven by separate patterns has been the source of much historical debate (see Brooks, 1988; Erwin, 2000). Regardless of the processes underlying macroevolution, there are patterns which may be observed across the phylogenetic history of higher taxa, and these patterns may provide insights into the complex associations of taxa with their environment. Modern parasite systematists use phylogenetic histories of taxa to examine macroevolutionary patterns of two main categories: host-parasite cospeciation and historical biogeography.

### Host-parasite associations

The hypothesis that parasites coevolve with their host lineages is an important and influential concept in modern evolutionary biology (see Brooks & McLennan, 2003). The long history of assocation between parasites and their hosts leads to adaptations in both lineages; this process is known variously as "coevolution" (Page & Holmes, 1998; Ronquist, 1998b), "coadaptation" (Brooks & McLennan, 1993), or "coaccommodation" (Brooks, 1979). The Parasitological Method of Hennig (1966) asserted that the phylogeny of parasites, which exhibited strong host-specificity, must parallel that of the host taxa. The Parasitological Method was rejected by Hennig, and later by Brooks (1979). Brooks (1979; 1981) found that parasites may evolve independent of host phylogeny and that the relationships between

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parasites and their hosts could be plesiomorphic, synapomorphic, autapomorphic, or homoplasious. Brooks suggested that points of congruence between host and parasite phylogenies reflected "cospeciation" events, presumably the result of vicariant speciation (see also Brooks & McLennan, 1993); Page & Holmes (1998) interpreted cospeciation as "contemporaneous cladogenetic events" in host and parasite lineages. Points of incongruence, representing homoplasious relationships between parasites and hosts, may be indicative of a number of evolutionary processes. Host-switching and extinction events may account for disparities between host and parasite phylogenies (Brooks, 1981; Page & Holmes, 1998). Modern analysis techniques, such as Maximum Cospeciation (Page, 1994), aim to explore the extent to which cospeciation, or host-switching, can explain observed patterns of parasite distribution and host-specificity.

## Historical biogeography

The concept that the fauna of the earth can be divided into distinct zoogeographic groups with defined boundaries, which correspond to the continental land masses and their geological history of drift, was first postulated by Alfred Russell Wallace. In the mid 19th Century, Wallace was living in the Malay Archipelago and corresponded extensively with his colleague, Henry Bates; Wallace wrote to Bates of his observations:

> "*In the Archipelago there are two distinct faunas rigidly circumscribed, which differ as much as those of South America and Africa, and more than those of Europe and North America. Yet there is nothing on the map or on the face of the islands to mark their limits... I believe the western part to be a separated portion of continental Asia, the eastern the fragmentary prolongation of a former pacific continent.*" (George in Whitmore, 1981, p. 3).

Throughout the latter part of the century, Wallace made many more observations and refined his hypothesis, drawing a boundary among the islands to separate the Oriental and Australian faunas, which became known as "Wallace's line". The observations of Wallace incited much interest in, and debate regarding, the biogeography of the region. Ornithologists, malacologists, botanists, mammalologists and ecologists interpreted the distribution of plants and animals in the Malay Archipelago and drew further hypothetical boundaries. Regardless of which of these many lines represents a real faunal boundary in the region, an important concept in biology had emerged: that slowly evolving animals and plants had been rafted by the historical drifting of continents to

reach their present distributions. The theory of plate tectonics and continental drift has had substantial implications for the study of present day biogeography. Like Wallace, the modern school of evolutionary biogeography asserts that regional biogeography is a reflection of a combination of interacting factors including the geological, floral and faunal history of an area as well as the contemporary distribution and ecology (Whitmore, 1981).

## Methods in macroevolutionary studies

Over the past decade, there has been much debate regarding methodology and best practice for the investigation of macroevolution, in particular, host-parasite cospeciation. Brooks (1979; 1981; 1998) developed a parsimony-based method, known as Brooks Parsimony Analysis (BPA) (and also secondary BPA, Brooks & McLennan, 2003), which implements the distribution of parasites as independent characters for use in phylogeny elucidation; it is the "fit" between known host phylogeny and the parasite-based phylogeny that indicates cospeciation events. Alternative methodologies to BPA have been developed, including Maximum Cospeciation (Page, 1994), which aims to reconcile independently derived host and parasite phylogenies by maximising the number of cospeciation events (see also Page, 1995; Page & Charleston, 1997, 1998; Ronquist, 1998a, b). In this method, cospeciation is preferred over other events, such as host-switching, extinctions, duplications or sorting-events, and costs are assigned, via a step-matrix, which make it more "expensive" for parasites and hosts to not cospeciate; randomisation of host and parasite phylogenies is used to test confidence intervals for cospeciation. Maximum Cospeciation, or event-based parsimony analysis, has been strongly criticised (*eg*: Brooks & McLennan, 2003; Dowling, 2002; but see also Morrone & Carpenter, 1994) as an inadequate method which overestimates the number of duplications and sorting events that may account for the distributions of widespread parasite taxa. It should be noted, however, that Brooks & McLennan (2003) state that secondary BPA is a preferred method only when parasite-based estimates of host phylogeny are sought. Proponents of event-based parsimony analysis contend, however, that Maximum Cospeciation is a heuristic tool for exploring the likelihood of cospeciation and, when molecular phylogenies are reconciled, has the advantage of incorporating estimated divergence times to choose among equally parsimonious solutions for parasite distributions (Page & Charleston, 1997; Page & Charleston, 1998; Page & Holmes, 1998; Ronquist, 1998b). The theoretical basis of both of these methods is employed also in the exploration of historical biogeography.

There is a lively and ongoing debate in the literature about methodology and best practice for investigating host-parasite associations and historical biogeography. Despite the lack of consensus among workers, biogeography and host-parasite coevolution have become important areas of research in systematics and comparative biology. Brooks (1979; 1981) enunciated the concept that parasites may evolve independently of their hosts, debasing the long held belief that parasites were entirely restricted, in their distribution and phylogenetic history, by their hosts. The hosts of parasites represent both their physical and biological environment, and independent evolutionary events in either will influence the phylogenetic histories of both host and parasite (Page & Holmes, 1998). It is the nature of this interaction, and the sequence of historical events, rather than simply counting cospeciation events, that makes the investigation of macroevolutionary patterns of hosts and parasites biologically interesting. In the case of the Gyliauchenidae, there are recognisable and distinct patterns of host-specificity and biogeographical distribution, which suggest that there may be strong macroevolutionary pressures being enacted upon these worms and their host fishes.

The following sections review the biology and phylogenetic history of herbivorous fishes and introduce the environment of the Indo-West Pacific. This review is important in the context of exploring the nature of host-parasite associations between gyliauchenids and fishes and the geographical co-occurrence of these groups in the waters of the Indo-West Pacific.

## Herbivorous Fishes

### Herbivory in fishes

Herbivory has evolved numerous times within the radiation of teleosts. Gerking (1984) estimated that at least 44 families of fishes have independently adopted plant-eating, and that in coral reef environments approximately half of all fishes are herbivorous (see Table II for a list of reef-dwelling herbivorous fishes). The intense herbivory on coral reefs by fishes has a large ecological impact, has been an important factor influencing the structure of reef communities and has acted as a selective pressure on the evolution of marine plants (Hay, 1991). Both the fishes and plant-life (including algae and higher plants) have adapted to this challenging environment. Herbivorous fishes exhibit various digestive strategies and morphological adaptations, particularly in their dentition and mandibular structure, and marine

algae differ in their growth rates and ability to deter or withstand herbivory through their structure, chemical composition and nutritional value.

**Table II. A list of reef-dwelling teleosts that incorporate large amounts of plant material in the diet (after Gerking, 1984).**

| Taxon (Scientific Family Name) | Common Name |
| --- | --- |
| Acanthuridae | surgeon fishes |
| Balistidae | trigger fishes |
| Blenniidae | blennies |
| Canthigasteridae | sharp-beaked puffer fishes |
| Chaetodontidae | butterfly fishes |
| Gobiidae | gobies |
| Hemiramphidae | half-beaks |
| Kyphosidae | chub, drummers (sometimes pilot fishes) |
| Labridae | wrasses |
| Leiognathidae | silver perch |
| Monocanthidae | file fishes, leather-jackets |
| Mugilidae | mullets |
| Ostraciontidae | trunk fishes |
| Pomacanthidae | angel fishes |
| Pomacentridae | damsel fishes |
| Scaridae | parrot fishes |
| Siganidae | rabbit fishes |
| Sparidae | breams, porgies |
| Tetraodontidae | puffer fishes |

Herbivorous marine fishes may be described as either grazers or browsers depending on their method of food procurement (Hiatt & Strasburg, 1960). Grazers are defined as biting algae very close to the surface from which it grows, often ingesting part of the substrate as they rasp at the plant growth. In contrast, browsers bite definite pieces of algae above the level of the substrate. Some fishes, such as *Microspathodon dorsalis* Gill, 1862 (Pomacentridae), employ a combination of both techniques during feeding, whereas other species are more or less consistently browsers or grazers (Gerking, 1984). Herbivory exacts high demands in terms of digestion and teleosts have evolved numerous strategies to obtain the nutrients locked within the cellulose walls of plant cells (Gerking, 1984):

- Chemical digestion exemplified by a thin-walled, low pH stomach, *eg*: some Acanthuridae and Pomacentridae.
- Chemical digestion facilitated by hindgut fermentation, *eg*: Kyphosidae.

- Mechanical processing within a high pH, muscular stomach with ingested sediment (trituration), *eg*: some Acanthuridae and Sparidae.
- Mechanical processing by a pharyngeal mill, *eg*: Scaridae.

Despite the efficiency of each of these methods of digesting plant material, no adult marine teleosts are known to be exclusive herbivores (Gerking, 1984). Whether animal material is incorporated into the diet of these fishes incidentally during grazing or because of energy requirements, is unclear. Regardless, it is important, from a parasitological perspective, to note that all marine fishes are zooplantivores as larvae and maintain omnivory to some degree throughout the entire life history.

## Dominant hosts of the Gyliauchenidae

Gyliauchenids are overwhelmingly restricted to reef-dwelling herbivorous marine fishes. There are intermittent reports in the literature of non-herbivorous fishes, such as synodontids and engraulids playing host to gyliauchenids (Srivastava, 1938; Gupta & Tandon, 1983); indeed the type species for gyliauchenids, *Gyliauchen tarachodes*, was first recorded from the pilot fish "*Tachysurus* n. sp." (Ariidae) by Nicoll (1915). With the passage of time it becomes increasingly difficult to ascertain the accuracy of such records. The significance of these records as representative of the entire group diminishes as further records and new species emerge. Of all the described gyliauchenids to date, only *G. indicum* Gupta & Tandon, 1983 has never been reported from a herbivorous fish. The remaining species span a wide range of host groups, including both herbivorous and non-herbivorous fishes, however, one assemblage in particular can be seen as the dominant host group; the Acanthuroidea (including the Acanthuridae, Siganidae and Zanclidae) is host to 13 (52%) of the 25 described gyliauchenids of the Indo-West Pacific. Further, some genera are strongly associated with some host groups. Species of *Apharyngogyliauchen* have been recorded only from labroid fishes and species of *Paragyliauchen* and *Flagellotrema* are commonly recorded only from chaetodontid and pomacanthid fishes.

## Suborder Acanthuroidei

The Suborder Acanthuroidei is a speciose group of percoid fishes, containing approximately 125 species (Tang *et al*., 1999) and, according to the classification of Winterbottom (1993), is comprised of six families: Ephippidae, Scatophagidae, Siganidae, Luvaridae, Zanclidae and Acanthuridae. The group consists primarily of shore-dwelling herbivorous marine fishes, with the exception of the monotypic, pelagic *Luvarus*. The Acanthuroidei has a cosmopolitan distribution, however, the majority of species are concentrated within the Indo-West Pacific Region, with some groups, such as the siganids, being entirely restricted to these waters.

Historically, the Acanthuroidei was a small and compact group, containing only the rabbit fishes (Siganidae), the moorish idol (Zanclidae) and the surgeon fishes (Acanthuridae). Recent phylogenetic studies based on osteological, myological and molecular evidence (Johnson & Washington, 1987; Tyler *et al*., 1989; Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al*., 1999), however, have expanded the group to include bat fishes (Ephippidae), scats (Scatophagidae) and the unusual and monotypic *Luvarus* (Luvaridae). These studies indicate also that siganids, *Luvarus*, *Zanclus* and the acanthurids form a monophyletic assemblage, the Superfamily Acanthuroidea, with scatophagids and ephippids as sequential sister-groups to this clade. Within the Acanthuroidea, there is broad conservation of diet (herbivory), life-cycle (long-lived pelagic larvae which settle in shore, generally on reefs) and distribution (general restriction to the Indo-West Pacific), although there are examples of exceptions to these conditions. As previously mentioned, these fishes represent the dominant host group for gyliauchenids, and consequently, this discussion of host fishes will be restricted to the Acanthuroidea.

## Superfamily Acanthuroidea

Monophyly of the Acanthuroidea (= Acanthuroidei *sensu stricto*: Tyler *et al*., 1989) is supported by the possession of a unique, highly specialised planktonic larva called the acronurus (Leis & Rennis, 1983). The acronurus larva is unique among percoid fishes in as much as it is one of the largest, reaching lengths of up to 60 mm, and has the longest planktonic duration (Tyler *et al*., 1989). In addition to the extended pelagic duration of the acronurus, Tyler *et al*. (1989) found a combination of eight characters of the morphology of the larvae and 11 adult morphological characters which unite the Acanthuroidea as a

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monophyletic assemblage.  The most basal taxon within the group is the Siganidae, which forms the sister to a group containing the Luvaridae, Zanclidae and Acanthuridae (Johnson & Washington, 1987; Tyler *et al.*, 1989; Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al.*, 1999).  The monotypic *Luvarus* Rafinesque, 1810 and *Zanclus* Cuvier, 1831 are sequential sister-groups to the derived and speciose Acanthuridae, although the molecular phylogeny presented by Tang *et al.* (1999) indicated a possible relationship between the acanthurid genus *Naso* Lacepède, 1801 and *Zanclus*.  Monophyly of *Zanclus* + *Naso* was dismissed by these authors as being an artefact of long-branch attraction, and was unsupported by a total evidence analysis including morphological data.

## Siganidae

Modern rabbit fishes are a dominant herbivorous component of the fish fauna of the Indo-West Pacific (Woodland, 1983; Tyler & Bannikov, 1997) and are primarily associated with coral reefs, although they are also found in other algae-rich environments such as mangroves and rocky shores.  Adult fishes are moderate in size, reaching lengths of up to 53 cm (for *S. javus*; Allen, 1981).  The family is monogeneric, with all 27 extant species (Woodland, 1983) housed in *Siganus* Forsskål, 1775 (see Table III).  Some authors (Woodland, 1983; 1990; 2001) divide *Siganus* into two subgenera, *Siganus* and *Lo* Seale, 1906, based on the possession of a tubular snout by the latter; *Siganus Siganus* contains 22 species, with the remaining five housed in *Siganus Lo*.  Monophyly of the Siganidae is supported by 18 morphological characters, including the unusual divided palatine, unique among modern teleosts, and the possession of two spines separated by three soft rays on the pelvic fins (Tyler & Bannikov, 1997).  There is only one autapomorphy of *Siganus*: the possession of a posterior barb on the procumbent pterygial spine.  The individual species of *Siganus* are all broadly similar morphologically, and are distinguished by patterns of coloration and differing body proportions.

The morphology of recent and fossil siganids is conserved; fossil specimens of *Ruffoichthys* Sorbini, 1983 differ slightly from the modern *Siganus*, but can be distinguished by the possession of only one spine on the pelvic fin (Tyler & Bannikov, 1997).  This fossil record indicates that modern rabbit fishes arose from ancestors dating back to the middle Eocene, approximately 47 mya.  During the Eocene and Oligocene (~47-24 mya) there was greater generic diversity among siganids; there are three other fossil genera, *Eosiganus* Tyler

& Bannikov, 1997, *Siganopygaeus* Danilchenko, 1968 and *Protosiganus* Whitley, 1935, which, in addition to *Ruffoichthys* and *Siganus*, comprise the Siganidae. Tyler & Bannikov (1997) could not estimate how speciose these early extinct genera were, however, they suggested that the broad morphological conservation in appearance of siganids from the time of the Eocene indicates that the ancestral species may have emerged as early as the Paleocene or Upper Cretaceous (~66 mya).

The diet of modern siganids consists principally of fleshy macroalgae supplemented by small amounts of filamentous algae, although the pelagic larvae and post-larvae are planktivorous (Woodland, 1990). This restriction in diet to herbivory after settlement has confined the distribution of *Siganus* to littoral (shallow) and sublittoral zones (Woodland, 1983); 13 species exclusively inhabit coral reefs, one species is estuarine, and the remainder are known to frequent reefs for at least some of their lifespan. Modern siganids are restricted entirely to the Indo-West Pacific, although two economically important species have been introduced to the Mediterranean Sea via the Suez Canal (Woodland, 1990). Species richness of *Siganus* is highest within the "fertile triangle" (*sensu* Briggs, 1974) of Indo-Malaya, whereas few species are found in French Polynesia, and the waters of East Africa are inhabited by an intermediate level of species. This pattern of species abundance and distribution within the Indo-West Pacific region is mirrored by similar patterns of other marine species such as fiddler crabs, corals and other fishes (*eg*: Allen, 1972; Crane, 1975; George & Jones, 1982), and has led to the suggestion by Briggs (1974; 1992) that Indo-Malaya is an evolutionary hotspot which displays high rates of speciation. Regardless of centralisation of species richness, Woodland (1983) believed that the confluence of the Indian and Pacific oceans has led to greater species richness in Indo-Malaya and that this richness is an artefact of pooling species from two oceans.

Speciation and radiation of *Siganus* may have been promoted by the geographical barrier of the Indo-Malayan peninsula and environmental barriers of the Malacca Strait and Java Sea. Woodland (1983) cited five species pairs, which are identical in body proportion, but exhibit different patterns of coloration, as evidence for allopatric speciation in *Siganus* by vicariance. The extreme morphological similarity of these sibling species suggests a recent separation. Allozyme electrophoretic studies (Lacson & Nelson, 1993) support this hypothesis. The modern distribution of the sibling species is virtually parapatric, with the boundary for four of the pairs passing through the Indo-Malayan region. Woodland

hypothesised that the Pleistocene glaciations (~0.7 mya) induced conditions in Indo-Malaya which promoted allopatric, or possibly parapatric, speciation. During the Pleistocene, the Malacca Strait and Java Sea were characterised by conditions which persist today: low salinity and muddy bottoms, high turbidity and poor coral development (Defant, 1961; Tija, 1966; Van Andel *et al.*, 1967). These conditions may have acted as partial barriers to corallophilic and shelf species, leading to speciation events.

The Pacific Ocean appears to have been more important in the speciation of the 17 remaining modern siganids; Woodland (1983) attributed their radiation to allopatric separation related to the colonisation ability of the fishes. Only four siganids are widely distributed throughout the entire Indo-West Pacific, and of these, only two, *S. argenteus* and *S. spinus*, range as far east as French Polynesia. This range appears to be a consequence of the behaviour of the pre-settlement larvae. The larvae of *S. argenteus* are uniquely adapted for extended pelagic existence, reaching 6-8 cm in length at sea before settlement and metamorphosis in reef environments. The larvae of *S. spinus* lack this pre-juvenile adaptation, however, the larvae are pelagic and display a synchronised migration to reefs for settlement. Despite ranging throughout the Indo-West Pacific, *S. argenteus* appears to retain gene-flow throughout the entire region, thereby inhibiting speciation (Woodland, 1983). In contrast, *S. spinus* has a sibling species, *S. luridus*, with an allopatric distribution in the West Indian Ocean.

Species of the subgenus *Lo*, in contrast to the widely distributed *S. argenteus* and *S. spinus*, are closely associated with *Acropora* coral and have restricted distributions. The unusual morphology of these fishes, which have specialised tubular snouts which are used to probe crevices in coral, suggests they are recently derived (Woodland, 1983). *S. Lo vulpinus* is centrally and widely distributed in the reefs of South-East Asia and the Western Pacific Ocean. *S. L. uspi* is restricted to Fiji, although it is occasionally sighted in New Caledonia; Woodland (1983) suggested that *S. L. uspi* may have diverged approximately 9 mya when Tonga and Fiji were isolated by sea floor spreading. The three remaining species in the subgenus, *S. L. magnificus*, *S. L. niger* and *S. L. unimaculatus*, have narrow and restricted ranges, suggesting that each has evolved in allopatry from small isolated populations of *S. L. vulpinus* (Woodland, 1983; 1990).

**Table III.  Composition of *Siganus* (after Woodland, 1990; 2001).**  Type-species for each genus are denoted with an asterisk (*).

| Genus | Subgenus | Species | Authority |
|---|---|---|---|
| *Siganus* | *Siganus* | *argenteus* | (Quoy & Gaimard, 1825) |
| | | *canaliculatus* | (Park, 1797) |
| | | *corallinus* | (Valenciennes, 1835) |
| | | *doliatus* | Cuvier in Guérin-Méneville, c. 1830 |
| | | *fuscescens* | (Houttuyn, 1782) |
| | | *guttatus* | (Bloch, 1787) |
| | | *javus* | (Linnaeus, 1766) |
| | | *labyrinthoidea* | (Bleeker, 1853) |
| | | *lineatus* | (Valenciennes, 1835) |
| | | *luridus* | (Rüppell, 1829) |
| | | *randalli* | Woodland, 1990 |
| | | *puelloides* | Woodland & Randall, 1979 |
| | | *puellus* | (Schlegel, 1852) |
| | | *punctatissimus* | Fowler & Bean, 1929 |
| | | *punctatus* | (Schneider in Bloch & Schneider, 1801) |
| | | *rivulatus* | Forsskål, 1775 |
| | | *spinus** | (Linnaeus, 1758) |
| | | *stellatus* | Forsskål, 1775 |
| | | *sutor* | (Valenciennes, 1835) |
| | | *trispilosus* | Woodland & Allen, 1977 |
| | | *vermiculatus* | (Valenciennes, 1835) |
| | | *virgatus* | (Valenciennes, 1835) |
| | *Lo* | *magnificus* | (Burgess, 1977) |
| | | *niger* | Woodland, 1990 |
| | | *unimaculatus* | (Evermann & Seale, 1907) |
| | | *uspi* | Gawel & Woodland, 1974 |
| | | *vulpinus** | (Schlegel & Müller, 1845) |

## Luvaridae

The louvar, *Luvarus imperialis* Rafinesque, 1810 is a strange and rarely collected cosmopolitan fish found in both tropical and temperate waters. The unusual morphology and bizarre metamorphosis obscured the affinities of this monotypic family until recent phylogenetic studies (Tyler *et al*., 1989; Tang *et al*., 1999) supported an association with the Acanthuroidea. Earlier workers hypothesised various relationships, placing *Luvarus* with the scombrids and carangids, however, there is strong morphological and molecular evidence which indicates that *Luvarus* is the sister-group to the Zanclidae + Acanthuridae. In the study by Tyler *et al*. (1989), eight synapomorphies based on adult morphology were found that support monophyly of *Luvarus*, *Zanclus* and the acanthurids, and seven autapomorphies of *Luvarus*; a further seven larval characters unite the group.

Little is known of the biology of *L. imperialis*; the morphology of the gut is dominated by a long, coiled intestine and is similar to that of other acanthuroid fishes, suggesting a herbivorous diet. *Luvarus* is unique among acanthuroids, however, in having adopted an entirely pelagic existence. Tyler *et al*. (1989) suggested that the conservation of gut morphology indicates that *Luvarus* has simply taken the browsing and grazing feeding habits of acanthuroids to the open sea, foraging on coelenterates and ctenophores rather than algae. They suggest further that the unusual series of larval metamorphosis is the result of heterochrony; the ancestral and pelagic juvenile stage may have experienced delays in settlement, due the absence of physical stimuli, and sexual development occurred. The pressures of a pelagic existence exerted extreme selective pressures on the ancestral luvarids, resulting in the remarkable and unusual extant species.

## Zanclidae

The Zanclidae (moorish idols) is represented by only one species, *Zanclus cornutus* (Linnaeus, 1758), which is distributed widely throughout the Indo-Pacific and Eastern Pacific in association with tropical reefs. The development of *Zanclus* is similar to other acanthuroid fishes and the larvae experience a prolonged pelagic maturation. Although the diet of *Zanclus* includes substantial amounts of algae, the moorish idol has adapted to omnivory, foraging on benthic invertebrates, such as sponges (Winterbottom & McLennan, 1993). Early classifications included the moorish idol within the Acanthuridae (*eg*: Nelson, 1984);

numerous specialisations of the larvae (Johnson & Washington, 1987) and adult osteological characters, however, indicate that *Zanclus* is best placed within its own family.

Monophyly of *Zanclus* and the acanthurids is supported by a total of 13 synapomorphies from larval and adult morphological characters (Tyler *et al*., 1989). The larvae of *Zanclus* and *Luvarus* share three common features to the exclusion of acanthurids; Johnson & Washington (1987) interpreted these characters as homoplastic, suggesting independent acquisitions in *Zanclus* and *Luvarus*, or reversal in the ancestral acanthurids. Tang *et al*. (1999) presented a molecular phylogeny, based on mitochondrial genes, which indicated a strongly supported monophyletic relationship between *Zanclus* and *Naso* (Acanthuridae). They rejected this relationship, however, speculating that their analysis was confounded by factors such as long-branch attraction. Total evidence phylogenies, generated using morphological and molecular data, rejected the relationship between *Zanclus* and *Naso* (Tang *et al*., 1999), and corroborated the placement of *Zanclus* as a sister to the Acanthuridae, as postulated by Guiasu & Winterbottom (1993) and Winterbottom (1993).

## Acanthuridae

Acanthurids are commonly observed fishes and represent a dominant component of the herbivorous fish fauna of coral reefs. They are colloquially known as surgeon fishes in reference to the scalpel-like modified scales, which are believed to function in defence, on the caudal peduncle of all species. Surgeon fishes have a wide distribution; they are found in all tropical and subtropical waters, with the exception of the Mediterranean Sea. The Acanthuridae is the most speciose family within the Acanthuroidea, containing 72 species within six genera: *Naso* Lacepède, 1801 (17 species), *Prionurus* Lacepède, 1804 (6 species), *Zebrasoma* Swainson, 1839 (6 species), *Paracanthurus* Bleeker, 1863 (monotypic), *Ctenochaetus* Gill, 1884 (6 species) and *Acanthurus* Forsskål, 1775 (36 species) (see Table IV for family composition). Although surgeon fishes represent a large, diverse and specialised group of fishes, monophyly of the Acanthuridae was supported by 11 morphological synapomorphies (three based on adult morphology and eight larval characters) in the study by Tyler *et al*. (1989). More recent phylogenetic analyses of the group (Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al*., 1999) have corroborated the findings of Tyler *et al*. (1989) and have found additional support for monophyly based on osteological and myological evidence.

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The internal relationships of the Acanthuridae have been well studied. Early taxonomic hypotheses (Aoyagi, 1943) suggested a linear or gradual relationship among the genera whereby *Naso* was basal, followed by *Prionurus*, *Paracanthurus*, *Zebrasoma*, and *Acanthurus*. *Ctenochaetus* was suggested to be derived from *Acanthurus* as a specialised offshoot. The basal position of *Naso* was rejected by Tyler (1970); the extreme morphological specialisation of the group suggested to Tyler that *Naso* were specialised *Prionurus*. Although he did not perform a phylogenetic analysis, Tyler (1970) hypothesised, based on osteological observations, that the acanthurids were derived from a *Prionurus*-like ancestor and had diverged into two groups. The first group contained the modern *Prionurus* and *Naso*. The second group contained the remaining genera, however, the interrelationships remained unclear; Tyler (1970) agreed with Aoyagi (1943) that *Ctenochaetus* were specialised *Acanthurus*, but could not determine the branching order of the two intermediate genera, *Zebrasoma* and *Paracanthurus*. Recent phylogenetic studies (Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al*., 1999) provided some support for the earlier hypothesis of Aoyagi. All three analyses were congruent, placing *Naso* as a sister-group to the remaining genera. Further, these phylogenies indicated that *Prionurus* is the sister to a crown group containing (*Zebrasoma* + *Paracanthurus*) and (*Acanthurus* + *Ctenochaetus*). Winterbottom (1993) signalled that the taxonomy of *Acanthurus* may require revision, by his tentative suggestion of paraphyly. *Acanthurus* was found to be paraphyletic with respect to *Ctenochaetus* in the molecular analysis by Tang *et al*. (1999), further supporting the hypothesis of Aoyagi that *Ctenochaetus* are a specialised subgroup within *Acanthurus*.

**Table IV.  Composition of the Acanthuridae (after Randall, 1955a; 1956; Winterbottom, 1993).**  Type-species for each genus are denoted with an asterisk (*).

| Subfamily | Genus | Species | Authority |
|---|---|---|---|
| Nasinae | *Naso* | *annulatus* | (Quoy & Gaimard, 1825) |
| | | *brachycentron* | (Valenciennes, 1835) |
| | | *brevirostris* | (Valenciennes, 1835) |
| | | *caesius* | Randall & Bell, 1992 |
| | | *caeruleacauda* | Randall, 1994 |
| | | *fageni* | Morrow, 1954 |
| | | *hexacanthus* | (Bleeker, 1855) |
| | | *lituratus* | Forster, 1801 |
| | | *lopezi* | Herre, 1927 |
| | | *maculatus* | Randall & Struhsaker, 1981 |
| | | *minor* | (Smith, 1966) |
| | | *reticulatus* | Randall, 2001 |
| | | *thorpei* | Smith, 1966 |
| | | *thynnoides* | (Valenciennes, 1835) |
| | | *tuberosus* | Lacépède, 1801 |
| | | *unicornis** | (Forsskål, 1775) |
| | | *vlamingii* | (Valenciennes, 1835) |
| Acanthurinae | *Prionurus* | *biafraensis* | (Blache & Rossignol, 1961) |
| | | *laticlavius* | (Valenciennes, 1846) |
| | | *maculatus* | Ogilby, 1887 |
| | | *microlepidotus** | Lacépède, 1804 |
| | | *punctatus* | Gill, 1862 |
| | | *scalprus* | Valenciennes, 1835 |
| | *Paracanthurus* | *hepatus** | (Bloch & Schneider, 1801) |
| | *Zebrasoma* | *flavescens* | (Bennett, 1828) |
| | | *gemmatum* | (Valenciennes, 1835) |
| | | *rostratum* | (Günther, 1875) |
| | | *scopas* | (Cuvier, 1829) |
| | | *veliferum** | (Bloch, 1795) |
| | | *xanthurum* | (Blyth, 1852) |
| | *Ctenochaetus* | *binotatus* | Randall, 1955 |
| | | *hawaiiensis* | Randall, 1955 |
| | | *marginatus* | (Valenciennes, 1835) |
| | | *striatus* | (Quoy & Gaimard, 1825) |
| | | *strigosus** | (Bennett, 1828) |
| | | *tominiensis* | Randall, 1955 |
| | *Acanthurus* | *achilles* | Shaw, 1803 |
| | | *albipectoralis* | Allen & Ayling, 1987 |
| | | *auranticavus* | Randall, 1956 |
| | | *bahianus* | Castelnau, 1855 |
| | | *bariene* | Lesson, 1830 |
| | | *bleekeri* | Günther, 1861 |
| | | *blochii* | Valenciennes, 1835 |

| Subfamily | Genus | Species | Authority |
|-----------|-------|---------|-----------|
| | *Acanthurus* | *chirurgus* | (Bloch, 1787) |
| | (cont.) | *coeruleus* | Bloch & Schneider, 1801 |
| | | *doreensis* | Cuvier & Valenciennes, 1835 |
| | | *dussumieri* | Cuvier & Valenciennes, 1835 |
| | | *fowleri* | de Beaufort, 1951 |
| | | *gahhm* | (Forsskål, 1775) |
| | | *glaucopareius* | Cuvier, 1829 |
| | | *grammoptilus* | Richardson, 1843 |
| | | *guttatus* | Bloch & Schneider, 1801 |
| | | *leucocheilus* | Herre, 1927 |
| | | *leucopareius* | (Jenkins, 1903) |
| | | *leucosternon* | Bennett, 1832 |
| | | *lineatus* | (Linnaeus, 1758) |
| | | *maculiceps* | (Ahl, 1923) |
| | | *mata* | Cuvier, 1829 |
| | | *mindorensis* | Herre, 1927 |
| | | *monroviae* | Steindachner, 1876 |
| | | *nigricans* | (Linnaeus, 1758) |
| | | *nigricauda* | Duncker & Mohr, 1929 |
| | | *nigrofuscus* | (Forsskål, 1775) |
| | | *nigroris* | Cuvier & Valenciennes, 1835 |
| | | *nubilis* | (Fowler & Bean, 1929) |
| | | *olivaceus* | Bloch & Schneider, 1801 |
| | | *polyzona* | (Bleeker, 1868) |
| | | *pyroferus* | Kittlitz, 1834 |
| | | *sohal** | (Forsskål, 1775) |
| | | *tennenti* | Günther, 1861 |
| | | *thompsoni* | (Fowler, 1923) |
| | | *triostegus* | (Linnaeus, 1758) |
| | | *xanthopterus* | Cuvier & Valenciennes, 1835 |

**_Naso_ Lacepède, 1801**

Members of the genus *Naso* are distinguished from other acanthurids by the presence of a horn or protuberance on the head of some of species, and it is for this feature that are commonly named the unicorn fishes.  The presence or absence of the characteristic protuberance has carried taxonomic importance for some authors (see Randall, 1955a) who split the group into two or more genera.  Randall (1955a) suggested that the basis for this distinction was weakened by sexual dimorphism and arbitrary definitions of a horn versus a bony prominence, and rejected any subdivision of the group.  Winterbottom (1992) suggested a sister-group relationship between two species, *N. minor* and *N. thynnoides*, on the basis of the possession of only one plate on either side of the caudal peduncle, unlike their congeners, which have two.  Randall (1994) described a third single-plated species, *N. caeruleacauda*, which he placed with the other two species within the subgenus *Axinurus*.  Phylogenetic analysis using myological data (Borden, 1998) indicated monophyly of the single-plated species; *N. caeruleacauda* was basal to *N. minor* + *N. thynnoides*, supporting the relationship hypothesised by Winterbottom (1992).  The phylogeny of Borden (1998) was poorly resolved and any attempts at taxonomic revision on the basis of this tree would have been premature.

Unicorn fishes are generally herbivorous and are entirely restricted to the reefs of the tropical Indo-West Pacific, extending in range from East Africa to the Hawaiian Islands (Borden, 1998).  Optimisation of phylogenetic hypotheses of the interrelationships of the Acanthuridae (Winterbottom & McLennan, 1993) indicated that macrophytic herbivory represents the ancestral condition within *Naso*.  This suggestion was corroborated by character optimisation of the phylogeny of Borden (1998).  The basal unicorn-fishes, *N. unicornis*, *N. lituratus*, *N, brachycentron* and *N. tuberosus* all graze on leafy, fleshy algae such as *Sargassum* and brown algae.  In contrast, zooplanktivory has been adopted once by the remaining members of *Naso*, which form a monophyletic assemblage.  The alteration in diet and feeding behaviour in *Naso* is associated with changes to the morphology of the maxilla, enabling these fishes to create buccal vacuums for ingesting and filtering large volumes of zooplankton (Borden, 1998).  The dietary switch in *Naso* has been a remarkably effective strategy.  Winterbottom & McLennan (1993) suggested that the adoption of zooplanktivory has been more successful in *Naso* than other acanthurid genera because the ancestral *Naso* were not as specialised to herbivory than the more derived acanthurids, such as *Acanthurus*, and this lack of pre-adaptation promoted their success and subsequent radiation.

### *Prionurus* **Lacepède, 1804**

Species of *Prionurus* are similar to those of *Naso* in that they possess spinulose body scales and have fixed plates on each side of the caudal peduncle, however, they can be distinguished by the presence of three or more pairs of fixed plates, as opposed to the one or two pairs of *Naso*. *Prionurus* is distributed throughout the Eastern Atlantic and East and West Pacific, however, is entirely absent from the Indian Ocean (Winterbottom & McLennan, 1993). *Prionurus* predominantly feed on macrophytic algae and are found in shallow waters on tropical reefs and subtropical rocky shores. Morphologically, *Prionurus* is the most generalised of the acanthurid fishes; Tyler (1970) postulated that the possession of many plesiomorphic characters by *Prionurus* indicated a basal position in the phylogeny of the Acanthuridae. Tyler hypothesised that the modern acanthurid fishes arose from a *Prionurus*-like ancestor. More recent phylogenetic studies (Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al*., 1999) have indicated that *Naso* is the most basal group, followed by *Prionurus* which is the sister-group to the remaining acanthurid genera. Current classifications (*eg*: Winterbottom, 1993) place *Prionurus* and the four remaining genera within the Acanthurinae to the exclusion of *Naso*, which is housed within Nasinae. Tyler *et al*. (1989) supported monophyly of the Acanthurinae with two synapomorphies from adult morphology and three from larval characters.

### *Zebrasoma* **Swainson, 1839 and** *Paracanthurus* **Bleeker, 1863**

The species of *Zebrasoma* are deep-bodied herbivorous fishes restricted to the tropical reefs of the Indo-West Pacific, although there is one, presumably erroneous, record of *Z. scopas* from waters surrounding Cuba (Randall, 1955b). All species of *Zebrasoma* have long snouts, which are an adaptation for the ingestion of fine, filamentous algae. They are further distinguished from other acanthurid fishes by the patch of dense setae on the base of the tail. *Paracanthurus* has only one representative, *P. hepatus*, which is distributed throughout the Indo-West Pacific in association with coral reefs and can be distinguished from *Zebrasoma* by the presence of nine, rather than four or five, dorsal spines (Tyler, 1970). The absence of a snout and a decrease in the length of the gut in *Paracanthurus* is correlated with the adoption of zooplanktivory (Winterbottom & McLennan, 1993). The sister-group relationship between *Zebrasoma* and *Paracanthurus* is well supported by six synapomorphies

(Winterbottom, 1993) and is corroborated by other morphological (Guiasu & Winterbottom, 1993) and molecular (Tang *et al.*, 1999) studies.

The intrageneric relationships of *Zebrasoma* have been investigated by Guiasu & Winterbottom (1998) using morphological data. The inferred phylogeny was poorly resolved, however, the preferred tree indicated that *Z. veliferum* was the most basal taxon within the genus. The remaining species were distributed on a pectinate tree in the sequence: *Z. gemmatum*, *Z. xanthurum*, *Z. rostratum*, *Z. scopas*, *Z. flavescens*. The sister relationship between *Z. scopas* and *Z. flavescens* is perhaps unsurprising given the historical taxonomic confusion with these species; fin ray counts of the two species designate an overlapping bimodal range and some authors have considered the two forms to represent a single polymorphic species with two colour variants. Randall (1955b) dismissed this hypothesis and recognised them as two distinct species, however, he suggested that the two forms may represent incipient, or sub-, species. Examination of the gut pattern of *Z. scopas* and *Z. flavescens* indicated that the two species are able to be differentiated; *Z. flavescens* has an apomorphic simplification of the first loop of the intestine (Mok, 1977). Optimisation of dietary preference on the phylogeny of Guiasu & Winterbottom (1998) indicated that herbivory, in particular, feeding on macroalgae, was ancestral for *Zebrasoma*. Within the clade, macroalgae have been replaced by fine, filamentous algae as the preferred diet. The basal *Z. veliferum* prefers macroalgae, in contrast to the derived species *Z. flavescens* and *Z. scopas*, which feed on filamentous algae (Guiasu & Winterbottom, 1998). The authors hypothesised that selective pressures, such as resource competition, have driven the diet switching and that the adoption of filamentous algae is correlated with the apomorphic development of small pharyngeal teeth in the derived species.

### *Ctenochaetus* Gill, 1884 and *Acanthurus* Forsskål, 1775

The species of *Ctenochaetus* and *Acanthurus* represent the most derived surgeon fishes. *Acanthurus* has an almost cosmopolitan distribution, with fishes found in Indo-West Pacific, East Pacific, East Atlantic and Caribbean waters (Winterbottom & McLennan, 1993). In contrast, the range of *Ctenochaetus* is entirely restricted to the Indo-West Pacific. *Ctenochaetus* and *Acanthurus* are distinguished from the other acanthurids by the combination of the possession of a moveable spine on the caudal peduncle (a feature shared with *Zebrasoma* and *Paracanthurus*) and the presence of true ctenoid scales (Tyler, 1970); the

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larval scales are resorbed during metamorphosis and new adult scales develop *de novo* (Johnson & Washington, 1987). *Ctenochaetus* can be differentiated from *Acanthurus* by the presence of numerous elongate and moveable teeth with denticulations on one side only. The unusual dentition of *Ctenochaetus* gives rise to the common name of bristle-tooth. Monophyly of *Ctenochaetus + Acanthurus* is well supported by many phylogenetic analyses (Guiasu & Winterbottom, 1993; Winterbottom, 1993; Tang *et al*., 1999), however, these studies indicate that the taxonomy of the two groups does not reflect their natural relationships. Winterbottom (1993) was unable to isolate a single synapomorphy for *Acanthurus* and tentatively suggested that it may be a paraphyletic assemblage. Osteological studies (Guiasu & Winterbottom, 1993) found a synapomorphy (numerous, feathery gill-rakers) which united *A. nigroris* with *Ctenochaetus*, in addition to global similarities in a number of other morphological specialisations. The phylogeny presented by Guiasu & Winterbottom (1993) indicated that *A. nigroris* shared a sister-group relationship with *Ctenochaetus* to the exclusion of other *Acanthurus* species. The phylogenetic analysis of mitochondrial gene sequence data by Tang *et al*. (1999) indicated that *A. guttatus* was more closely related to *Ctenochaetus* than to *A. xanthopterus*, while supporting the monophyly of *Ctenochaetus* and *Acanthurus + Ctenochaetus*. These recent phylogenetic studies provide substantial and convincing evidence that *Acanthurus* is paraphyletic with respect to *Ctenochaetus*.

The intrageneric relationships of both *Ctenochaetus* and *Acanthurus* are currently not understood and are further confounded by the paraphyly of *Acanthurus*. Randall (1956) proposed seven subgroups within *Acanthurus*, which, although superficially consistent, he did not regard as sufficiently distinct to demarcate as subgenera. Despite the lack of insight into the relationships among the 42 species, patterns of dietary shift from the ancestral acanthurid condition can be observed. Optimisation of dietary behaviour onto the cladogram presented by Winterbottom (1993) indicated that whilst some members of *Acanthurus* are herbivorous (*eg*: *A. nigrofuscus* feeds on mats of microalgae as juveniles and fleshy macroalgae as adults, Fishelson *et al*., 1987), others have switched from the plesiomorphic condition, to zooplanktivory, independent of some *Naso* species and *Paracanthurus* (Winterbottom & McLennan, 1993). Detritivory is also observed in some *Acanthurus* species; members of Group 7 of Randall (1956) are distinguished by the presence of a large, thick-walled, gizzard-like stomach. The elongate and highly denticulate teeth of this group further preadapt them

for foraging on diatomaceous detritus. Interestingly, *A. xanthopterus*, implicated as a sister-group to *Ctenochaetus*, is classified within Group 7 (Randall, 1956). Winterbottom (1993) suggested that this subgroup of *Acanthurus* may be revealed to be the sister to *Ctenochaetus*. The monophyletic *Ctenochaetus* are all detritivores, indicating that this shift from herbivory has occurred once with the assemblage. Further, the uniquely adapted teeth of *Ctenochaetus*, coupled with their recent phylogenetic emergence, suggest that this switch in diet has occurred relatively recently (Purcell & Bellwood, 1992).

## Suborder Labroidei

## Scaridae

The Scaridae (parrot fishes) is an ecologically important and distinctive group of herbivorous, coral reef associated fishes. Although the group has a relatively low diversity compared to other percoid fishes (Choat & Randall, 1986), comprising 89 species in nine genera (see Table V for taxonomic composition of the family), their vibrant colour patterns and conspicuous feeding habits have attracted much attention from biologists and recreational divers alike. The parrot fishes are classified within the Labroidei (= Labridae, Scaridae and Odacidae) which is characterised by the possession of modified pharyngeal apparatus. This apparatus functions as a second pair of jaws in the oesophagus and is used to crush and grind ingested particles of plant and calcareous substrate (Choat & Bellwood, 1994). The distribution of parrot fishes extends throughout the tropics and is almost exclusively associated with reef environments; some species associated with seagrass beds are most abundant in the Caribbean. Only one genus, *Scarus* Gronow, 1763, is common to all tropical seas (Randall, 1992a, b). In the Atlantic Ocean, parrot fishes are also represented by members of *Sparisoma* Swainson, 1839, *Nicolsina* Fowler, 1915 and *Cryptotomus* Cope, 1871. The remaining five genera of scarids are restricted to the Indo-Pacific region.

Modern scarids evolved from labrid fishes during the middle Miocene and have changed little in morphology since their divergence (Bellwood & Schultz, 1991). The scarids have been classified by some workers (Kaufman & Liem, 1982) as a subfamily of the Labridae, however, comparative larval morphology (Richards & Leis, 1984) and ecological differences suggest that the scarids form a natural group and should be maintained as a distinct family (Choat & Randall, 1986). The Scaridae is principally defined by fusion of the teeth to form beak-like dental plates, the absence of a true stomach and herbivory. A small number of

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species have secondarily evolved carnivory, feeding on live corals (Choat & Randall, 1986). The pharyngeal apparatus, common to all labroids, is further specialised in scarids. The teeth in the pharynx are arranged in rows and used to grind ingested algae and coral chunks into a fine, easily digested paste (Choat & Bellwood, 1994). This triturating action turns large pieces of coral into sand, which passes to the reef floor with the faeces. Randall (1992) suggested that parrot fishes are the single most important source of sand production in reef environments. The evolution of the unusual feeding mechanism of parrot fishes has recently been investigated using molecular techniques (Bernardi *et al*., 2000). Results from this analysis agreed with the hypothesis of Bellwood (1994) that recent scarids arose from browsing ancestors in seagrass habitats which invaded coral reef environments. Here, the fishes adopted feeding strategies which initially disturbed the substrate and later involved scraping and biting the coral, as manifest in the feeding ecology of modern scarids.

Parrot fishes are remarkable not only for their unusual feeding adaptations, but also for their conserved body shape and size. Morphological uniformity has confounded accurate taxonomy of the group (Randall, 1963). Most taxonomic studies have relied heavily on patterns of coloration for specific identification (see Schultz, 1969 for discussion). Dependence on colour, however, was found to be of further hindrance to scarid taxonomy subsequent to the elucidation of the life history of parrot fishes. Scarids are protogynous hermaphrodites which commonly display two colour patterns during different stages of their life history. Terminal phase (sexually mature) males are brightly coloured in contrast to females and smaller, immature males. This colour variation is known as sexual dichromatism. The understanding of sexual dichromatism in parrot fishes has led to the revision of their taxonomy, which has realised much synonymy and duplication of species names and the dubious status of poorly preserved types (Randall, 1963; Schultz, 1969).

**Table V. Composition of the Family Scaridae (Labroidei).** Type-species for each genus
are denoted with an asterisk (*).

| Genus | Species | Authority |
|---|---|---|
| *Bolbometopon* | *muricatum** | (Valenciennes, 1840) |
| *Calotomus* | *carolinus** | (Valenciennes, 1840) |
| | *japonicus* | (Valenciennes, 1840) |
| | *spinidens* | (Quoy & Gaimard, 1824) |
| | *viridescens* | (Rüppell, 1835) |
| | *zonarchus* | (Jenkins, 1903) |
| *Cetoscarus* | *bicolor** | (Rüppell, 1829) |
| *Chlorurus* | *atrilunula* | (Randall & Bruce, 1983) |
| | *bleekeri* | (De Beaufort, 1940) |
| | *bowersi* | (Snyder, 1909) |
| | *capistratoides* | (Bleeker, 1847) |
| | *cyanescens* | (Valenciennes, 1940) |
| | *enneacanthus* | (Lacepède, 1802) |
| | *frontalis* | (Valenciennes, 1840) |
| | *genazonatus* | (Randall & Bruce, 1983) |
| | *gibbus** | (Rüppell, 1829) |
| | *japanensis* | (Bloch, 1789) |
| | *oedema* | (Snyder, 1909) |
| | *perspicillatus* | (Steindachner, 1879) |
| | *pyrrhurus* | (Jordan & Seale, 1906) |
| | *rhakoura* | Randall & Anderson, 1997 |
| | *sordidus* | (Forsskål, 1775) |
| | *strongylocephalus* | (Bleeker, 1854) |
| | *troschelii* | (Bleeker, 1853) |
| *Cryptotomus* | *roseus** | Cope, 1871 |
| *Hipposcarus* | *harid** | (Forsskål, 1775) |
| | *longiceps* | (Valenciennes, 1840) |
| *Leptoscarus* | *vaigiensis** | (Quoy & Gaimard, 1824) |
| *Nicholsina* | *denticulata* | (Evermann & Radcliffe, 1917) |
| | *usta collettei* | Schultz, 1968 |
| | *usta usta** | (Valenciennes, 1840) |
| *Scarus* | *altipinnis* | (Steindachner, 1879) |
| | *arabicus* | (Steindachner, 1902) |
| | *bataviensis* | Bleeker, 1957 |
| | *caudofasciatus* | (Günther, 1832) |
| | *chameleon* | Choat & Randall, 1986 |
| | *coelestinus* | Valenciennes, 1840 |
| | *coeruleus* | (Bloch, 1786) |
| | *collana* | Rüppell, 1835 |
| | *compressus* | (Osburn & Nichols, 1916) |
| | *dimidiatus* | Bleeker, 1859 |
| | *dubius* | Bennett, 1828 |
| | *falcipinnis* | (Playfair, 1868) |
| | *ferrugineus* | Forsskål, 1775 |
| | *festivus* | Valenciennes, 1840 |
| | *flavipectoralis* | Schultz, 1958 |
| | *forsteni* | (Bleeker, 1861) |
| | *frenatus* | Lacepède, 1802 |
| | *fuscopurpureus* | (Klunzinger, 1871) |
| | *ghobban* | Forsskål, 1775 |
| | *globiceps* | Valenciennes, 1840 |

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| Genus | Species | Authority |
|---|---|---|
| *Scarus* (cont.) | *guacamaia* | Cuvier, 1829 |
| | *hoefleri* | (Steindachner, 1881) |
| | *hypselopterus* | Bleeker, 1853 |
| | *iserti* | (Bloch, 1789) |
| | *javanicus* | Bleeker, 1854 |
| | *koputea* | Randall & Choat, 1980 |
| | *longipinnis* | Randall & Choat, 1980 |
| | *microrhinos* | Bleeker, 1854 |
| | *niger* | Forsskål, 1775 |
| | *obishime* | Randall & Earle, 1993 |
| | *oktodon* | (Bleeker, 1861) |
| | *oviceps* | Valenciennes, 1840 |
| | *ovifrons* | Temminck & Schlegel, 1846 |
| | *perrico* | Jordan & Gilbert, 1882 |
| | *persicus* | Randall & Bruce, 1983 |
| | *prasiognathos* | Valenciennes, 1840 |
| | *psittacus** | Forsskål, 1775 |
| | *quoyi* | Valenciennes, 1840 |
| | *rivulatus* | Valenciennes, 1840 |
| | *rubroviolaceus* | Bleeker, 1847 |
| | *russelii* | Valenciennes, 1840 |
| | *scaber* | Valenciennes, 1840 |
| | *schlegeli* | (Bleeker, 1861) |
| | *spinus* | (Kner, 1868) |
| | *taeniopterus* | Desmarest, 1831 |
| | *tricolor* | Bleeker, 1847 |
| | *vetula* | Bloch & Schneider, 1801 |
| | *viridifucatus* | (Smith, 1956) |
| | *xanthopleura* | Bleeker, 1853 |
| | *zufar* | Randall & Hoover, 1995 |
| *Sparisoma* | *atomarium* | (Poey, 1861) |
| | *aurofrenatum* | (Valenciennes, 1840) |
| | *chrysopterum* | (Bloch & Schneider, 1801) |
| | *cretense* | (Linnaeus, 1758) |
| | *radians* | (Valenciennes, 1840) |
| | *rubripinne* | (Valenciennes, 1840) |
| | *strigatus* | (Günther, 1862) |
| | *viride** | (Bonnaterre, 1788) |

## Suborder Percoidei

## Chaetodontidae and Pomacanthidae

Butterfly fishes (Chaetodontidae) are a conspicuous and colourful component of the circum-tropical reef fauna, predominantly in the Indo-Pacific region (Johnson & Gill, 1992). A small number of species is known to occur in cooler, temperate waters. Of the 125 species of chaetodontids, only 12 are recorded from tropical Atlantic waters (Allen, 1981). Although the highest species diversity is observed in the central Indo-Pacific, this diversity declines markedly eastward and westward from this region, as does their biomass (Motta, 1989); the Great Barrier Reef harbours the highest diversity of butterfly fishes (Findley & Findley, 1989).

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The group is defined by the presence of small, bristle-like teeth which are adapted to a wide range of food sources. Five major dietary preferences have been observed for butterfly fishes (Allen, 1981):

1. hard coral feeders;
2. soft coral (with small amounts of hard coral) feeders;
3. benthic invertebrate (polychaetes, crustaceans) feeders;
4. zooplantivores;
5. opportunistic omnivores.

The final assemblage of omnivores feeds on a wide range of food items, including substantial quantities of algae. Sano (1989) reported that although scleractinian coral are the dominant food resource of Japanese butterfly fishes, algae, polychaetes, alcyonarians and sea anemones constitute significant components of the diet. Sano indicated that this dietary range was similar to that of butterfly fishes from other geographic regions. Although dentition patterns can be correlated to food resources (*eg*: *Forciper flavissimus* Jordan & McGregor, 1898 has rows of small, recurved teeth on an elongate jaw which it uses for probing into small crevices and tearing at prey items in contrast to the small robust jaws and teeth of *Chaetodon unimaculatus* Bloch, 1787 which it uses for grazing corals), the dietary preferences of Atlantic butterfly fishes appear to be related to behaviour rather than morphology (Motta, 1989).

The angel fishes (Pomacanthidae) occur in all tropical waters, and their greatest diversity is seen in the Indo-Pacific region (Allen, 1981). The group consists of 84 species within nine genera, all of which are associated with coral reefs. Angel fishes are predominantly herbivorous, although some Atlantic species supplement the diet with small amounts of benthic invertebrates including sponges, bryozoans and tunicates. The angel fishes are phylogenetically close to the butterfly fishes; early taxonomists classified the Pomacanthidae as a subfamily of the Chaetodontidae. The group is distinguished, however, by the absence of the unusual and distinctive tholichthys larva of chaetodontids. The tholichthys larva is characterised by a deep, round body with prominent head spination; the head is covered by body plates which are fused and extend posteriorly over the body (Leis, 1989). Pomacanthids are further defined by the presence of a large genal spine at the angle of the precopercle (Johnson & Gill, 1992).

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## The tropical Indo-West Pacific

The tropical Indo-West Pacific region is one of the most speciose regions known, with the diversity of the region exceeding twice that of all other tropical waters combined (Briggs, 1999).  The Indo-West Pacific region is spread across more than half the surface of the world in terms of longitude, and extends through about 60˚ from north to south (Briggs, 1974).  The diversity of fauna in this region has been a rich source of material for biogeographical studies (*eg*: Chenoweth *et al*., 1998).  Wallace's line has long been established as an important barrier in the Malay Archipelago, which separates Australian and Oriental terrestrial fauna and flora. The distribution of marine fauna throughout this region, however, is not delineated along this line.  The largest concentration of diversity of the marine fauna is observed in the region known as the East Indies Triangle (Briggs, 1974), which encompasses the Malay Archipelago, New Guinea and the Philippines.

The origins and factors driving the diversification of the marine fauna of the Indo-West Pacific are unclear.  Ancient geological events, such as the closing of the Tethys Sea and break-up of Gondwana have been hypothesised as major factors in the diversification of highly dispersed marine species (see Palumbi, 1997).  Briggs (1999) suggested that the modern fauna of the Indo-West Pacific has been derived from residual Tethyan populations, which were well established and diversified before the closing of the Tethys Sea during the Miocene.  There are significant molecular data, however, that indicate that speciation in the Indo-West Pacific is a much more recent phenomenon.  Palumbi (1997) cited molecular data, which indicated that species of echinoderms diverged in the Indo-West Pacific during the last 1-3 mya.  Further, molecular data suggests that some species of butterfly fishes (Chaetodontidae) have separated within the last 1 mya (McMillan & Palumbi, 1995).  The Pleistocene glaciations (~ 0.7 mya), and concomitant fluctuations in sea-level in the East Indies Triangle, have been suggested by Woodland (1983) as playing a major role in the speciation of *Siganus* (Siganidae).  Further, Chenoweth *et al*. (1998) conluded that sea-level changes had significantly contributed to the molecular diversity of marine fishes.  The authors cited molecular evidence which indicated that populations of fishes on the East coast of Australia experienced significant bottlenecks due to the drying of coastal lagoons during the Pleistocene glaciations.  Palumbi (1997) suggested that speciation in the Indo-West Pacific has been an ongoing process, spanning the Miocene through the Pleistocene to modern times.

## 1.5  Conclusions

The review of the literature suggests that the Gyliauchenidae is a small family of trematodes, which is found commonly in species of the Acanthuroidei in the Indo-West Pacific. The species of the Gyliauchenidae display remarkable adaptations to the environment of herbivory imposed by the hosts. The restriction of the group in terms of both host associations and biogeography, coupled with its unique morphology, makes it a rich source of inspiration for a study of the taxonomy, systematic relationships and evolutionary biology.

# Chapter 2
# Aims of Study

## 2.0  Introduction

Review of the taxonomic literature pertaining to the Gyliauchenidae indicates that there is a great deal of confusion surrounding the accurate diagnosis of species.  It is clear that the features characterising individual species are poorly understood, especially with respect to the conformation of the oesophagus; in fact, opinion varies as to the relative taxonomic importance of specific characteristics and structures.  It is important, prior to any new survey research being performed, that the accurate diagnosis of species can be confirmed.  In addition, the overlapping generic diagnoses confound species diagnosis; the reliance on characters such as the location of the ovary relative to the testes is variable and subject to distortion through specimen preparation.  The use of such unstable characters in generic diagnosis is unsatisfactory.  Furthermore, the use of host information by many authors to verify the taxonomic identity of species, can not be considered useful nor appropriate.  The current status of the family must be disentangled and consolidated as a priority before the biodiversity, host associations and evolutionary biology of the Gyliauchenidae can be investigated.  Bearing this caveat in mind, the aims for this present study can be divided into goals in both the taxonomy and systematics and the evolutionary biology of the Gyliauchenidae.

## 2.1  Taxonomic and Systematic Aims

- **Investigate the biodiversity of the gyliauchenid fauna of fishes of the Great Barrier Reef, Queensland, Australia.**
  There have been few studies of the gyliauchenid fauna on the Great Barrier Reef; in fact, there have been few comprehensive surveys conducted of other families of Digenea from this region (see Cribb *et al*., 1994).  The Gyliauchenidae is a little known and poorly understood family, which is endemic to the Indo-West Pacific.  The Great Barrier Reef accommodates an enormous diversity of fish fauna.  Russell (1983)

estimated that there are approximately 1300 species of fishes from 110 families on the Great Barrier Reef. Cribb *et al.* (1994) predicted that the diversity of the digenean fauna is much greater than that of the fishes and conservatively estimated that the total number of digeneans of fishes on the Great Barrier Reef approximates 2270 species. Of this number, Cribb *et al.* stated that only one tenth has been collected.

- **Investigate the endemicity of the gyliauchenid fauna of the Great Barrier Reef by comparison with the fauna from other coral reef environments**.

- **Consolidate the status of the Gyliauchenidae**.

  The type material for nearly all species of gyliauchenids requires examination and all descriptions must be reviewed in order to establish clear character diagnoses of all species.

- **Examine the internal phylogenetic relationships of the Gyliauchenidae**.

  Modern systems of classification are intended to reflect the natural associations of the taxa they identify. It is imperative to any systematic study that the inter-relatedness of the taxa is understood.

- **Reconstruct the generic classification such that it accurately reflects the phylogenetic hierarchy of the taxa**.

- **Stable characters, which are useful for generic diagnosis, need to be isolated such that the current genera can be correctly identified**.

  New genera may be established for specimens that do not conform to the revised diagnosis of any of the known genera.

- **Explore patterns in host-specificity and the geographical distribution of gyliauchenids in their fish hosts**.

## 2.2  Evolutionary Biology Aims

The observed patterns of distribution of the Gyliauchenidae raises interesting evolutionary questions.  Not only do these worms possess a highly derived morphology adapted to herbivory, they are also entirely restricted the Indo-west Pacific and found within a limited range of hosts.  These factors, together with the manageable size of the group, make them an ideal model group for investigating macroevolutionary questions.  In this thesis, I ask the following questions:

- What is the approximate age of the gyliauchenid lineage?  When did the Gyliauchenidae diverge from a lepocreadoid ancestor?

- How did the observed distribution of gyliauchenids in herbivorous reef-fishes of the Indo-West Pacfic arise?  Is the association of gyliauchenids with acanthuroids ancestral?

- How did the observed specialised morphology of the digestive and reproductive systems of the Gyliauchenidae evolve?  Has parasitism of herbivorous fishes provided a selective pressure on the morphology and reproductive strategies of the gyliauchenids?

# Chapter 3

# General Materials and Methods

## 3.1 Specimen Collection

**Specimen Collection and Fixation**

Fishes were caught by either by spear or seine-net, taken live to shore and death was effected by euthanasia via benzocaine (10% ethanol solution) or via dorsal notochordotomy. A complete list of all fishes collected and examined is included as Appendix II. Figure 1 shows a map of the Indo-West Pacific, which marks the locations sampled in this study, and a list of all collecting localities is shown below (Table VI). A small number of fishes were obtained from market (Brisbane, Australia; Perpignon, France; Moorea, New Caledonia). The entire gut was removed from the dead fishes and maintained in vertebrate saline (0.85%). I used dissecting microscopes to look for worms in the large intestine and rectum. The gut was then washed in saline until the supernatant was clear and the gut was then re-examined for worms. Precipitated gut content was also examined. Digeneans were removed from the gut and washed in saline, then killed in near-boiling saline and preserved in 10% formalin for morphological examination. Additional specimens were killed and preserved in 98-100% ethanol for use in phylogenetic studies using molecular methods. Where molecular samples were made, morphotype vouchers were collected for microscopy by the boiling saline/formalin method. Small amounts of host tissue (including striated muscle, heart muscle and liver tissue) were preserved in 98-100% ethanol for later genetic analysis (not this study)
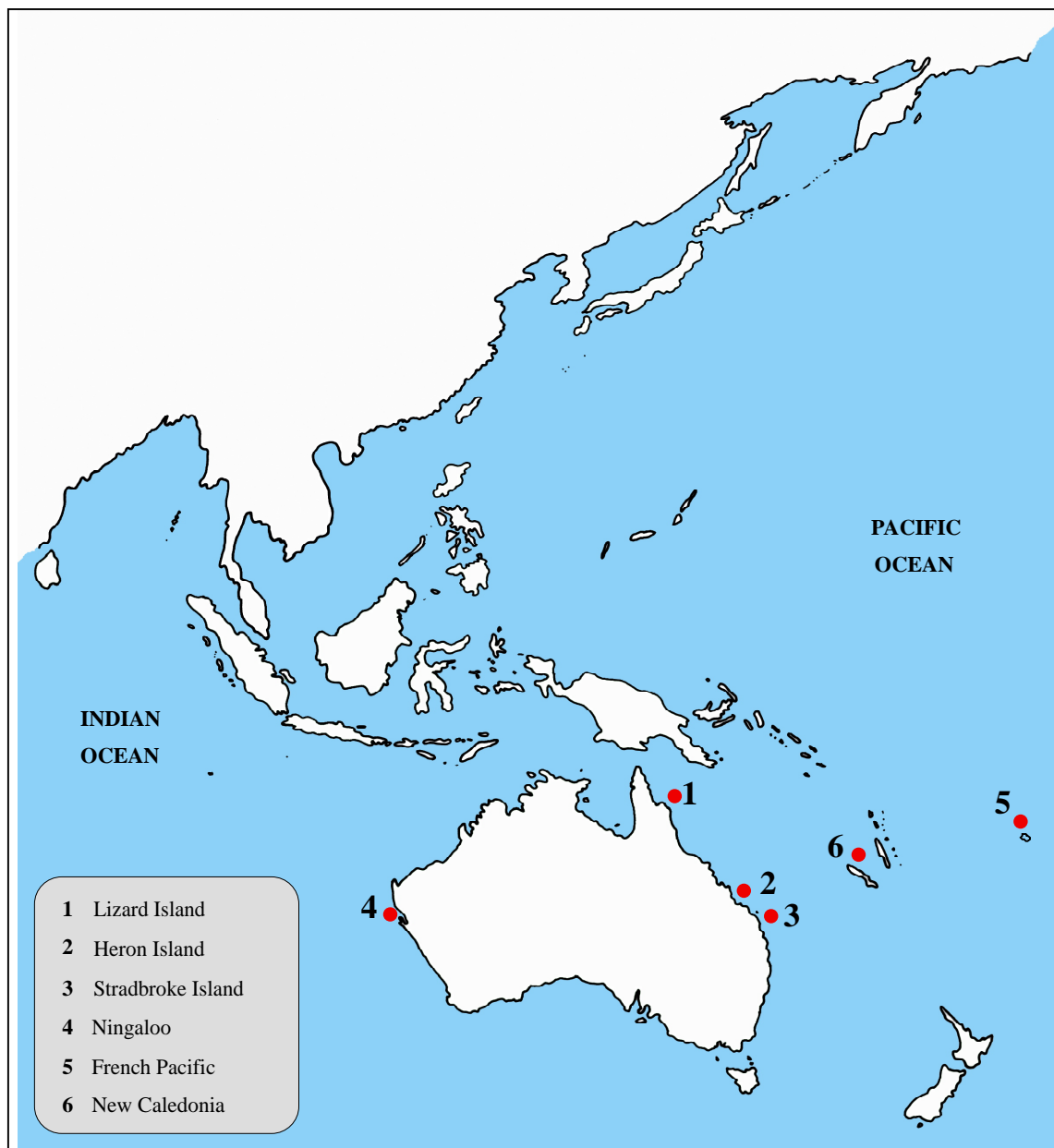
**Figure 1. Map of the Indo-West Pacific region, showing key collecting sites sampled in this study.** Note: only sites from which gyliauchenid specimens were obtained are depicted.