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Taxonomy and phylogeny of the buccal-attaching Cymothoidae (Crustacea: Isopoda) of Australia

Melissa Beata Martin

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Taxonomy and phylogeny of the buccal-attaching Cymothoidae from Australia

By Melissa Beata Martin

BSc Marine Biology (Hons)

Supervisor: ____

Professor Barbara F. Nowak

DECLARATIONS BY THE AUTHOR

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CO-AUTHORSHIP

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Melissa B. Martin (MBM), IMAS, University of Tasmania (candidate)
Barbara F. Nowak (BFN), IMAS, University of Tasmania
Niel L. Bruce (NLB), MTQ, Queensland Museum

We the undersigned agree with the stated proportion of work undertaken for each of the published peer-reviewed manuscripts contributing to this thesis.

Signed: ______________________________ Date: 7.12.2015

Professor Barbara Nowak
Supervisor
Institute for Marine and Antarctic Studies, Launceston
University of Tasmania

Signed: Date: 7.12.15

Associate Professor John Purser
Deputy Director, Fisheries and Aquaculture
Institute for Marine and Antarctic Studies, Launceston
University of Tasmania

Author details and their roles:

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ABBREVIATIONS

AIM	Auckland Institute and Museum, Auckland, New Zealand
AM	Australian Museum, Sydney
BMNH	The Natural History Museum
HSM	Hayama Shiosai Museum; Institute of Oceanology
IOCAS	Chinese Academy of Sciences, Shandong, China
MNHN	Muséum Nationale d’Histoire Naturelle, Paris
MTQ	Museum of Tropical Queensland, Townsville
NHMW	Naturhistorisches Museum, Vienna
NSMT	National Museum of Nature and Science, Tokyo, Japan
QM	Queensland Museum, Brisbane
SAM	South Australian Museum
SMNH	Swedish Museum of Natural History, Stockholm
TINRO	Russian Pacific Federal Fisheries Research Institute; Vladivostok
TMAG	Tasmanian Museum and Art Gallery, Hobart
TOYA	Toyama Science Museum, Toyama, Japan
USNM	National Museum of Natural History, Smithsonian Institution
ZMHB	Zoologisches Museum, Museum für Naturkunde, Humboldt-Universität Berlin, Germany

NOTE REGARDING THESIS STRUCTURE

The first and second chapters of this thesis were written as a general introduction and review of relevant literature that relate to the research of this thesis. Chapters 3 to 6 are taxonomic chapters that have been published or submitted and therefore follow the journal style. Chapter 3 of the genus *Ceratothoa* consist of two parts (Part I and II) since it has been published into two papers. Since all result chapters (Chapters 3 to 7) have been discussed comprehensively, Chapter 8 is a summary which includes the outcome of this research, general remarks and discussion of the implications and future undertakings from this research. Figure and table captions from the original publications have been modified in accordance to the respective thesis chapters. Other publications (as first and co-author) which are indirectly related to this research are presented in the Appendix I. The reference style of *Zootaxa* journal has been adopted for this thesis, and a list of references of all chapters are included at the end of the thesis.

EXECUTIVE SUMMARY

The family Cymothoidae consists of crustacean parasites of marine, brackish and freshwater fishes, with 383 species in 43 genera. Different genera attach to different site on its host (externally, inside the flesh, branchial and buccal cavity) and display high host and site specificity. In the Australian context, one flesh-burrowing species is known, and the external and gill-attaching species have been thoroughly reviewed. The buccal-attaching genera remain unrevised, thus this research focuses on the revision of four genera: *Ceratothoa* Dana, 1852, *Glossobius* Schioedte & Meinert, 1883, *Cymothoa* Fabricius, 1793 and *Smenispa* (Bleeker, 1857).

Ceratothoa is represented in Australia by nine species, including two new species (*Ceratothoa barracuda* sp. nov. and *Ceratothoa globulus* sp. nov) and two new records (*Ceratothoa carinata* (Bianconi, 1869) and *Ceratothoa oxyrrhynchaena* Koelbel, 1878). Three widely recorded species: *Ceratothoa imbricata* (Fabricius, 1775), *Ceratothoa banksii* (Leach, 1818) and *Ceratothoa trigonocephala* (Leach, 1818) were impossible to separate or synonymize with any degree of confidence. *Ceratothoa imbricata* is here redescribed, with *Ceratothoa trillesi* (Avdeev, 1979) and *Ceratothoa huttoni* Filhol, 1885 placed into junior synonymy; the preferred hosts are species of the genus *Trachurus* (Carangidae). *Ceratothoa banksii* (Leach, 1818) is here validated and brought out of synonymy with *Ceratothoa imbricata*. *Ceratothoa trigonocephala* (of unknown host identity and type locality) is excluded from the Australian fauna, including *Ceratothoa lineata* Miers, 1876a, which is here transferred to the genus *Mothocya* Costa, 1851, with *Mothocya ihi* Bruce, 1986 placed into junior synonymy. This research regards *Ceratothoa contracta* (Miers, 1880), *Ceratothoa novaezelandiae* Filhol, 1885 and *Ceratothoa gaudichaudii* (Milne Edwards, 1840) as *species inquirenda*.

Glossobius is represented by seven species worldwide, with *Glossobius anctus* Bruce & Bowman, 1989 being the only known species in Australia. This research contributes to the redescription of *Glossobius impressus* (Say, 1818), new to Australian and southern African waters. *Glossobius arimae* Nunomura, 2001 is incorrectly placed in the genus and transferred to *Ceratothoa*; whereas *Glossobius ogasawarensis*

Nunomura, 1994 is here placed in synonymy with *Glossobius auritus* Bovallius, 1885, reducing the number of accepted species in the genus to five. The identity of *Glossobius crassa* (Dana, 1853) is impossible to resolve, and is here removed from synonymy with *G. auritus* and placed into *nomen dubium*.

Cymothoa hermani Hadfield, Bruce & Smit, 2011, previously known from Tanzania, is new to Australian waters. *Cymothoa carangi* Avdeev, 1979; *Cymothoa epimerica* Avdeev, 1979; *Cymothoa parupenei* Avdeev, 1979; *Cymothoa propria* Avdeev, 1979; *Cymothoa rotunda* Avdeev, 1979; *Cymothoa pulchrum* Lanchester, 1902; *Cymothoa curta* Schioedte & Meinert, 1884 and *Cymothoa plebeia* Schioedte & Meinert, 1884 are here redescribed, the latter two excluded from the Australian fauna. *Cymothoa limbata* Schioedte & Meinert, 1884 is placed into junior synonymy with *Cymothoa eremita* (Brünnich, 1783). *Cymothoa eremita* and *Cymothoa indica* Schioedte & Meinert, 1884 are both known to have high morphological variability and display low host specificity, occurring on 12 and 10 host families respectively.

Smenispa irregularis (Bleeker, 1857) is one of the smaller and least known of the cymothoid genera, with only two species listed in The World Register for Marine Species. There have been few records of *Smenispa* since its original description and its host preferences and geographical distribution remain little known. This research discusses the nomenclatural change from *Enispa* Schioedte and Meinert, 1884 to *Smenispa* Özdikem, 2009, and a full description provided for female and male of the species.

This study also aims to investigate the phylogeny of both morphological (outgroup comparison) and molecular (using mitochondrial genes 16S rRNA and cytochrome oxidase 1) relationships within the Cymothoidae by expanding morphological and limited genetic data sets. Both morphological and molecular analyses showed that 1) the family is monophyletic, 2) neither analyses support the view of a linear evolutionary pathway based on site attachment (from the external-attaching cymothoids to a derived buccal and gill-attaching cymothoids) 3) neither analyses revealed that the host-specificity is higher in the more derived genera. The morphological cladistics resulted in two fairly distinct clades: 1) the predominantly buccal and gill attaching cymothoid clade and 2) the South American freshwater

cymothoid clade. The Anilocrinae is basally unresolved, which also includes non-external attaching genera such as *Livoneca*, *Norileca* and *Smenispa*. The molecular cladistics for 16S rRNA formed congruent generic clades (*Anilocra* and *Ceratothoa*) whereas COl cladistics formed weakly supported generic clades for *Nerocila* and *Cymothoa*. It is likely that cymothoid taxonomic classifications reflect convergence due to similar life styles (morphological adaptations).

This project has contributed to the knowledge of the buccal-attaching cymothoids by increasing species numbers and providing full accounts of the species synonymy, species and generic diagnosis, distribution, known hosts and species keys. This thesis also highlights the complexities of the Cymothoidae phylogenetics and that the preliminary results suggest complex history (e.g. parasitic diversification strategies, pathogenicity, life history) within the family.

CHAPTER 1

General Introduction

1.1. THE ISOPODA

The Isopoda are malacostracan crustaceans (relatives of the shrimps, crabs and lobsters) commonly known as wood lice, pill bugs and slaters (Bruce 2001). Species within the Isopoda belong to various feeding categories: free-living predators, scavengers, grazers, parasites (temporary or obligatory) and filter feeders (Poore 2002; Poore & Bruce 2012).

The World Register of Marine, Freshwater and Terrestrial Isopod Crustaceans lists more than 10,000 species in eleven suborders for Isopoda: Oniscidea Latreille, 1802 (4,261 species, 38 families); Cymothoida Wägele, 1989 (2,769 species, 30 families); Asellota Latreille, 1802 (2,038 species, 30 families); Sphaeromatidea Wägele, 1989 (871 species, eight families); Valvifera Sars, 1882 (613 species; 11 families); Phreatoicidea Stebbing, 1893 (86 species, seven families); Limnoriidea Brandt & Poore, 2002 (63 species; three families); Microcerberidea Lang, 1961 (48 species; 2 families); Tainisopidea Brandt & Poore, 2003 (seven species, one family); Calabozoida Van Lieshout, 1983 (three species, two families); and Phoratopidea Brandt & Poore, 2003 (one species, one family) (WoRMS 2014).

The Isopoda are represented by marine, estuarine, freshwater and terrestrial species. Currently the world's described estuarine and marine isopods total approximately 6,250 species (Poore & Bruce 2012; Wetzer 2015); and nearly 500 species are known from freshwater (e.g. Tainisopidea [see Brandt & Poore 2003] and some sphaeromatids, [see Wetzer 2015]). The Oniscidea are exclusively terrestrial (Poore 2002; Wetzer 2015). The isopod families Cirolanidae Dana, 1852; Cymothoidae Leach, 1814; Anthuridae Leach, 1814; Expanathuridae Poore, 2001 and Leptanthuridae Poore, 2001 are dominant in the tropics (Poore & Bruce 2012); families within Valvifera are predominantly found in temperate and cool waters and the family Sphaeromatidae Latreille, 1825 occur in temperate waters (Poore & Bruce 2012).

1.2. BODY MORPHOLOGY OF THE ISOPODA AND THE FAMILY CYMOTHOIDAE

Marine isopods have the most morphologically diverse body plan of all crustaceans (Brusca 1997; Wetzer 2001; Poore 2002; Poore & Bruce 2012). Isopods range in size from 0.3 mm (e.g. some Microcerberidae) to nearly 50 cm in length (e.g. deep-sea *Bathynomus* spp.) (Schotte *et al.* 2008 onwards). Several taxa are characterized by a unique body shape, such as anthurideans with their elongated and cylindrical bodies, asellotes with parallel-sided bodies, phreatoicideans with laterally flattened bodies, and the generally oval and vaulted bodies of oniscideans and cymothoids (Brandt & Poore 2003).

The body of a typical isopod is divided into three regions: the cephalon (head); the pereon (thorax) and the pleon (abdomen) (Poore & Bruce 2012). The cephalon of an isopod bears a pair of eyes that are visible from the dorsal view; and two pairs of antennae (refered to as antennula, antennula or antennae1, and antenna or antennae 2) and mouthparts visible from the ventral view. It is also referred to as the cephalothorax as the first pereonite is fused with the cephalon (Kensley & Schotte 1989; Stachowitsch 1992) and in some cases, fused with the second pereonite (e.g. Gnathiidae) (Wetzer *et al.* 1997). In primitive isopods (e.g. species of Phreatoicidea and Calabozoidea), the cephalon is attached anteriorly to pereonite 1, with slight or moderate forward extensions of the first coxae, and is relatively immobile (Brandt & Poore 2003). Cymothoids have a cephalon that is barely laterally overlapped by the coxae of pereopod 1, free from pereonite 1 and is able to flex dorsoventrally (but not rotate sideways freely). The eyes of isopods, including most cymothoids (Poore *et al.* 2002) are mostly sessile. The two pairs of antennae are uniramous, and consist of a basal peduncle and a distal flagellum (Stachowitsch 1992).

The isopod mouthparts, similar to other peracarid crustaceans, consist of a pair of maxillipeds (*singular* maxilliped), mandibles (*singular* mandible), maxillulae (*singular* maxillula) and maxillae (*singular* maxilla). The maxillipeds are the largest pair of mouthparts (Stachowitsch 1992). The maxillipeds represent a highly modified pair of appendages (thoracopods) of the first pereonal segment (which is fused to head) and consist of a basal section (coxa, basis) bearing a five-segmented (ischium, merus, carpus, propodus, dactylus) palp (Stachowitsch 1992). Most carnivorous and parasitic isopods have reduced maxillipedal endite (Brandt & Poore 2003). The oblique segmentation and hooks of the maxillipeds are characteristics of cymothoids and aegids (Brandt & Poore 2003). Developed lamina vibrans and oostegites attached to the maxillipeds are a characteristic found in ovigerous female cymothoids (Brusca 1981). In the family Cymothiodae, the maxilliped palp is reduced to three segments.

The mandible is the first pair of mouthparts and is often sclerotized (Kensley & Schotte 1989). The mandibular incisors are primitively multidentate, with usually four to five cusps (e.g. Phreatoicidea) whereas in carnivorous and parasitic isopods, the incisors are acute (Brandt & Poore 2003). The small blade-like molar process is apparently used for slicing and piercing host tissue to penetrate blood vessels (Brusca 1981; Lester 2005). In cymothoids, the molar process lacks setae and serrations, and is small and fleshy. The mandibular palp in cymothoids comprises.

The maxillula (also refered to as maxillule, maxilla 1 or first maxillae) is immediately posterior to the mandibles. In cymothoids, the maxillula is a slender appendage with several styles or spines (generally three to five) to facilitate transfer of host blood, mucus or tissue to the gut (Brusca 1981). Brandt & Poore (2003) found the characters describing maxillula uninformative for phylogenetic analysis.

The maxillae (also referred to as second maxillae or maxillae 2) are immediately posterior to the maxillulae. The maxillae are bilobed appendages bearing several strong, minute, terminal spines which aid gripping of the host flesh (Brusca 1981). Primitively, the maxillae have rows of complex setae arranged along oblique apices, are without endopod and the basal endites are reduced to two, one or absent (Brandt & Poore 2003). In cymothoids, the two lobes are interpreted as coxal and basal endite and the solitary lobe as a coxal endite (Brandt & Poore 2003).

The isopod pereon, the division between the cephalon and pleon (more appropriately between the cephalothorax and pleon), consists of seven pereonites (Stachowitsch 1992). The pereon is morphologically not equivalent to a thorax because the first (and occasionally the second) thoracic segment bears maxillipeds and is fused to the cephalon (Stachowitsch 1992). Each pereonite ventrally bears a pair of uniramous pereopods (seven pairs in total) that are morphologically uniform, consisting of coxa, basis, ischium, merus, carpus, propodus and dactylus. The coxae are the proximal segment of the appendage, often expanded to form projecting coxal plate (Stachowitsch 1992). The first coxal plates are fused to pereonite 1 in cymothoids, with the remaining coxae 2–7 distinct on the lateral margins of the respective pereonites. In most isopods, pereopods 1–3 are directed anteriorly whereas 4–7 are directed posteriorly. Patterns of setation are difficult to score for phylogenetic analysis as the patterns of setation varies considerably between taxa (Brandt & Poore 2003).

The pleon is the posterior or abdominal region of the body, consisting of six pleonites (Kensley & Schotte 1989; Stachowitsch 1992). The first five pleonites are free and articulating (Brandt & Poore 2003) as seen in most cymothoids, each pleonite bearing a pair of biramous pleopods (ventrally visible). In most isopods, the sixth pleonite is fused to the telson to form a pleotelson (Wetzer *et al.* 1997) and instead of pleopods, is represented by uropods (Stachowitsch 1992). In the plesiomorphic state, the ventral side of the pleon is flat and pleopods are restricted laterally by the pleonite. The unmodified pleopod consists of a basal segment known as the peduncle (or protopod) and two distal rami called the endopod and the exopod (Wilson 1989). The pleopods of the Cymothoida allow simultaneous swimming and respiration (Brandt & Poore 2003). Pleopod 2 rami of a male isopod have modified copulatory structures (appendix masculina). Pleopods 3–5 have very thin cuticle and function as gills (Wilson 1989). The presence of pleopod setae on one or both of the pleopod rami is a synapomorphy in other cymothooidan taxa.

The telson is the terminal end of the body which bears the anus (Kensley & Schotte 1989). In isopods, the telson is fused with one or more of the pleonites (6 or 5 and 6) to form the ‘pleo-telson’ (Stachowitsch 1992). In some cases (e.g. sphaeromatids), pleonites 2–4 may be fused to each other but are free from fusion of the remaining pleotelson (Brandt & Poore 2003). The lateral and posterior margins of the pleotelson vary from lanceolate, ovate, pointed, sub-parallel, to concave and convex (Stachowitsch 1992). The uropods are paired pleonal appendages of the last pleonite (Kensley & Schotte 1989), situated at the anterolateral margins of the pleotelson (Bruce *et al*. 2002) and are used for steering (Brandt & Poore 2003). Like the pleopods, it consists of the basal peduncle, and the endopod and exopod rami. The styliform uropods are thought to be plesiomorphic, as in asellotes, oniscideans and phreatoicideans (Brandt & Poore 2003) and present in most cymothoids.

1.3. PHYLOGENY OF THE ORDER ISOPODA AND THE FAMILY CYMOTHOIDEA

Isopods belong to the superorder Peracarida Calman, 1904, because species brood their eggs and young in a marsupium on the ventral side of their bodies (Poore 2002). The developed embryos then released from the brood pouch are termed mancae (singular= manca) (Poore 2002); thus the order is not known to have ‘true’ larval form (Brusca & Wehrtmann 2009). The morphological synapomorphies (shared derived characteristics) which define isopods are: 1) the eyes sessile (when present); 2) the carapace reduced to a cephalic shield; 3) antenna 1 and 2 uniramous (although antenna 2 may bear small exopod); 4) all pereopods uniramous; 5) all pleopods are biramous; 6) pleonite 6 is fused to the telson to form the pleotelson; and 7) uropodal rami uniarticulate. Depending on the species, male isopods have paired penes located either on coxal plate 7, pereonite 7 or pleonite 1; and an appendix masculina on either the mesial or distal margin of pleopod endopod 2 (Poore 2002). Female isopods have gonopores located on pereonite 5 (Poore 2002).

The Isopoda is a monophyletic group (Brusca & Wilson 1991; Brandt & Poore 2003; Wilson 2009; Poore & Bruce 2012), which differs from other peracarid orders by the biphasic moulting (Vernet & Charmantier-Daures 1994; Wilson 2009) and the specialized heart musculature (Nylund *et al.* 1987; Wilson 2009). Early studies of the phylogeny of Peracarida found the sister taxon of the Isopoda is either Tanaidacea Hansen, 1895 (Siewing 1963; Hessler 1983; Pires 1987), or Amphipoda (Wagner 1994; Schram & Hof 1998; Wheeler 1998; Wills 1998). Poore's (2005: Tab. III) morphological analysis found Amphipoda to be a sister taxon to Isopoda based on eight apomorphic character states. The amphipod–isopod clade was supported by later studies (Spears *et al.* 2005; Jenner *et al.* 2009; Wills *et al.* 2009). Wilson (2009) argued that Poore's (2005) clade was poorly supported due to the overgeneralized character states, inaccurate scoring of specific taxa, some characters that disprove the isopod-amphipod clade is not used and that molecular studies did not produce well-defined results due to limited numbers of peracarid taxa or relevant sequences. Wilson's (2009) analysis found that tanaidaceans remain the best sister group to isopods although the 18S molecular analysis partially supports this theory. Wilson (2009) warned that these results were inconclusive and requires further investigation (e.g. further investigation of the morphological results of the spelaeogriphacean-isopod sister group relationship).

Many taxonomists have attempted to attain a more robust classification for isopod phylogeny at a suborder and family level (e.g. see Sars 1882; Hansen 1916; Monod 1922; Tabacaru & Danielopol 1999), particularly for the suborder Flabellifera (now replaced by Cymothoida and Sphaeromatidea). Morphological cladistics of the Isopod was not resolved due to poor dataset and certain characters were misinterpreted or revealed little to reflect monophyletic relationships. On a basal level, most authors have agreed that Phreatoicidea, Asellota and Oniscidea are sister groups to the remaining isopod suborders (Wägele 1981, 1989; Schmalfuss 1989; Brusca & Wilson 1991; Wilson & Keable 2001; Schimdt 2008; Wilson 2009). Wägele's (1981, 1989) study regarded Phreatoicidea, Asellota and Oniscidea as polyphyletic and derived from an ancestral type while Brusca & Wilson (1991) treated these groups as a derived type (representatives with expanded pereopodal coxal plates and broad uropods).

Sars (1882) differentiated the Flabellifera from other isopods by the presence of lateral or anterolateral uropods that form a tailfan with the pleotelson (other isopods have styliform and terminal uropods). Wägele (1981, 1989) proposed that 1) the ancestral isopod was a long-tailed form and 2) Flabellifera was polyphyletic and replaced by the suborders Cymothoida Wägele 1989 and Sphaeromatidea Wägele 1989. Brusca & Wilson's (1991) review of the isopod systematics showed that the ancestral isopod was a short-tailed form and that Flabellifera was paraphyletic, thus not supporting Wägele's (1989) new suborders. Brusca & Wilson (1991) did admit their data were unable to hypothesize sister group relationships for all isopod taxa and would require better resolution. The review of Brandt & Poore (2003) is to date the most significant contribution to the understanding of isopod phylogeny using morphological characters. Some of the key contributions are 1) the proposal of three suborders (Tainisopidea, Phoratopidea and Limnoriidea) because of the numerous supporting synapomorphies, 2) the two suborders Cymothoida and Sphaeromatidea are re-introduced and the orders Flabellifera and Epicaridea are dispensed, 3) the suborder Anthuridea is reduced to superfamily rank and Epicaridea is reduced to two superfamilies within Cymothoida, 4) the new family Basserolidae is proposed.

Attempts to resolve isopod relationships using molecular genetics are few. Wetzer (2001) evaluated three loci from the mitochondrial genome (12S and 16S ribosomal RNA) and one protein-coding (COI) for relevance in inferring isopod phylogeny at the suborder level. All three loci resulted in similar patterns, with the most speciose suborders of isopods having the most divergent mitochondrial nucleotide sequences (Wetzer 2001). Wetzer (2002) later explored the three loci from Wetzer (2001) separately and in combination, but these resulted in conflicting trees. The results were promising because: 1) valviferans had a sphaeromatid ancestor, and 2) oniscids and sphaeromatids are possibly more closely related than previously thought. As for the Cymothoidae, the parsimony analysis of 16S rDNA (see Fig. 4 in Wetzer (2002) and CO1 (see Fig. 5, 91% bootstrap in Wetzer (2002) formed a well-supported clade, although only two genera were used (*Lironeca* and *Olencira*) and therefore reveals very little information of the family's relationship.

In the same year, Dreyer & Wägele (2002) used nuclear ssu rRNA gene from several isopods to study the order's phylogeny. Their molecular phylogeny resulted in the proposal of a monophyletic group named Scutocoxifera tax. nov. (unranked), and was supported by morphological characters from a previous work (Dreyer & Wägele 2001). Scutocoxifera is composed of the Oniscidea, Valvifera, Sphaeromatidea, Anthuridea and Cymothoida. However, much of the relationships within Scutoxifera are not resolved due to the occurrence of a rapid radiation, followed by a long period of divergent evolution of the suborders (Dreyer & Wägele 2002). Thus the Scutoxifera is not used in modern classification.

The relationship of isopod suborders still needs to be revised because of: 1) certain key taxa missing or lack information (e.g. species from the families Microcerberidae, Calabozoidae, and most families in the Asellota), 2) weak scores for certain morphological characters (e.g. detailed scoring of internal anatomy) and 3) the need of molecular sequences for representatives of Isopoda families (e.g. Calabozoidae, Microcerberidae) (Wilson 2009). In order to further understand peracarid phylogeny, other markers (coding and non-coding), nuclear and mitochondrial, should be examined (Wilson 2009).

1.3.1 The suborder Cymothoida and the family Cymothoidae

The Cymothoida have evolved from the scavenging and predatory feeding strategy towards parasitism on fish and other crustaceans (Poore 2002). The Cirolanidae is the most basal family within the suborder, defined by the tridentate mandibular incisor (see clade 14, Fig. 6C in Brandt & Poore 2003). Cymothoida is defined by the following characters: (1) the absence of a mandibular lacinia mobilis, (2) the maxillipedal endite shorter than palp article 1, and (3) pereopodal meri 1–3 having short, blunt and robust setae (see clade 5, Fig. 6C in Brandt & Poore 2003). The Cymothoida is sister to the suborder Limnoriidea, both taxa defined by the broad mandibular molar, a state which apparently meets both plesiomorphic and apomorphic conditions (see clade 4, Fig. 6C in Brandt & Poore 2003). The Cymothoida+Limnoriidae is sister taxon to *Phoratopus*, the clade defined by the mandibular molar being triangular blade-like or conical process; pleopods 4 and 5 have plumose setae on the margins of both rami (Brandt & Poore 2003). The basal position of the Cymothoida remains contradictory (Wilson 2009). Within the Isopoda, the suborder Cymothoida is rejected by the molecular data (see Fig. 2 in Wilson 2009) but supported by the morphological data (see Fig. 4 in Wilson 2009). Wilson's (2009) optimization parsimony analysis of molecular 18S and morphological data showed that the suborder Cymothoida formed a polyphyletic clade (Fig. 5). Until further research can resolve both morphological and molecular issues of the suborder, the existing classification of Brandt & Poore (2003) is retained.

1.3.2 The family Cymothoidae

Molecular and morphological phylogenetic results show that the Cymothoidae is the sister group of the derived parasitic Bopyridae (Wägele 1989; Dreyer & Wägele 2001). It was also suggested that the Cymothoidae are derived from the Aegidae (Menzies *et al.* 1955; Dreyer & Wägele 2001; Brandt & Poore 2003). The Aegidae and Cymothoidae share small pars incisiva of the mandibles (Dreyer & Wägele 2001), a strongly curved pereopod 1 and a maxillipedal palp with terminal articles set obliquely with hooks (Brandt & Poore 2003).

Brusca (1981) hypothesized that cymothoids are composed of three eco-morphological adaptive lineages: the externally-attaching genera, the flesh-burrowing genera, and the buccal and branchial cavity genera. Brusca (1981) also suggested that the externally-attaching cymothoids had further evolved and adapted into the opercular cavity. Williams & Bunkley-Williams (1994a) however, suggested overcrowding of the buccal cavity caused isopods to move from the buccal regions to attach to the external surfaces.

Jones *et al.* (2008) and Ketmaier *et al.* (2008) used a molecular phylogenetic framework to determine the evolution of these parasitic cymothoid isopods. The use of mitochondrial genes (large ribosomal DNA subunit, 16S rRNA, and cytochrome oxidase I) showed that the more specialized mouth/gill-attaching species were not necessarily derived from the externally-dwelling cymothoids. These results indicate a complex evolutionary history and suggest that gill and buccal parasitic habits may have evolved independently. The 'linear evolutionary pathway' `theory of buccal and gill-attaching cymothoids derived from externally attaching cymothoids is not supported in this study. However, both Jones *et al.* (2008) and Ketmaier *et al.* (2008) acknowledge the small dataset used in their molecular studies and therefore should be considered preliminary results.

Hadfield (2012) revised the relationship of the Cymothoidae on a generic level using morphological analysis. The resultant trees of the 50% majority rule tree and the strict consensus tree revealed that the 'Anilocrinae' formed a well-supported clade. The buccal-attaching genera formed a well-supported clade (*Ceratothoa*, *Glossobius, Cinusa*, *Lobothorax*, and *Cymothoa*) upheld by pereonite 1 anterolateral margins encompassing the cephalon, pereopods 5–7 basis with large blade-like carina, and maxilla medial lobe partly fused (see Hadfield 2012; Fig. 7.4). However, Hadfield's (2012) morphological dataset was fairly small, consisting of 24 taxa and 40 characters states, and were not compared to any new molecular dataset to better understand evolutionary relationships.

1.4. MARINE ISOPODS WORLDWIDE AND IN AUSTRALIA

Isopods are the most diverse crustaceans (Martin & George 2001; Poore & Bruce 2012) and are ubiquitous, occupying most habitat types such as mountainous regions (Ianc & Ferenţi 2014), deep underground aquifers in caves (Botosaneanu 1986; Schotte *et al.* 2008 onwards), mangrove roots (Ellison & Farnsworth 1990), seagrasses (Kang & Yun 1988), coral reefs (Delaney 1984) and the deep sea, from the supralittoral and intertidal depths of more than six kilometres (Wetzer 2001; Poore & Bruce 2012). Regional isopod faunas have been documented for the Caribbean Sea (see Brusca & Iverson 1985; Kensley & Schotte 1989), North America (see Richardson 1905a; Schultz 1969), Southern America (see Menzies 1962a, b; Menzies & George 1972), Europe (see Sars 1837–1927; Hansen 1906, 1916; Naylor 1972) Southern African region (see Kensley 1978), Japan (see Saito *et al.* 2000), the Indian Ocean (see Kensley 2001), Singapore (see Bruce & Helen 2015), New Zealand (see Hurley 1961) and Australia (see Poore 2002).

The Australian isopod fauna is relatively well-documented in comparison to that of other nearby regions (e.g. western Indian Ocean, temperate South America and eastern Africa). To date, the Australian isopod fauna has 1, 209 species (ABRS 2009). Species of the Microcereberidea and Calabozoidea are not known from Australia (ABRS 2009). Isopods from Australia are distributed along two MEOW realms (Marine Ecoregions of the World; see Spalding *et al.* 2007). Tropical Central Indo-Pacific currently has 475 described species and temperate Australia has 541 described species (Poore & Bruce 2012). The continental slope of temperate southeastern Australia has 357 known species (Poore *et al.* 1994). The western and northern regions of Australia are far less explored compared with other Australian regions and will yield new species. Recent sampling in Western Australia yielded 47 isopod species as potentially new (Poore & Bruce 2012).

1.5. PARASITIC ISOPODS WORLDWIDE AND IN AUSTRALIA

Parasitism is the close association of two organisms, of which the parasite is dependent on a host and derives some form of benefit (Rohde 2005). Some parasites can display predation, although a true predator kills and eats prey whereas a parasite feeds but often does not kill the host. Some parasitic effects may be detrimental in the long term (Rohde 2005). Parasites also generally form a permanent association with its host, while micropredators rarely form a permanent association (Bruce 2009).

Of the 144 isopod families, few are parasitic and are found in the superfamilies Cryptoniscoidea Kossmann, 1880; Bopyroidea Rafinesque, 1815 and Cymothooidea Leach, 1814. The superfamilies Cryptoniscoidea and Bopyroidea represent approximately 7.7% of described isopods (Williams & Boyko 2012), with nearly 90% known species contained within the Bopyroidea (Markham 1985; Boyko *et al.* 2014). The Cymothooidea (of the suborder Cymothoida Wägele, 1989) is a polyphyletic taxon which includes all predatory, parasitic, blood-sucking or scavenging isopods. The parasitic cymothooideans include the cymothoids, aegiids, tridentellids, corallanids and gnathiids (Smit *et al.* 2014; Wetzer 2015). As the level of parasitism increases, setae are fewer (particularly the pereopods and mouthparts), mouthparts develop abrading serrate scales and body segments are smoother (Poore & Bruce 2012).

1.5.1 The parasitic superfamilies Cryptoniscoidea and Bopyroidea

It is estimated that 3% of all crustacean species are parasites of other crustaceans (Kuris 1974). Bopyroidea and Cryptoniscoidea, referred to as epicarideans, are obligate parasites of other crustaceans (Espinosa-Pérez & Hendrickx 2001). Epicarideans differ from other isopods by the highly modified mouthparts forming a suctorial cone for hemolymph feeding of their crustacean hosts (Boyko *et al.* 2014). The three families of Bopyroidea are Bopyridae Rafinesque, 1815; Dajidae Sars, 1882; and Entoniscidae Kossmann, 1881. The families are centered on the Indo-West Pacific and likely have the greatest number of undescribed species (Williams & Boyko 2012). The Bopyridae is currently divided into nine subfamilies comprising 605 described species that are exclusively parasitic on decapod hosts (Williams & Boyko 2012). Cryptoniscoids are ectoparasitic on copepods, ostracods, isopods, amphipods, barnacles and mysids (Nielsen & Strömberg 1965). The Cryptoniscoidea contains seven families with approximately 100 described species (Williams & Boyko 2012).

The biodiversity and taxonomy of the epicarideans are still unresolved and require attention. For example, there is evidence that the bopyrid fauna of the Indo-West Pacific may be double the number presently known in that region (Markham 1986; An 2009; An *et al*. 2010, 2012; Markham 2010; Williams & Madad 2010) and it is anticipated that many more species occur in northern Australia (Poore 2002). Of the 37 known entoniscids (WoRMS 2014), two species occur from Australia, with many species still awaiting description (Shields & Earley 1993). The taxonomic framework of cryptoniscoids is unresolved as families and genera are based on host association rather than morphological characters (Nielsen & Strömberg 1965, 1973).

1.5.2 The cymothooidan parasites

The seven families included in Cymothooidea according to the classification of Brandt & Poore (2003) are Aegidae White, 1850; Anuropidae Stebbing, 1893; Corallanidae, Hansen, 1890; Cymothoidae Leach, 1818; Gnathiidae Leach, 1814; Protognathiidae Wägele & Brandt, 1988 and Tridentellidae Bruce, 1984. One further family, the Barybrotidae Hansen, 1890, was re-validated by Bruce (2009). The two non-parasitic families are the Protognathiidae and Anuropidae, whereas the five other families show progressive development towards parasitism. The protognathiids (two species in the genus *Protognathia*) are mesopelagic species found in the Antarctic and Southern Oceans (Wägele & Brandt 1988). The anuropids (10 species in the single genus *Anuropus*) are bathypelagic species found in the Caribbean and Pacific Ocean (Beddard 1886).

There are 155 species of Aegidae, of which 33 are known from Australian waters (ABRS 2009). Aegids are micropredators rather than parasites of fishes, generally feeding on blood meal and are not permanently attached to their hosts (Bruce 2009). *Alitropus typus* Milne Edwards, 1840 is probably the most common aegid reported attacking fishes in India and Southeast Asia in fresh and brackish waters (Rohde 2005). Species from the genus *Rocinela*, may attach for longer periods to their hosts (see examples in Novotny & Mahnken 1971; Wing & Moles 1995; de Lima *et al.* 2005).

The Tridentellidae consists of 21 species in the genus *Tridentella* and is known from the Pacific and Atlantic Oceans (WoRMS 2014). *Tridentella saxicola* (Hale, 1925) is the only known species to occur in Australia (ABRS 2009), although six species are present in the nearby Indo-Pacific. Several tridentellids are fish ectobionts in their free-living state (Bruce 1984). The mouthparts appear well adapted for rasping and piercing into host flesh (Bruce 1984). Species within the genus can be separated by differences in cephalon, posterior pereonites, pleonites and pleotelson morphology (Bruce 1984, 2008).

The Gnathiidae is cosmopolitan with 222 species in 12 genera (WoRMS 2014), of which 56 species in seven genera occur in Australia (ABRS 2009). Holdich & Harrison (1980) and Cohen & Poore (1994) reviewed the family for the Australian fauna, with further species contribution from Coetzee *et al.* (2008, 2009), Ferreira *et al.* (2009, 2010), Farquharson *et al.* (2012) and Svavarsson & Bruce (2012). The adults are benthic isopods whereas the juvenile ‘pranizas’ are ectoparasitic on fishes (Svavarsson 2006). The taxonomy relies on male characters as females and juveniles are not easily identified (Brusca *et al*. 2001). Recently though, Wilson *et al.* (2011) were able to illustrate taxonomically useful traits from juvenile species to aid identification (e.g. shape of the cephalon, eyes and uropod setation) as well as morphometrics of total body expansion of the pereon after blood feeding.

The Corallanidae consists of 80 species in six genera, with the genus *Corilana* considered to be *nomen dubium*. Delaney (1989) provided a world review of the family, which included keys to the genera, phylogeny of the family and the biogeography. The Australian corallanids consist of 15 species from five genera (ABRS 2009), mostly reviewed by Bruce (1982 a, b). Cirolanids are chiefly marine species that are benthic, with few that inhabit freshwater and estuarine environments. Corallanidae includes micropredators and temporary parasitic species, mostly on fishes. The species *Tachaea caridophaga* (Riek, 1953) and *Excorallana tricornis occidentalis* Richardson, 1905 are to date the only known corallanids to parasitize crustaceans (Riek 1967; Guzman *et al*. 1988).

The Cymothoidae consists of 383 species within 40 genera (Smit *et al*. 2014), of which 71 species in 17 genera are found in Australia (ABRS 2009). Cymothoids are obligate parasites of fishes and exhibit high host and site specificity (Trilles 1969, 1994; Brusca 1981; Bunkley-Williams & Williams 1998a). The family is ubiquitous except in the polar waters and has a high diversity in tropical regions (Brusca 1981; Bakenhaster *et al*. 2006). The Australian fauna was reviewed by Bruce (1986, 1987a, b, c, 1990, 1991) which resolved the complex taxonomy of the Anilocrinae and gill- and branchial-attaching genera. The buccal-attaching genera, particularly in Australian waters, remained unrevised, with many species of doubtful or unclear identity (Poore 2002).

1.6. CYMOTHOIDS IN AUSTRALIA

The first known cymothoid species to be recorded from Australia was *Cymothoa bopyroides* Lesueur, 1814 (=*Ourozeuktes bopyroides*), without the known type status or its current whereabouts (Bruce *et al.* 2002). The first described buccal-attaching cymothoid species from Australian waters was *Codonophilus argus* Haswell, 1881, the type species (by monotypy) for the genus *Codonophilus* Haswell, 1881. The following year, Haswell (1882) recorded two species, *Ceratothoa trigonocephala* (Leach, 1818) and *Ourozeuktes owenii* (Milne Edwards, 1840) and proposed the new species *Ourozeuktes pyriformis* Haswell, 1882 for the Australian fauna. Currently, *Ourozeuktes owenii* and *O. pyriformis* are junior synonyms of *Ourozeuktes bopyroides* (Lesueur, 1814) (see Poore 2002); *Codonophilus argus* is here regarded as *species inquirenda* (see Chapter 3 part II); and *Ceratothoa trigonocephala* is excluded from the Australian fauna (see Chapter 3 part II). Although most of the taxonomy of Australian cymothoid has improved since Haswell's work, it is nonetheless an important footing for the understanding of Australian cymothoids.

Prior to Haswell's cymothoid contribution in 1881, records of Australian species of cymothoid date back as early as 1775 [e.g. *Ceratothoa imbricata* (Fabricius, 1775), see Chapter 2]. Most of these records were from the Indo-Pacific region. Important contributions prior to Haswell's works of species now known from Australia include those of Milne Edwards (1840; three species), Bleeker (1857; five species), Koelbel (1878; two species), Miers (1880; two species) and Schioedte & Meinert (1881, 1883, 1884; 10 species), bringing a total of 22 species in the 1880s.

The first comprehensive review of the Australian Cymothoidae included works of Hale (1926, 1940), who described two new species and provided a key to the family. Hale's (1926, 1940) contribution brought the total number of species in Australia to 14 within seven genera at the time. Later contributions by Avdeev (1975a, b, c, 1978 a, b, 1979a, b) and Bruce (1986, 1987a, b, c, 1990, 1991) described 70 species within 16 genera. Bruce (1986, 1987a, b, c, 1990, 1991) reviewed the externally-attaching genera (*Anilocra, Nerocila, Creniola, Pleopodias, Norileca* and *Renocila*) and gill-attaching genera (*Mothocya*, *Livoneca* and *Elthusa*) for Australia and provided keys for the genera and species of that family. The Australian buccal-attaching genera (*Ceratothoa*, *Cymothoa*, *Glossobius* and *Smenispa*) prior to this study remained unrevised.

1.6.1 Buccal-attaching cymothoids

The marine buccal-attaching genera (based on host site attachment and not morphological characteristics) are: *Cymothoa* (51 species), *Ceratothoa* (33 species), *Glossobius* (nine species), *Catoessa* (four species), *Paracymothoa* (three species), *Lobothorax* (three species), *Smenispa* (two species), *Olencira* (two species), *Tetragonocephalon* (one species) and *Cinusa* (one species) (see Bruce & Schotte 2015 for valid species within each genus). All species of *Braga* (Thatcher 2000, 2002; Thatcher *et al.* 2009) and *Paracymothoa* (Lemos de Castro 1955; Taberner 1976; Bowman 1986) are known only from South America, *Cinusa* occurs in southern Africa (Hadfield *et al.* 2010), and species of *Olencira* occur in the northwest Atlantic Ocean. The generic diagnoses for most buccal-attaching genera have recently been revised, whereas some genera still require some attention such as *Olencira* (see Hoffman 1999) and *Tetragonocephalon*.

Prior to this study, the Zoological Catalogue of Australia lists 22 buccal-attaching species in the genera *Catoessa*, *Ceratothoa*, *Cymothoa*, *Smenispa*, *Glossobius* and *Tetragonocephalon* (Bruce *et al.* 2002). A comprehensive genus diagnosis is available for *Cymothoa* (Hadfield *et al.* 2013), *Ceratothoa* (Hadfield *et al.* 2014a), *Glossobius* (Bruce & Bowman 1989) and *Smenispa* (*Enispa* in Bruce 1990). In the Australian context, there is still considerable confusion over species within certain genera. Prior to this study, it was still unclear if *Ceratothoa imbricata* and *Ceratothoa trigonocephala* (Leach, 1818) should remain valid species or synonymised (discussed in Chapter 3 part II). There were also no keys available for species of the genera *Ceratothoa* and *Cymothoa* of the Australian fauna. With the access of type material and an extensive cymothoid collection from various museums, this enabled me to 1) resolve complex species synonymies, 2) provide new insight into host usage and 3) provide new keys for the genera *Ceratothoa* (see Chapter 3 part II), *Glossobius* (see Chapter 4) and *Cymothoa* (see Chapter 5).

1.7. THE CYMOTHOIDAE AND THE TAXONOMIC CHALLENGES

Species within Cymothoidae show high levels of polymorphism due to intra-specific and inter-specific variations (Brusca 1981; Smit *et al*. 2014). For example, *Mothocya melanosticta* (Schioedte & Meinert, 1884) was previously regarded as a highly variable species of low host specificity which later proved to be nine similar species with high host specificity and narrow geographical distribution (see Table 3 in Bruce 1986). Another example is seen in the species *Cymothoa eremita* (Brünnich, 1783), *C. mathoei* and *C. leschenaultii* that were thought to be separate species but later identified as conspecific (see Hadfield *et al.* 2013). The species *Ceratothoa retusa* (Schioedte & Meinert, 1883), although well characterized by the cephalon immersed in pereonite 1, anterolateral margins of pereonite 1 projecting forward and the broader than long pleotelson, is now known to have intra-specific geographic variation based on nine characters (see Table 1 in Hadfield *et al.* 2014b).

Cymothoid illustrations from the 19th century and early 20th century are frequently inadequate and minimal, often consisting of only a dorsal view of a single adult female. This poses a challenge since key morphological characteristics and species variations are not often illustrated, and thus uncertainty arises if a species name should remain valid, or brought into or out of synonymy. This can be resolved if type specimens can be located and redescribed (examples presented in Chapters 3 to 6 of this thesis). Another possibility for rejecting or confirming species without types is to resample from the type locality and ideally also the type host (if mentioned). Sampling for cymothoids in its own right is a challenge, since cymothoid infestation rates on host and species abundance from a single sample are often low (personal observation).

Current morphological characteristics used in descriptions of cymothoid isopods include: cephalon anterior margin and pleotelson; size and position of the eyes; length and width of the cephalon, pereonites and pleotelson; shape of the body; number of articles in the antennae; shape and size of the coxal plates (and their dorsal visibility); configuration of the posterolateral angles of the pereonites; relative length of the uropod rami; and presence and absence of carinae on the pereopod basis (Brusca 1981;

Hadfield *et al.* 2010). Part of the aim of this thesis is identifying new morphological characteristics to improve the morphological data matrix for the Cymothoidae phylogeny and to deduce if the buccal-attaching clade is monophyletic.

1.8 AIMS AND OBJECTIVES

The aims and objectives of this research are listed below:

AIM 1: To provide a complete taxonomic revision of the buccal-attaching cymothoid genera, with emphasis on the Australian region.

Objectives of aim 1:

1. To revise the genera *Ceratothoa*, *Cymothoa*, *Glossobius* and *Smenispa*.
2. To resolve complex nomenclature and species identity problems (e.g *Ceratothoa imbricata*, *Ceratothoa banksii* and *Ceratothoa trigonocephala*).
3. To provide detail species descriptions from new and type material (where applicable).
4. To provide comprehensive keys to the Australian buccal-attaching genera and to the species of *Ceratothoa*, *Cymothoa,* and *Glossobius*.
5. To provide host account details to better understand host specificity.
6. To map the distribution of cymothoid species from new and available material.

AIM 2: To revise the phylogeny of the family Cymothoidae.

Objectives of aim 2:

1. To test the monophyly of the family Cymothoidae.
2. To test if the phylogeny of Cymothoidae is independent of site attachment (e.g. gills, mouth, flesh).
3. To expand on Hadfield’s (2012) dataset with additional genera and characters and analyse the morphological phylogeny of the Cymothoidae.

4. To further expand the molecular phylogeny from Jones's *et al.* (2008) and Ketmaier's *et al.* (2008) study based on 16S rRNA and COI.

1.9 THESIS STRUCTURE

This review is deemed an important contribution because it: 1) addresses nominal species of doubtful and unclear identity within the genera *Cymothoa*, *Ceratothoa* and *Glossobius*, inclusive of species identification keys; 2) provides better understanding of species distribution from new material, particularly species of new records to Australia; 3) provides better understanding of host usage among different species within Australia; 4) identifies intra-specific and inter-specific morphological variations; 5) introduces new species sequence from different taxa using two mitochondrial genes (16S rRNA and cytochrome oxidase I), 6) evaluates the combination of 16S rRNA and cytochrome oxidase I from previous literature and using current research to produce similar evolutionary trees, and 7) re-examines Hadfield's (2012) morphological dataset with new characteristics and taxa; and 8) evaluates the outcome of the resulting tree.

Chapter 1 (current chapter) is a general introduction of the Isopoda and the family Cymothoidae focusing on the biodiversity of parasitic isopods (particularly the buccal-attaching cymothoids) and morphological and molecular phylogenetics of various isopods and the Cymothoidae. Chapter 2 provides a literature review of the Cymothoidae history, current classification and summaries of current knowledge of the ecology and economical impacts of the Cymothoidae. Chapters 3, 4, 5 and 6 are devoted to taxonomic reviews of the buccal-attaching genera *Ceratothoa*, *Glossobius*, *Cymothoa* and *Smenispa* respectively, with keys provided for the first three chapters. Chapter 7 explains the cladistics analysis of the family Cymothoidae using morphological and molecular data, bearing in mind that most specimens used in the molecular study were obtained from museum collections and most materials were not preserved properly for genetic analysis. Chapter 8 provides a summary of this research's significant findings and outputs; and suggestions for future work.

CHAPTER 2

Literature Review

2.1 THE HISTORY OF CYMOTHOID TAXONOMY

Records of arthropod parasites on fish date back to 300 BC when Aristotle observed parasitic isopods (=cymothoids) on the fins of fishes, and ‘lice’ (possibly ergasilid copepods) in the gills of bleak (Lester & Hayward 2006). The first early records of cymothoids were in the works of Belon (1553) and Rondelet (1554). Subsequent publications were mostly sporadic and covered broader aspects of biology and species dynamics. Smit *et al*. (2014) showed a spike in the documentation of cymothoid species in the periods 1810–1820, 1850–1860, 1870–1890 and 1970–1990 (Fig. 2.1), attributable to Leach (1814, 1815, 1818), Bleeker (1857), Schioedte & Meinert (1881, 1883, 1884), Bovallius (1885), Richardson (1905a, b), Brusca (1981), Williams & Williams (1979, 1981, 1985a, b), Williams & Bunkley-Williams (1982), Thatcher (2000) and Bruce (1986, 1987a,b,c, 1990, 1991).

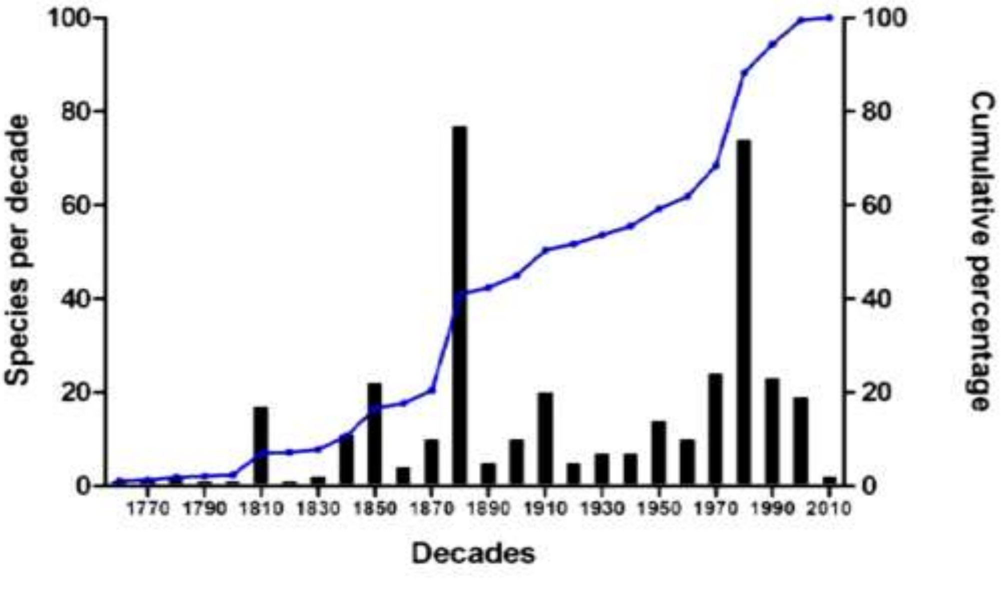

Figure 2.1 Absolute numbers and cumulative percentages of 383 Cymothoidae species published per decade (from Smit *et al*. 2014).

Although many species of Cymothoidae date from the earliest period of crustacean taxonomy (e.g. references such as Linnaeus, Leach, Milne Edwards), much of our knowledge of the Cymothoidae is still poor. A number of authors have paved the way in laying the foundation of the taxonomy, evolution and zoogeography of the family. The family name Cymothoidae Leach, 1818 was established by William Elford Leach, who also named nine new species at the time (see Leach 1813–1814, 1815, 1818). Milne Edwards (1840), another noteworthy contributor, provided the first world-wide review of crustaceans and proposed 30 species names for the Cymothoidae at the time. Jørgen Christian Schioedte and Frederik Vilhelm August Meinert were probably the most significant contributors of the Nineteenth Century, when the Danish researchers provided the world's most comprehensive revision of what is now the superfamily Cymothooidea, which includes the families Corallanidae, Aegidae, Tridentellidae and Cymothoidae (see Schioedte & Meinert 1881, 1883, 1884 for references on Cymothoidae). The contributions of Schioedte & Meinert are important because unlike most early publications, theirs were genuinely worldwide revisions, and they specified both the provenance and the holding institutions of the specimens examined (see examples in Schioedte & Meinert 1881, 1883, 1884). Schioedte and Meinert also gave detailed classification for the family, including sub-family and tribe names. Other important works prior to the 1900s include Risso (1816), Say (1818), Otto (1828), Perty (1833) Bleeker (1857), Miers (1877, 1880) and Haller (1880).

During the Twentieth Century, there was considerable taxonomic activity on the family, particularly in the 1980s. Authors who worked on a global basis are Stebbing (two new species, see examples in Stebbing 1910a, 1923, 1924), Bowman (13 new species; see examples in Bowman 1962, 1986) and Trilles (seven new species, see examples in 1964b, 1968, 1969, 1972a, b, c, 1975, 1994). Authors that have contributed to the knowledge of cymothoids on a regional basis are Nierstrasz (five new species, see Nierstrasz 1915, 1931) for the Malay Archipelago; Richardson (24 new species, see Richardson 1905, 1910a, b, 1911) for cymothoids of the West Atlantic and Eastern Pacific; Williams and Bunkley-Williams (27 new species, see examples in Williams & Williams 1979, 1981, 1985, 1982, 1994a, b, 1999, 1985a, b; Bunkley-Williams & Williams 1996, 1998 a, b, 2003; Bunkley-Williams *et al.* 1998, 1999, 2006) for the

Caribbean, Japan and Thailand fauna; Thatcher (15 new species, see examples in Thatcher 1988, 1993a, b, 1996, 1997, 2000, 2002; Thatcher *et al.* 2003 a, b, c, 2007, 2009) for freshwater cymothoids of the South American freshwaters; Brusca (2 new species, see examples in Brusca 1978a, b, 1981) for cymothoids of the tropical East Pacific; and Hale (three new species, see Hale 1926, 1927, 1929, 1940), Avdeev (15 new species, see examples in Avdeev 1975a, b, c, 1978a, b, 1979a, b, 1985a, b, 1992) and Bruce (39 new species; see examples in Bruce 1986, 1987a, b, c, 1990, 1991) for cymothoids from Australian waters.

2.2 CYMOTHOIDAE DIVERSITY

Cymothoidae is cosmopolitan with 383 species in more than 40 genera (Smit *et al.* 2014). The family is dominated by marine cymothoids, with few genera containing more than 20 species: *Anilocra* (49 species), *Nerocila* (42 species), *Cymothoa* (50 species), *Ceratothoa* (33 species), and *Mothocya* (29 species) (Smit *et al.* 2014). Other genera have one to ten species: e.g. (*Glossobius*: five; *Joryma*: four species; *Olencira*, *Ourozeuktes* and *Tetragonocephalon*: one species each). The freshwater cymothoids consist of the genera *Anphira*, *Artystone*, *Asotana*, *Braga*, *Ichthyoxenus*, *Isonebula*, *Paracymothoa*, *Riggia*, *Telotha* and *Vanamea*, mostly occurring in South America and parts of Africa and Asia. *Ichthyoxenus* (24 species) is the most specious freshwater genus and also includes some marine species (e.g. *Ichthyoxenus puhi* Bowman (1962). Table 2.1 includes a list of all valid genera and the numbers of valid species for each genus from the World Marine, Freshwater and Terrestrial Isopod Crustaceans database. These figures are estimates since many nominal species and genera are still of questionable validity, particularly if types are not available. At the time of Smit's *et al.* (2014) record, 16 genera and 83 species were in synonymy, and a further seven are regarded as *nomen dubia*. This thesis further adds two new species, two species as *nomen dubia*, two species as *nomen nuda*, five species in synonymy and two species transferred to separate genera.

Table 2.1 Current list of valid cymothoid genera and species numbers (Bruce & Schotte 2015).

No	Genus	Authority	Species numbers
1	*Aegathoa*	Dana, 1853	2
2	*Agarna*	Schioedte & Meinert, 1884	4
3	*Amblycephalon*	Pillai, 1954	2
4	*Anilocra*	Leach, 1818	49; 1 nomen dubium
5	*Anphira*	Thatcher, 1993	4
6	*Artystone*	Schioedte, 1866	3
7	*Asotana*	Schioedte & Meinert, 1881	3
8	*Braga*	Schioedte & Meinert, 1881	7
9	*Catoessa*	Schioedte & Meinert, 1884	4
10	*Ceratothoa*	Dana, 1852	31; 1 nomen nudum
11	*Cinusa*	Schioedte & Meinert, 1884	1
12	*Creniola*	Bruce, 1987	3
13	*Cterissa*	Schioedte & Meinert, 1884	2
14	*Cymothoa*	Fabricius, 1787	49; 1 nomen nudum, 1nomen dubium
15	*Elthusa*	Schioedte & Meinert, 1884	28
16	*Emetha*	Schioedte & Meinert, 1883	2
17	*Smenispa*	Özdikem, 2009	2
18	*Glossobius*	Schioedte & Meinert, 1883	9
19	*Ichthyoxenus*	Herklots, 1870	23
20	*Idusa*	Schioedte & Meinert, 1884	3
21	*Isonebula*	Taberner, 1977	2
22	*Joryma*	Bowman & Tareen, 1983	4
23	*Kuna*	Williams & Williams, 1985	1
24	*Lathraena*	Schioedte & Meinert, 1881	1
25	*Livoneca*	Leach, 1818	3 valid; 1 taxon inquirendum; 14 nomen dubia
26	*Lobothorax*	Bleeker, 1857	3
27	*Mothocya*	Hope, 1851	30 valid, 1 nomen dubium
28	*Nerocila*	Leach, 1818	42 valid, 1 nomen dubium
29	*Norileca*	Bruce, 1990	3
30	*Olencira*	Leach, 1818	2
31	*Ourozeuktes*	Milne-Edwards, 1840	1
32	*Paracymothoa*	Lemos de Castro, 1955	3
33	*Philostomella*	Szidat & Schubart, 1960	1
34	*Pleopodias*	Richardson, 1910	4
35	*Plotor*	Schioedte & Meinert, 1881	1
36	*Pseudoirona*	Pillai, 1964	1
37	*Renocila*	Miers, 1880	18
38	*Rhiothra*	Schioedte & Meinert, 1884	1
39	*Riggia*	Szidat, 1948	5
40	*Ryukyua*	Williams & Bunkley-Williams, 1994	2
41	*Telotha*	Schioedte & Meinert, 1884	2
42	*Tetragonocephalon*	Avdeev, 1975	1
43	*Vanamea*	Thatcher, 1993	1

2.3 CYMOTHOIDAE DISTRIBUTION

Cymothoids are found worldwide, with high records in tropical and subtropical areas. Few cymothoid species (e.g. species of *Anilocra* and *Elthusa*) occur at depths over 200 meters (see Bruce 1987a, 1990). Smit *et al.* (2014) provided a worldwide distribution map of cymothoids using Spalding's *et al*. (2007) Marine Ecoregions of the World (Fig. 2.2). The highest diversity is found within the tropical regions, with 79 species from the Central Indo-Pacific, followed by the Western Indo-Pacific (44) and the Tropical Atlantic (41). The high occurrence of Cymothoidae in the Central Indo-Pacific is in agreement with the findings of other authors (Brusca 1981; Avdeev 1985b; Poore & Bruce 2012). The eastern Pacific, southern Africa and Polar regions have the lowest species diversity (reflected in Fig 2.2).

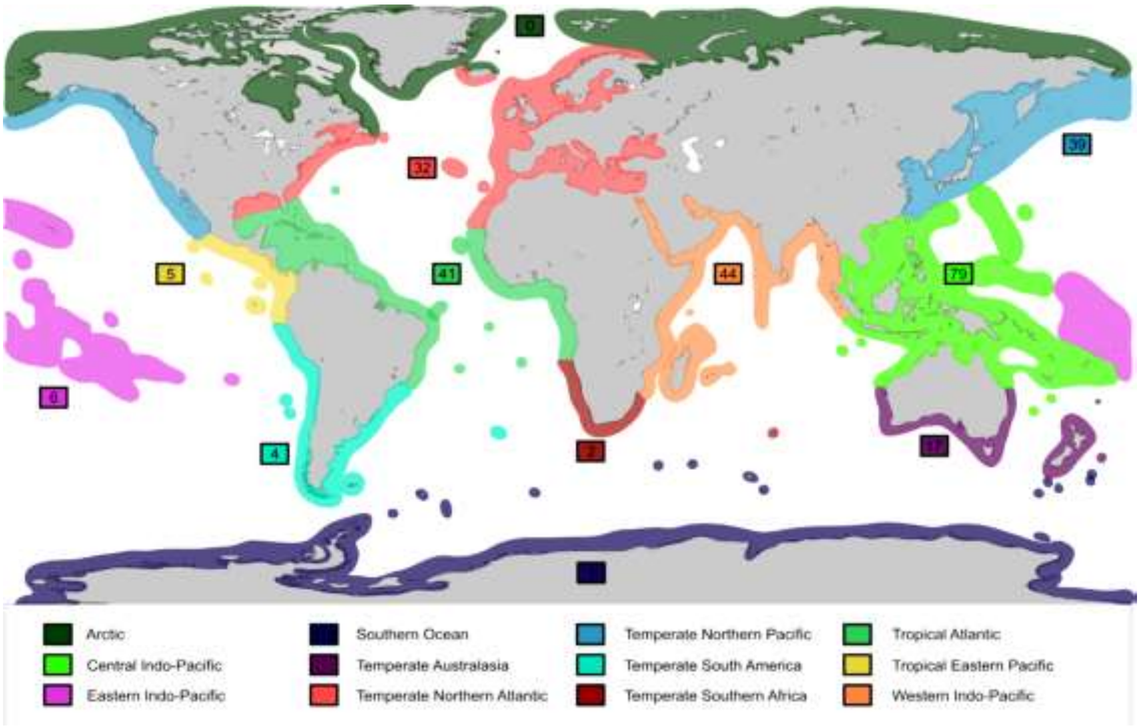

Figure 2.2 Numbers of marine cymothoid species in different biogeographic regions (Marine Ecoregions of the World). Data mapped using Spalding's *et al*. (2007) Marine Ecoregions of the World (MEOW) from Smit *et al.* (2014).

The above species numbers for different biogeographic regions ought to be approached with caution as many of the species identity and taxonomy need to be clearly sorted and accurately characterized. For example, *Ceratothoa trigonocephala* (Leach, 1818) was initially thought to have a worldwide distribution parasitizing 18 species in 17 genera of fish hosts. The recent work by Hadfield *et al.* (2014a) and this research (Chapter 3) show that most previous host records are incorrect and these have now been excluded from the South African and Australian waters. Since host specificity remains uncertain, previous records of the distribution of *C. trigonocephala* remain uncertain.

Brusca (1981) suggested that the low numbers of species in the Americas was due to poor documentation within the region. In South America, most of our current understanding of freshwater cymothoids comes from the works of Thatcher and colleagues (see examples in Thatcher 1988, 1993a, b, 1996, 1997, 2000, 2002). Brusca's (1981) observation may not entirely hold true, since in the 19th century, global research expeditions did include the Americas (e.g. The United States Exploring Expeditions of 1838 to 1842, The *Challenger* expedition of 1872 to 1876). However, it is known that sampling for cymothoids can be difficult. For example, Bruce (1987c) explained how his six weeks attempt of field work sampling for cymothoids from the Great Barrier Reef included 2–4 hours under water each day. Some cymothoids may be obtained through opportunistic fishing, fish markets or examination of by-catch from research expeditions (e.g. Bluefin research vessel, University of Tasmania) and from museum collections.

Marine cymothoids are not known to have high endemism. Brusca (1981) suggested that the low endemism of marine cymothoids has appeared to be established during the Permo-Triassic Tethyan Sea radiation and the nature of its host association influences the wide distribution. The freshwater cymothoids display high endemism. Nearly 10 freshwater genera occur in South America, whereas three species from the genus *Ichthyoxenus* occur in the central African region. In Asia, *Ichthyoxenus* is predominant in China, Indonesia, India and South East Asia (Brusca 1981; Fig. 3).

2.4 CYMOTHOIDAE LIFE CYCLE

Cymothoids are protandrous hermaphrodites (Hale 1926, 1927, 1929; Bullar 1876; Brusca 1981; Bunkley-Williams & Williams 1998a; Thatcher 1996; Legrand 1951; Trilles 1969), where the pullus 1 phase are all males and eventually develop into adult females. Most cymothoid species life history is poorly documented due to difficulties in keeping cymothoids in laboratory conditions (Smit *et al.* 2014). Some works that illustrate complete cymothoid life cycles refer to *Anilocra pomacentri* Bruce, 1987 (see Adlard & Lester 1995), *Glossobius hemiramphi* Williams & Williams, 1985 (see Bakenhaster *et al*. 2006), *Ceratothoa oestroides* (Risso, 1816) (see Mladineo 2003) and *Mothocya epimerica* Costa, 1851 (Bello *et al*. 1997). A cymothoid life cycle ranges from days [62 days for *Anilocra pomacentri* (see Adlard & Lester 1995)] to years [one year for *Glossobius hemiramphi* (see Bakenhester *et al*. 2006), nine years for *Ceratothoa imbricata* (Fabricius, 1775) (see Maxwell 1982)].

2.4.1 Marsupiumite (Figure 2.3A-D)

Bakenhaster *et al.* (2006) referred to marsupiumites as residents of the female cymothoid marsupium (brood pouch) and identified five ontogenetic stages (four of which are pictured in Fig. 2.3). Figure 2.3A are embryos nested within the marsupium of an ovigerous female cymothoid, with an ovoid to subspherical appearance and absence of structural segmentation (Bakenhaster *et al.* 2006). The marsupiumites would then develop into elongate or oval-like embryos (Fig. 2.3B), with the presence of cephalic capsule differentiation and vaguely visible limb bud development. The segmented embryos (Fig. 2.3C), also known as the prehatch I stage (Brusca 1978a, b; Adlard & Lester 1995) have evident eye formation, two rows of ventral limb buds and mouthpart morphology. The manca is considered the final marsupial and this development is also the first free swimming stage (Fig. 2.3D).

There has been an ongoing confusion with the terms “pullus” (*plural* pulli), manca (*plural* mancae) and juvenile (*plural* juveniles) which are loosely used in different publications (Bakenhaster *et al.* 2006). The developing eggs in the adult ovigerous female marsupium (brood pouch) will hatch and moult into the first pulli stage (also refered as the pre-mancae, pulli I, and pre-hatch II (see Adlard & Lester

1995; Mladineo 2003; Lester 2005; Bakenhaster *et al.* 2006), the pulli are all sexually non-differentiated, have six pairs of pereopods, strongly pigmented (Smit *et al.* 2014) and lack uropodal and pleotelsonic setae (Bakenhaster *et al.* 2006). The first pulli will only develop in the marsupium into mancae (also refered as the second pulli or pulli II stage). In contrast to Bakenhaster *et al.* (2006), Trilles (1964b) defined four different pulli stages in the development of *Ceratothoa oestroides* embryonated eggs. Pulli I develop into Pulli II and III (bearing seven pereopods) and the final Pullus IV stage, the beginning of postlarval development.

The mancae phase (in which all mancae are males) occurs when the mancae have left the female marsupium in search of a host. The mancae have well-developed eyes, lack an appendix masculina and have swimming pereopods with setae present on the margins of the appendages (Bakenhaster *et al.* 2006; Smit *et al.* 2014). The mancae remain free swimming for several days, [up to seven days according to Varvarigos (2003)] in search of a host until the yolk diminishes (Brusca 1978a). Free-swimming mancae are not permanently attached to a host, thus are capable of re-infecting new individuals. *Ceratothoa oestroides* mancae in experimental conditions were able to feed on different individuals of *Sparus aurata* Linnaeus, 1758 for up to a week without molting into sessile juveniles (Mladineo 2003). In contrast, mancae that were unable to find a suitable host did not survive a week (Menzies *et al*. 1955; Sandifer & Kerby 1983). Once an appropriate host is found, the mancae lose their swimming ability and swimming setae, and moult into juvenile males.

2.4.2 Juvenile male (Figure 2.3E) and adult male (Figure 2.3F)

Cymothoids undergo different developmental stages of male juveniles, transitory stage of males to females and adult ovigerous females (Trilles 1969). Many authors loosely apply the juvenile terminology to describe the mancae stage (Hale 1926; Sartor & Pires 1988; Bunkley-Williams & Williams 1998a; Jones *et al.* 2008). Thus it is important that authors clearly identify defining characteristics of each life stage (Bakenhaster *et al*. 2006), since morphological characteristics of different species from different genera are not treated equally.

The juvenile stage generally follows after the manca has attached itself to a host using robust and hooked dactylus on the pereopods. The cymothoid juvenile also develops a seventh pair of pereopod and appears morphologically similar to an adult female. Once a host is found, the juveniles begin hematophagic nourishment (Trilles 1969; Mladineo 2003), and continue to grow and moult while attached to its host (Trilles & Hipeau-Jacquotte 2012). Upon parasitizing a fish, the juvenile male will grow rapidly and transform into an adult female cymothoid without the competition of a similar cymothoid species. The first male to parasitise a fish changes into an ovigerous female whereas subsequent males attaching to the same fish remain as males (while the female is alive), either juvenile or adult (Rohde 2005). It is suggested that the presence of the adult female inhibits the transformation of other rival male cymothoids into females on the fish (Thatcher 2000). Sartor & Pires (1988) described *Cymothoa liannae* Sartor & Pires, 1988 juvenile males (referred to as immature males) as having well-developed eyes, penes present midventrally, and antenna 1 and uropod rami with setae, whereas the adult males are morphologically bigger, have less visible eyes, penes are wider and more developed, and the antennae and uropodal rami lack setae. Bakenhaster *et al*. (2006) described the adult males (referred to as functional males) of *Glossobius hemiramphi* occurring on the second and third gill arches in one branchial chamber of the host, and the presence of a well-developed penial lobe and pereopod 6 and 7 of similar size.

2.4.3 Adult male (Figure 2.3F) to adult female (Figure 2.3G)

The number of moults for sex reversal in different cymothoid genera varies. Williams & Williams (1981) found the transition from male to female required multiple moults whereas Brusca (1981) suggested that it roughly took one moult. The juvenile transition from male to female is dependent on factors such as: the presence of other available females or adult males that inhibit development (Lincoln 1971b; Smit *et al.* 2014), temperature and host availability (Mladineo 2003). Sexual transformation occurs with the regression of the male organs and the development of the female reproductive apparatus (Smit *et al.* 2014). The female and male sex organs are located ventrally on the pereon near the bases of the pleopods. The male possesses a penial process (bilobed projection) and an appendix masculina (the second pleopod bearing an elongate structure near the mesial margin of the endopod) that is used to transfer the spermatophore to the female (Thatcher 2000). Female cymothoids inhibit the sexual transformation of their associated males and thus maintain a stable female-male pair (Bunkley-Williams & Williams 1998a; Thatcher 2000). Pheromones released by females through the host blood (Legrand & Juchault 1970; Raibut & Trilles 1993) or host mucus of some species (Trilles & Hipeau-Jacquotte 1996) stimulate the neurosecretory system of conspecific males, resulting in extension of masculinity from prolonged androgen production.

2.4.4 Cymothoid mating (Figure 2.3H)

Male cymothoids of different species display different mating behaviour and attachments towards the female. The mating observation of *Anilocra* sp. by Legrand (1952) showed a cymothoid pair coming together from their immobile host positions and remaining in contact from 5 to 10 minutes before returning to their original positions. In other species, such as *Smenispa convexa* (Richardson, 1905), juvenile males were found moving back and forth on the host to fertilize the females, presumably in response to female pheromone (Rohde 2005). Gill-attaching cymothoids occur with equal frequency on either the left or right buccal chamber, with no more than one pair occurring per host (Williams & Williams 1985b). In flesh-burrowing cymothoids such as *Ourozeuktes*, small males are found in the pouch with the female (Rohde 2005).

Cannibalism has also been observed during copulation. Two forms of cannibalism in *Ichthyoxenus fushanensis* Tsai & Dai, 1999 were documented, where the male is consumed by the female after copulation; or the female is eaten by the male during or after the incubation of the eggs (Tsai & Dai 2003).

2.4.5 Development of ovigerous female (Figure 2.3I-L)

The adult female morphology changes after copulation. The ventral body develops oostegites, which are plates that protect and aerate the young developing embryos. The developed oostegites form the marsupium that contains the eggs (Menzies *et al.* 1955; Trilles 1964a; Brusca 1981; Maxwell 1982; Adlard & Lester 1995). Mancae are released from the brood pouch by the posterior lifting of the adult female's body away from the host using its seventh pereopod. The marsupium is disrupted and the mancae exit either posteriorly or through a central opening (Williams & Williams 1985b; Bullar 1878). There are two different types of brood pouch within the Cymothoidae (Bruce 1987c) that may play a significant role in the emergence of hatching of the cymothoids.

Sartor & Pires (1988) described the pre-ovigerous females of *Cymothoa liannae* having oostegites arising from the coxae of pereopods 1 to 5, increasing in size from the first oostegites pair to the last, similar to the *Ceratothoa imbricata* specimen in Fig. 2.3I. The pre-ovigerous is similar to the ovigerous females of Fig. 2.3J–L, with the exception of the fully developed and free oostegites plates of the ovigerous females, which enables it to bear marsupiumites.

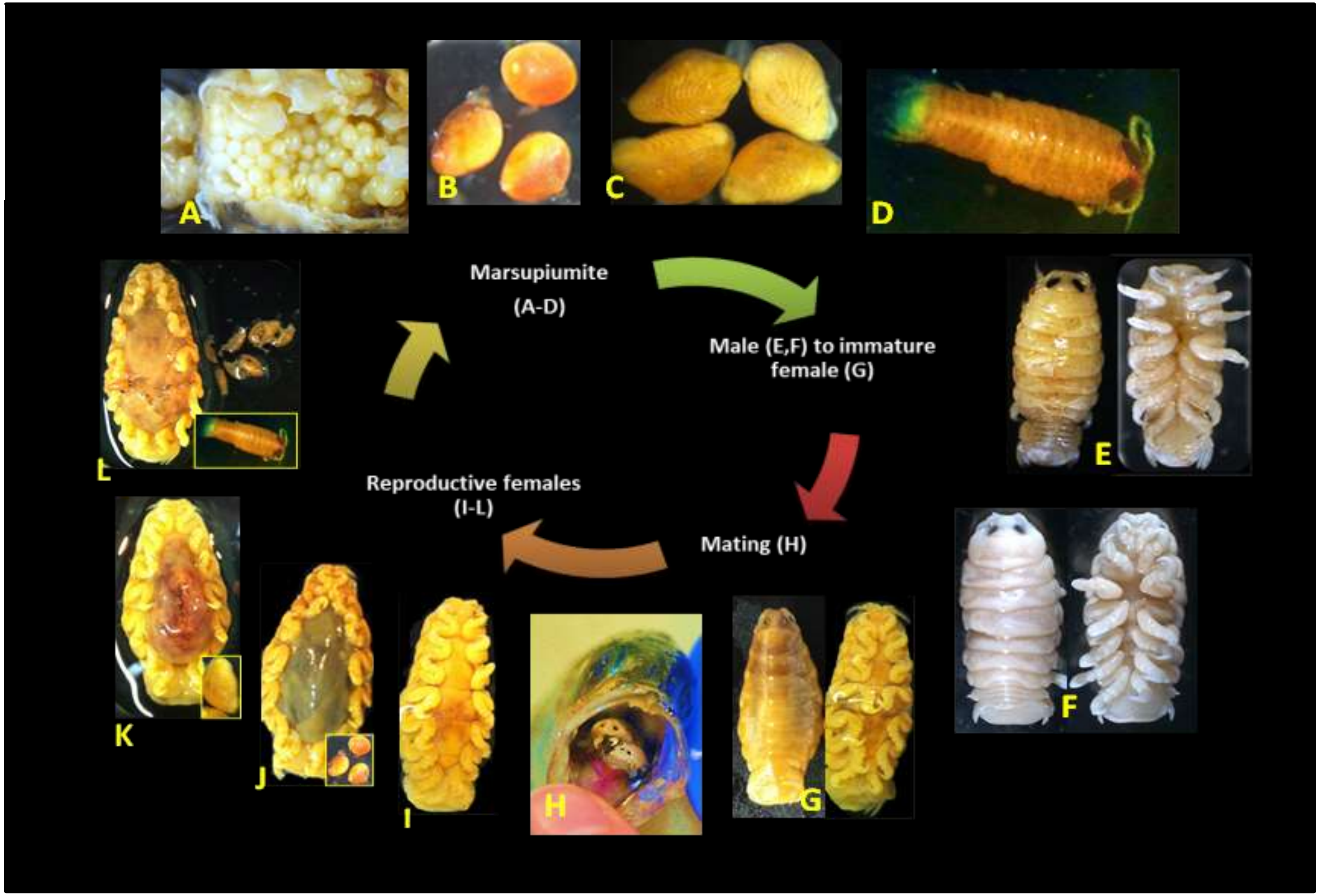

Figure 2.3 Cymothoid general life cycle (adapted from Bakenhaster *et al*. 2006).

2.5 CYMOTHOIDAE SPECIFICITY

Host specificity within Crustacea is diverse, ranging from low specificity (stenoxene or euryxene) to strict specificity (oioxene) (Trilles & Hipeau-Jacquotte 2012). Cymothoids are obligate parasites of fishes, although unusual attachments on non-teleost hosts have been reported in the past [e.g. crustaceans, sponges, cephalopods, amphibians (Haswell 1881; Baer 1952; Trilles 1972b; Trilles & Öktener 2004; Suat Ates *et al.* 2006)]. Previous literature suggests that host availability, life cycle and ecology are possible factors for the diverse spectrum displayed in cymothoid host-association (Trilles 1968, 1969; Brusca 1978a, 1981; Segal 1987; Fogelman 2005).

2.5.1 Host-specificity

Cymothoids generally display high host specificity (Trilles 1964a, b; Brusca 1981), although this varies at species, genus and host-family levels. *Cinusa tetrodontis* Schioedte & Meinert, 1884 is known only from the evil-eye pufferfish *Amblyrhynchotes honckenii* (Bloch, 1785) (see Hadfield *et al.* 2010) and *Tetragonocephalon lutianus* Avdeev, 1975 has only been reported from the emperor red snapper *Lutjanus sebae* (Cuvier 1816) (see Avdeev 1975c). On a host-generic level, *Anilocra leptosoma* Bleeker, 1857 has only been reported from the host genus *Nematalosa*, and *Olencira praegustator* (Latrobe, 1802) was reported from menhadens of the genus *Brevoortia* (Williams & Bunkley-Williams 1999; Trilles 2007). *Anilocra apogonae* Bruce, 1987, *Anilocra nemipteri* Bruce, 1987, *Anilocra pomacentri* Bruce, 1987 and *Anilocra chromis* Williams & Williams, 1981 are named after their associations with the host genera *Apogon, Nemipterus, Pomacentrus* and *Chromis* respectively (Bruce 1987a; Williams *et al.* 1982). On a host-family level, *Ichthyoxenus puhi* (Bowman, 1962) is reported on eels from the family Muraenidae (Bowman 1962; Martin *et al.* 2014b), *Ourozeuktes bopyroides* (Lesueur, 1814) is always found on leatherjackets from the family Monacanthidae (Hale 1926), and species of the genus *Renocila* are either genus or family specific (see Table 1 in Bruce 1987c).

Some cymothoid species use host species from different families. Trilles (1975), Brusca (1981) and Bruce (1987b) showed that some species of *Nerocila*, particularly those from cool–temperate waters, exhibited low preference for host genus or family. *Anilocra physodes* (Linnaeus, 1758) has been reported from 25 fish genera in 13 families (summarized by Trilles 1975).

It is not known why certain cymothoids species are more cosmopolitan and have low host preference than others. Brusca (1978b) suggest this may be linked to functional utility or ecological preference of the cymothoid rather than taxonomic preference of the host. Brusca (1978b) suggested that *Elthusa vulgaris* prefers demersal fishes (e.g. cod, flat fishes and rockfishes) and schooling fishes (e.g. perch and sanddab) that will facilitate the dispersal of mancae (see Table 1 pg. 13 in Brusca 1978b). Like *E. vulgaris*, *Nerocila californica* Schioedte & Meinert, 1881 has low specificity and is known to parasitize 12 different species of demersal or schooling fishes (Brusca 1978a, b). Brusca (1978a) noted that the host fish of *N. californica* are entirely unrelated phylogenetically but are similar in behavior. Brusca (1978a) did not mention if the fish behavior relates to feeding, adaptive or schooling behavior, but it would be an interesting aspect of host-parasite relationship to investigate.

Fish behaviour could influence host susceptibility towards cymothoid. Williams *et al.* (1982) conducted a field transfer of *Anilocra chromis* to brown chromis *Chromis multilineata* (Guichenot, 1853) and blue chromis *Chromis cyanea* (Poey, 1860) to test host susceptibility. Ovigerous *A. chromis* females were used because (quoted from Williams *et al.* (1982)) "…its size normally exceeds other life cycle stages, simplifying both transfers and field observations; and the inability to swim or feed simplifies the interpretation of experimental results". It was observed the blue chromis was more aggressive to the transferred *A. chromis*, whereas the brown chromis was placid with the presence of the isopod. This host reaction would suggest that predisposition may affect selection of a suitable host.

The wide selection of host species for a single cymothoid species may be a genuine misidentification of the cymothoid species. The taxonomic resolution of a cymothoid species usually sheds light on host preference, of which in most cases, the species would later prove to be host specific. For example, the Caribbean species *Anilocra laticauda* (now deemed *nomen dubium*) was initially reported from 11 host families (Trilles & Vala 1975). The revision of the Caribbean *Anilocra* by Williams & Williams (1981) revealed that *A. laticauda* refers to possibly nine species, of which eight species are limited to one host species (or one genus), while one other species parasitizes three host genera in two families. In another example, *Ceratothoa trigonocephala* was believed to parasitize 18 host species in 17 genera from 14 families (Smit *et al.* 2014). Recent work by Hadfield *et al.* (2014a) and the present research (see Chapter 3) showed that most records for *C. trigonocephala* were in fact misidentifications (or unrecognizable), leading to a completely misleading and incorrect perception of both distribution and host-cymothoid association. Revision of this species showed that the species does not occur in Australian or South African waters.

Cymothoids have been reported to form accidental or unusual attachments towards unlikely host fish. One reason could be the transfer of cymothoids from prey to predator. Williams & Bunkley-Williams (1994a) discovered the unusual attachment of *Anilocra acuta* Richardson, 1910b in the pharynx of scombrid mackerel *Scomberomorus cavalla* (Cuvier, 1829). The scombrid probably consumed the prey that already had the externally attached *Anilocra*, which later attached in the mackerel's pharynx (Williams & Bunkley-Williams 1994a). Similar prey-predator shifts have been observed on cymothoids re-occurring in the buccal cavity of the host's predator (e.g. jacks, barracuda and snappers) from the Caribbean (Williams & Bunkley-Williams 1994a) and even on a lantern shark (Williams *et al.* 2010). Another "opportunistic" attachment is the transfer of cymothoids from wild fish to farm cultured fish (for examples see Bragoni *et al*. 1983, 1984; Sievers *et al*. 1996; Šarušić 1999; Papapanagiotou *et al*. 1999). Sievers *et al.* (1996) reported such an incident, where the infective mancae of *Ceratothoa gaudichaudii* (here regarded as *species inquirenda*) did not reach reproductive maturity on Atlantic salmon *Salmo salar* Linnaeus, 1758. *Trachurus murphyi* Nichols, 1920 was most likely the source of infection, as schools of these fish passed by farms during summer for food. An infected *T. murphyi* could bear a gravid female cymothoid and release swimming mancae, parasitizing *Salmo salar* (see Sievers *et al.* 1996). Andrews *et al.* (2013) reported a similar scenario of *Ceratothoa banksii* (Leach, 1818) on striped trumpeter *Latris lineata* (Forster, 1801).

Not all cymothoids display a preference for uninfected hosts. Multiple cymothoid species have been known to occur on the same host and are able to co-exist. Williams & Williams (1985c) reported *Anilocra abudefdufi* Williams & Williams, 1981 and *Cuna insularis* (Williams & Williams, 1985) of a single sergeant major *Abudefduf saxatilis* (Linnaeus, 1758) from islands off the Caribbean coast of Panama. A similar case was reported by Williams & Williams (1981) where two cymothoid isopods, the external-attaching *Anilocra acanthuri* Williams & Williams, 1981 and the gill-attaching *Agarna cumulus* (Haller 1881) were found on one doctorfish *Acanthurus chirurgus* (Bloch, 1787). It was suggested that the presence of an existing cymothoid on the parasitized host facilitated the attachment of another cymothoid species (Williams & William 1985c).

2.5.2 Site preference

Cymothoids have unique morphological characteristics suited for the localized site-attachment on the host (Fig. 2.4), with certain genera displaying precise localization (Brusca 1981; Fogelman & Grutter 2008). Some cymothoid genera are branchial parasites (*Livoneca*, *Mothocya*, *Irona*), others attach to the external surfaces (*Anilocra*, *Nerocila*, *Renocila*) or in the buccal cavity (*Ceratothoa*, *Cymothoa*, *Emetha*) while a few genera burrow in the flesh (*Artystone*, *Icthyoxenus*, *Ourozeuktes*, *Riggia*) (Trilles 1969, 1994; Thatcher *et al*. 2003a). The body morphology of the external-attaching genera (e.g. *Anilocra* and *Nerocila*) are strongly vaulted, dorsoventrally flattened and streamlined (Lincoln 1971b), which allows less resistance in water.

The buccal-attaching genera have thick cuticle that protects the isopod in the chambers of their hosts (Trilles 1969) and long, slender dactyli of pereopods 4–7 to adhere to the fish’s tongue or buccal region. Gill-attaching cymothoids have an asymmetrical body morphology that follows the shape of the host’s operculum.

Overcrowding of cymothoids on a single host has been reported and may facilitate shift of site attachment among cymothoids. Williams & Bunkley-Williams (1994a) examined a crevalle jack *Caranx hippos* (Linnaeus, 1766) parasitized with 260 *Cymothoa oestrum* mancae (loosely termed juveniles). The fish was confined in a fish trap for an unknown period, with mancae in the gills, buccal chamber and head of the fish. This was due to the high availability of the mancae confined in a trap for a short period of time. A stressed fish (stress may be directly related to physiological or environmental factors, or a combination of both) may trigger an ovigerous female cymothoid to release mancae which can attach to the same host as their parents (Williams & Williams 1985b). Cymothoid migratory movements on its host vary in different life phases., The fins of fishes are usually the initial site of attachment for a juvenile male, of which the cymothoid will subsequently migrate to other sites of the host's body (Mladineo 2003; Trilles & Hipeau-Jacquotte 2012) and thereafter attach permanently as an adult female (Fogelman & Grutter 2008; Jones *et al.* 2008).

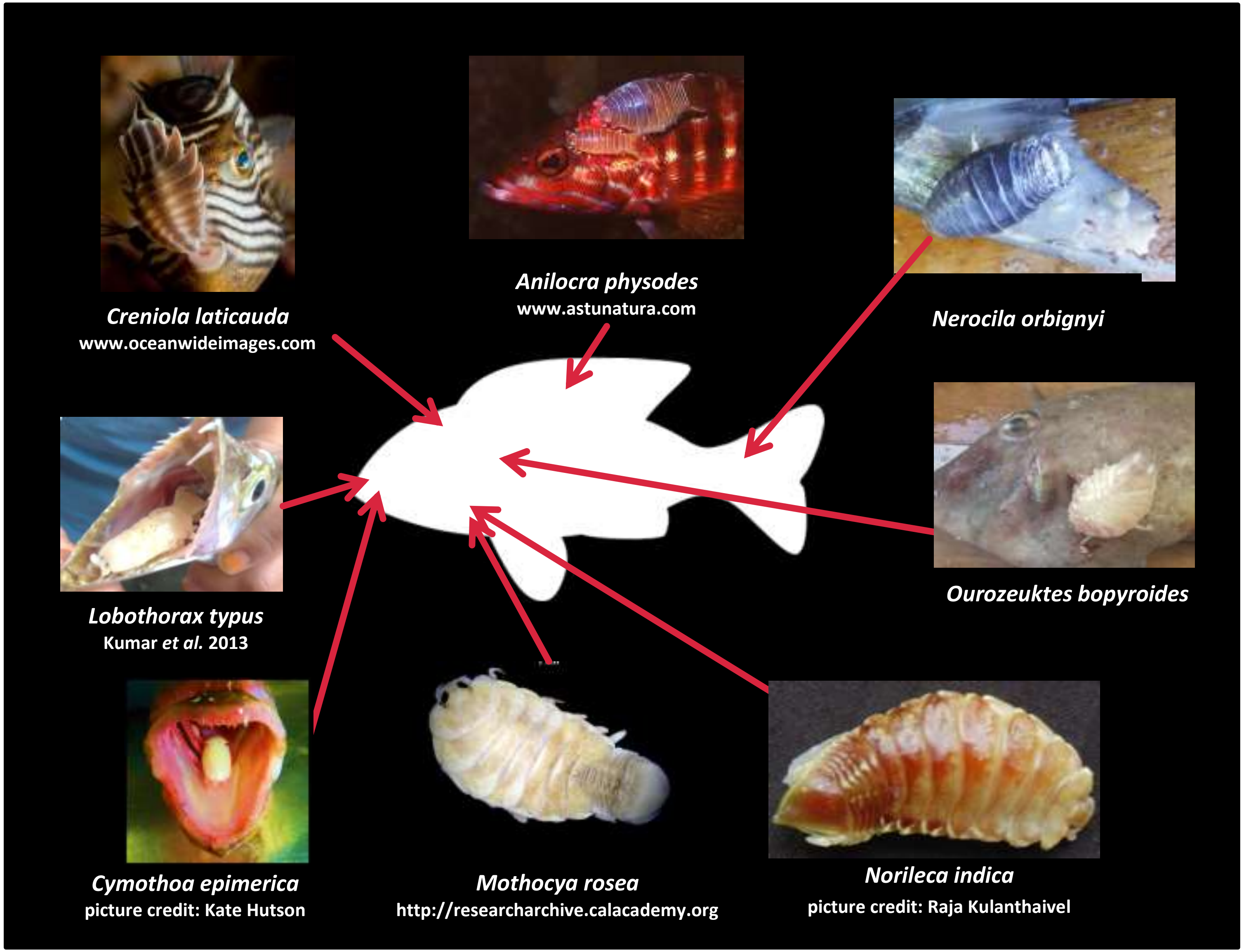

Figure 2.4 Examples of host sites attached by different cymothoid genera

2.5.3 Biological tagging

Parasites have been used as biological tags to study fish population biology, phylogeny, feeding and migration (see full list of literature in Avdeev 1992; Longshaw & Feist 2010; Moore *et al.* 2010). MacKenzie (1983, 2005) and Avdeev (1992) provided a number of criteria for the selection of a parasite as a biological indicator (direct quote):

1) The parasite should have significantly different levels of infection in different parts of the study area.
2) The parasite should have a long life-span in the target host.
3) Method of examination should involve minimum dissection.
4) The parasites should have a direct single-host life cycle.
5) The level of infection should preferably show no significant annual variation.
6) The parasite should be easily detected and identified.

The possibility of using cymothoids as biological tags should be approached with caution. Cuyas *et al*. (2004) considered *Ceratothoa steindachneri* Koelbel, 1878 as a suitable biological indicator for stock identification of *Serranus atricauda* Günther, 1874 from islands of La Palma and Gran Canaria. *C. steindachneri* infected a large number of *S. atricauda* from the western La Palma Island as compared to the eastern island of Gran Canaria (Cuyas *et al*. 2004). *Ceratothoa steindachneri* was used as a biological tag for spatial origin because of the well-defined scar on the host (after death) and the long life-span of the cymothoid on the host. Cuyas *et al*. (2004) claimed that under certain climatic conditions, the transfer and survival of mancae (loosely termed as larva or juvenile) from the west to the east islands are possible, but may not necessarily colonize the east island. Although Cuyas's *et al*. (2004) justification for the use of *C. steindachneri* is practical, this cymothoid has been known to parasitize multiple hosts. *Ceratothoa steindachneri* is able to parasitize different host species from five families (for list of literature see Hadfield 2012). It is possible that there are other infected fishes within the La Palma region capable of transferring the mancae due to the low host specificity of the cymothoid. Thus, in this scenario, the use of cymothoid as a biological tag is deemed unfeasible.

Cymothoids have been used as biological tags to study the migratory routes of horse mackerels in the Pacific Ocean (Avdeev 1992; Aldana *et al.* 1995; George-Nascimento 2000). Avdeev (1992) found that *Ceratothoa trigonocephala* and *C. gaudichaudii* were always associated with the host *Trachurus* and *Scomber* and that both cymothoids showed strong degree of endemism (*C. gaudichaudii* from the southwest Pacific and *C. trigonocephala* from the southeast Pacific. However, my current research (see Chapter 3 part II) revealed that the *C. trigonocephala* and *C. gaudichaudii* referred to in Avdeev (1992) are *C. imbricata*, which is host specific to the genus *Trachurus* and is likely to migrate vast distances with its host.

In another study, *Cymothoa indica* Schioedte & Meinert, 1884 was previously only known to occur in the Indo-Pacific region (Red Sea and the Indian Ocean) but was later reported from the Levantine basin (Trilles & Hipeau-Jacquotte 2012). It was suggested that *C. indica* was able to parasitize Sphyraenidae fishes that occurred from the Indo-Pacific region, which later migrated to the Mediterranean via the Suez Canal (Trilles & Bariche 2006; Bariche & Trilles 2008). Current study now shows that *C. indica* has low host specificity (see Hadfield 2012 and Chapter 5), and would not befit the criteria of a biological tag for host migration. Thus in this example (and that previously mentioned), cymothoids are not suitable biological tags for host migration unless there is clearly establish identity of the species.

A more recent and promising use of cymothoids for fish stock discrimination is the use of otolith chemistry. Otolith chemistry can provide insights into the fish population at finer spatial scales than has previously been achieved using genetic or tagging techniques (Campana & Thorrold 2001). The sagitta (the largest of the three otoliths in a fish) is used to determine the concentrations of various trace elements and stable isotopes present in the calcium carbon matrix. Environmental (e.g. ambient seawater, concentrations, temperature, salinity) and physiological factors are known to influence otolith chemistry. Heagney *et al.* (2013) studied the effect of parasitism of *Ceratothoa imbricata* on the otolith chemistry of host *Trachurus novaezelandiae* Richardson, 1843. The changes of trace elements lithium, calcium, magnesium, strontium and barium were measured for unparasitised and parasitized *T. novaezelandiae* of three subpopulations from Jervis Bay. The otoliths of parasitized fish from the three subpopulation were characterized by significantly lower Li: Ca and Mg:

Ca, and higher Sr: Ca than unparasitised fish (Heagney *et al.* 2013). Despite the similar outcome of trace elements across the three subpopulations, parasite assemblages and loads were not uniform within a population, resulting in differential effects of parasitism and creating differences in otolith chemistry within a single stock or subpopulation unit, as observed in Heagney *et al.* (2013). However, understanding the correlation between otolith chemistry and parasitism is difficult to interpret since differences in otolith chemistry can be the result of differences in parasite loads, or different geographic environments. This can only be resolved with better knowledge of host migration and its environment that would influence otolith chemistry signatures.

The use of cymothoids as biological tags for host population studies may not be suitable because of: 1) the infestation rates of different host population which will not necessarily show contrast; 2) the unpredictable annual variation of infection on host; and 3) the varying degrees of host specificity (Avdeev 1992). However, certain cymothoids are specific to a single host species, have a direct host life cycle and are capable of surviving for long periods on their host. It would be best to re-evaluate the use of cymothoids as biological tags by choosing species that are highly host specific, easily identified (e.g. *Cinusa tetrodontis, Ourozeuktes bopyroides*), and would not cause much harm or damage to its host. The use of adult female cymothoids would be preferable for species identification compared to that of mancae).

2.6 ECOLOGICAL AND ECONOMICAL IMPORTANCE

2.6.1 Cymothoidae in aquaculture and wild fish

In a recent report (Skirtun *et al.* 2013), the gross value of the Australian aquaculture production increased by $100 million to $1.1 billion from 2011 to 2012, accounting for 46% of the gross value of Australian fisheries production. The Australian fish aquaculture production increased by 10% to 84,605 tonnes, accounting for 36% of the total Australian fisheries production. Of this, salmonids (particularly *Salmo salar*) are the largest tonnage of the aquaculture-produced species. Other commercially important species include *Thunnus maccoyii* (Castelnau, 1872) (southern bluefin tuna), *Lates calcarifer* (Bloch, 1790) (barramundi) and *Bidyanus bidyanus* (Mitchell, 1838) (silver perch).

Aquaculture of finfish requires confining teleosts in large numbers to artificial settings such as fish cage(s). The act of introducing fish species to new geographical locations leads to new management problems, resulting in higher incidence of disease outbreaks and parasitic infections (Andrews 2010). Higher stocking density also results in increased contact between host fish and parasites (Kabata 1981; Murray & Peeler 2005). Parasitic crustaceans are just such examples, accountable for many outbreaks in finfish aquaculture and can act as vectors for bacterial and viral pathogens (Johnson *et al.* 2004; Catalano & Hutson 2010).

Cymothoids are economically significant parasites known to infect a variety of commercially important fishes (Brusca 1981; Bunkley-Williams & Williams 1998a; Fogelman & Grutter 2008) with increasing reports of aquaculture incidences (Table 2.2). In fisheries and aquaculture, profit is directly related to the condition of harvested fish (Athanassopoulou *et al.* 2009). Since some of these cymothoids show low fish specificity (see *Cymothoa eremita* (Brünnich, 1783) and *Cymothoa indica* Schioedte & Meinert, 1884 in Chapter 5), they are likely to be easily transferred from wild fish species to farmed fish, thus resulting in disease outbreaks. Furthermore, the cryptic nature and low abundance of these parasites make assessing their prevalence on wild fish populations challenging (Roche *et al.* 2013). The host of most adult cymothoids is usually a single individual host. However, this obligate cymothoid-host association does not apply to mancae which are opportunistic to different intermediate hosts before settling on the definitive host (Fogelman & Grutter 2008). Young and small sized fish are more susceptible to infestation than adult fish with evidence of some mancae injuring and killing larval and juvenile fish of commercially important species (Brusca 1981; Bunkley-Williams & Williams 1998a; Papapanagiotou *et al*. 1999; Horton & Okamura 2001; Mladineo 2002, 2003). Reported farmed fish mortalities due to infection with cymothoids ranged from 7–50% (Bargoni *et al.* 1984; Papapanagiotou *et al.* 1999; Šarušic 1999; Papapanagiotou & Trilles 2001).

Farmed fish are prone to interact with wild fish that get attracted to farm sites for food or refuge (Castro *et al.* 2002; Fernandez-Jover *et al.* 2008; Catalano & Hutson 2010), potentially resulting in enhanced disease transmission (Ogawa 1996; Papapanagiotou *et al.* 1999). Bragoni *et al*. (1984) noted the different periods of sea bass mortalities chiefly caused by *Nerocila orbignyi*. Wild mullets (Mugilidae) were the probable cause for the sea bass infection, as this parasite infection prevalence on mullets reached 90% in August and were seen feeding around the sea cages. In 1999, Papapanagiotou *et al.* (1999) reported *Emetha audouini* mancae causing 10.75% cumulative mortality in 30 g sea bass. There were no previous reports of *E. audouini* infecting sea bass in the wild, as its usual hosts are from the families Sparidae and Centracanthidae (Trilles 1968; Papapanagiotou *et al.* 1999).

There are different approaches to prevent the transmission of cymothoids from the wild into aquaculture. For fish stocking management, measures to take into account are to: 1) reduce fish densities in holding pens, 2) avoid placing pens and fishes of different ages or size grades in close proximity, 3) place pens in deeper waters with sufficient currents, and 4) use fine mesh nets to prevent wild fish from getting into marine pens (Bargoni *et al.* 1984; Papapanagiotou *et al.* 1999; Papapanagiotou & Trilles 2001; Rajkumar *et al*. 2005a, b). Different chemical treatments and baths have also been tested with varying degrees of success (Hatai & Yasumoto 1980, 1982; Brusca 1981; Sievers *et al.* 1995; Athanassopoulou *et al.* 2001; Papapanagiotou & Trilles 2001). Attempts to biologically control cymothoids have also been investigated. The cleaner shrimp *Periclimenes pedersoni* (Chace, 1958) was observed immediately removing and feeding on mancae of *Anilocra* sp. from French grunt *Haemulon flavolineatum* (Desmarest, 1823) (see Williams & William 1979; Bunkley-Williams & Williams 1998b). It may be hypothesized that cleaner shrimp in reefs can reduce cymothoids on fish, as observed by Thorsen & Trilles (2002) where *A. physodes* (written as *A. capensis*) was less abundant in reefs with cleaner fishes *Coris julis* (Linnaeus, 1758) and *Thalassoma pavo* (Linnaeus, 1758), than reefs with fewer cleaner fishes.

Table 2.2. Cymothoid issues in aquaculture worldwide

Cymothoid	Cultured fish	Possible host 'vectors'	Region	Comments	References
Ceratothoa gaudichaudii (*species inquirenda*)	Salmon *Salmo salar* Coho salmon *Oncorhynchus kisutch*	Chilean mackerel *Trachurus murphyi*	Chile	• Salmon with more than eight cymothoids had reduced weights • Cymothoids found infecting *Trachurus murphyi* (fish was found around sea cages in summer) • 29% cymothoid prevalence in high density coho salmon tanks	Alvarado *et al.* 1990; Roa 1992; Inostroza *et al.*1993; Sievers *et al.* 1996; Gonzalez *et al.* 1997
Ceratothoa oestroides	Sea bass *Dicentrarchus labrax* Sea bream *Sparus auratus*	*Boops boops*	Croatia Turkey	• *Boops boops* reported carrying cymothoid and feeding near sea cages • Mortalities of 10 to 20% for both bass and bream fingerlings up to 10 g • 20% reduction of growth rate in adult fish	Šarušic 1999; Horton & Okamura 2001
Ceratothoa banksii	Striped trumpeter *Latris lineata*	-	Tasmania	• Cymothoids were only found in sea cage systems • No cymothoids were found on Atlantic salmon, though the fish was in close proximity to the cages of striped trumpeter	Andrews *et al.* 2013
Ceratothoa cf. *imbricata* (=*Ceratothoa banksii*)	Atlantic salmon *Salmo salar*	12 species were captured, none carried cymothoids	Tasmania	• 76% of salmon pens contained wild fish species. • Fish mostly infected by monogeneans and *Trichodina* spp.	Nowak *et al.* 2004
Emetha audouini	Sea bass *Dicentrarchus labrax*	Fish from families Sparidae and Centracanthida	Greece	• Mancae caused cumulative mortality of 10.75% in 30 g sea bass.	Papapanagiotou *et al.* 1999 Papapanagiotou & Trilles 2001
Nerocila orbignyi	Sea bass *Dicentrarchus labrax*	Fish from family Mugilidae	Diana Pond, Corsica	• Decrease in condition factor and weight of parasitized fish • Mortality of sea bass • 90% cymothoid prevalence on wild mullets	Bragoni *et al.*1983, 1984

2.6.2 Cymothoidae feeding

Cymothoids are generally haematophagous and possess anti-coagulating enzymes (produced by the lateral oesophageal glands) to aid feeding on the blood of the host (Romestand & Trilles 1976a, b; Romestand 1979). Probable mechanisms to trigger blood-sucking in cymothoids are the osmotic pressure and ionic composition of the host blood, or the chemoreceptors that may be located in the mouthparts of the cymothoids (Thuet & Romestand 1981). The hepatopancreas, intestine and posterior intestinal diverticula provide haemolytic enzymes that become active in the erythrocytes of fishes. The absorption of the nutrition from the haemoglobin occurs in the hepatopancrease (Romestand 1979). This eventually reduces the levels of blood lipid, liver lipid, calcium, potassium and magnesium in the host (Trilles & Hipeau-Jacquotte 2012). Some cymothoids clearly abrade and maybe feed on muscle tissue. *Anilocra pomacentri* has been observed rasping the muscle tissue from its host with its mouthparts and forming lesions (Adlard & Lester 1995).

Different cymothoid life stages have different feeding requirements. Adult cymothoids were observed to be exclusively blood-feeders (Legrand 1952; Adlard & Lester 1995; Colorni *et al.* 1997), feeding erratically on their host and allowing healing of the lesions between long feeding intervals (Trilles 1968; Romestand 1979). The ovigerous females have only been known to feed before vitellogenesis and after the release of mancae (Romestand *et al*. 1982; Adlard & Lester 1995). Romestand *et al.* (1982) reported on the darkening of the ovigerous female's intestinal caeca at the start of the initial reproductive cycle and gradually becoming lighter during egg development. Feeding in adult females has not been observed during the development of eggs to mancae in marsupium. This is a likely result of the development of the oostegites that covers the mouthparts of the adult females. Feeding in non-gravid females and adult males has been reported to cause harm to its host (Bunkley-Williams & Williams 1998a). The mancae are immediately able to feed on blood, mucus, and epithelium once expelled from the marsupium (Adlard & Lester 1995; Fogelman *et al.* 2009).

2.6.3 Cymothoidae host effects

Cymothoids inflict varying degrees of harm to their host (Fogelman & Grutter 2008). Infections can be detrimental, either directly or indirectly affecting the physiological status of the host (Adlard & Lester 1995). Direct feeding on the host by cymothoids has been reported to cause: 1) decrease of erythrocyte count, lipid in liver and serum, blood protein and lipid; 2) anemia; 3) induced slow growth and weight loss on the host; 4) decrease in size or parasitic castration of gonads; 5) reduction of oocyte production by the host; 6) hypertrophy and hypervasculixation of the spleen; 7) various modifications in the leukocytes; and 8) mortality; though not all the listed effects are known to occur concurrently (Sadzikowsky & Wallace 1974; Lanzing & O'Connor 1975; Romestand & Trilles 1977a; Lindsay & Moran 1976; Romestand 1979; Bargoni *et al.* 1983; Sandifer & Kerby 1983; Adlard & Lester 1995; Lester & Roubal 1995; Šarušic 1999; Mladineo 2002; Horton & Okamura 2003; Fogelman *et al.* 2009).

Fishes attacked by cymothoids may undergo long-term behavioural changes. Guthrie & Kroger (1974) reported that menhadens parasitized by *Olencira praegustator* were less likely to avoid surface trawls in clear water as compared to unparasitised menhadens. In another report, pomacentrids infected by *Anilocra pomacentri* displayed abnormal behaviour including fast and erratic movements to stasis observed in the field (Adlard & Lester 1994). Pomacentrids with abnormal behaviour were more susceptible to predation than normally behaved pomacentrids. However, an established host-parasite association showed no difference in behaviour between infected and uninfected fish (Adlard & Lester 1994).

Cymothoids can cause localized damage on the site attachment of the host. External cymothoids (e.g. *Anilocra* spp., *Renocila* spp., *Nerocila* spp.) induce damage to scales, loss of pigmentation or colour change, hypertrophy of the epidermis, wounds or lesion, dissolution of basal membrane, bone deformation, disorganization of the connective tissues (Bowman & Mariscal 1968; Morton 1974; Williams & Williams 1981). Gill-attaching cymothoids are associated with loss of gill filaments, pressure atrophy, and erosion of gill cavity (Menzies *et al.* 1955; Guthrie & Kroger 1974; Sadzikowsky & Wallace 1974; Williams & Bunkley-Williams 1982, 1985a; Colorni *et* *al.* 1997). Effects to the gills of fishes may result in blood flow impediment, cardiac output and respiratory reduction and cause secondary infection (Leonardos & Trilles 2003; Trilles 2007). Flesh-dwelling cymothoids cause invagination in the host flesh; pressure atrophy to the liver, stomach and intestine; and possible loss of pectoral fin (Thatcher & Carvalho 1988; Schaefer 1993) though the cymothoid does not seem to cause secondary infection or inflict damage to the host (Thatcher 2000). The buccal-attaching cymothoids are able to inflict damage such as disorganization of the connective tissue, sloughing of epidermis and erosion cartilage, osteolysis and cranial modification (Trilles 1969, 1994; Romestand & Trilles 1977a, b; Brusca & Gilligan 1983). For further examples of buccal-attaching cymothoid effects on host refer to Table 2.3 and Fig. 2.5.

The level of damage on a fish host is likely to vary for different cymothoid life stages. The mancae are usually not host specific and are able to swim and feed on different intermediate hosts for short periods of time and possibly cause more damage or death to their temporary host (Lindsay & Moran 1976; Segal 1987; Adlard & Lester 1994; Thatcher 2000; Fogelman & Grutter 2008). Female adult cymothoids lose the ability to swim and are occasionally known to move from their original positions (in reference to external-attaching cymothoids) on the host for copulation. Adult female cymothoids are obligate parasites and require a permanent attachment with the host as a sustainable nutrient source. This may be a reason why most records of fish parasitized by adult cymothoids have few reports on secondary infection and are able to survive relatively long (Maxwell 1982; Colorni *et al.* 1997; Leonardos & Trilles 2003).

Table 2.3 Host pathology caused by buccal-attaching cymothoids

Signs reported	Comments	Isopod species examples	Host examples	References
Weight loss	• Increased intensity of infestation • Some cymothoids settled in the gills • Cymothoids caused predeposition of the farmed fish to infection of other diseases and resulted in lower market value • Empty stomach, distended guts filled with serous liquid • Enlarged bile vesicle	*Ceratothoa gaudichaudii* *Cymothoa indica* *Ceratothoa oestroides*	*Salmo salar* (Atlantic salmon) *Etroplus suratensis* (Pearl spot) *Sparus aurata* (Sea bream)	Sievers *et al.* 1996 Panikkar & Aiyar 1937 Mladineo 2003
Growth of vomerine teeth	• Evident on the first two thoracic segments of the isopods suggesting the isopod may function as a tongue	*Ceratothoa oestroides*	*Boops boops* (Bogue)	Vu-Tân-Tuê 1963
Disorganization and regression of the connective tissue, epidermis and cartilage of the tongue	• Blood feeding and decrease in blood circulation at the site of attachment • Length and weight of tongue decreased by 50% • Impeded closure of the fish's mouth, obstructing the inflow of water	*Ceratothoa oestroides*	*Boops boops* (Bogue)	Romestand & Trilles 1977a, b
Erosive lesion of the oral mucosa	• Attachment of parasite in the buccal cavity	*Ceratothoa gaudichaudii*	*Salmo salar* (Atlantic salmon)	Roa 1992
Poor oral formation	• Tongue replaced by adult isopod • Hypertrophy of tongue	*Cymothoa exigua*	*Lutjanus guttatus* (Spotted rose snapper)	Brusca & Gilligan 1983
Damaged gill lamellae	• Different developing parasitic stages at the gill region	*Ceratothoa gaudichaudii*	*Salmo salar* (Atlantic salmon)	Sievers *et al.* 1996

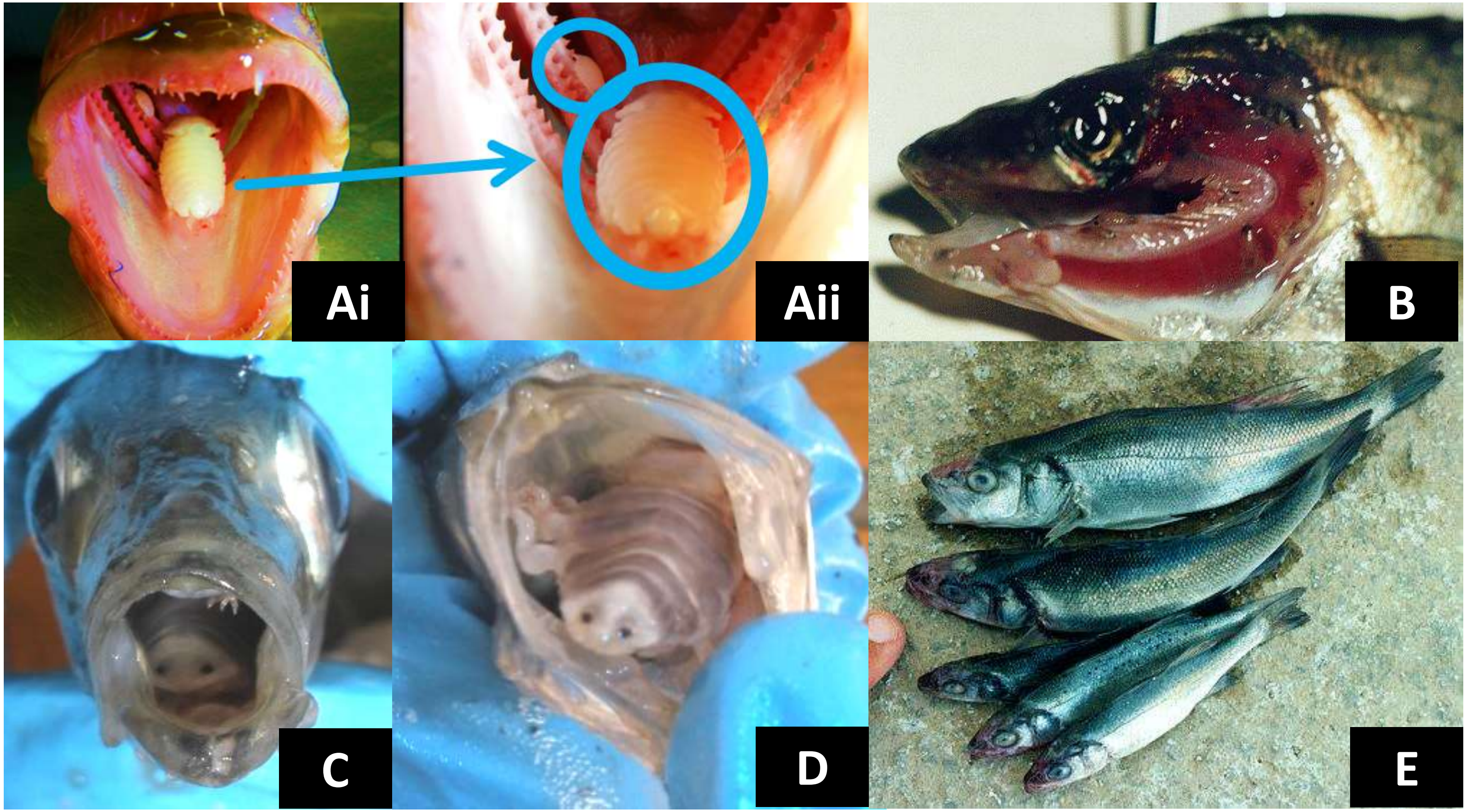

Figure 2.5 Effects of buccal-attaching cymothoids on their hosts. Ai, degeneration of the host tongue *Epinephelus coioides* (pic. credit K. Hutson); Aii, magnified picture of Ai with juvenile male attached to the gill-rakers; B, gill inflammation and focal necrosis of *Dicentrarchus labrax* by mancae (pic. credit P. Varvarigos); C & D, buccal deformation of host *Trachurus declivis* (pic. credit M. Stride); E, necrotic head and eye tissue on *Dicentrarchus labrax* by mancae (pic. credit P. Varvarigos).

2.7 MORPHOLOGICAL CHARACTERS OF TAXONOMIC UTILITY FOR THE CYMOTHOIDAE

To date, species and genus identification is largely restricted to mature female cymothoids and rarely juvenile males or mancae are used (Figs. 2.6 and 2.7). To some degree, host identity is useful for cymothoid species identification (e.g. species of *Mothocya*, *Anilocra*, *Nerocila* and *Ourozeuktes*). Cymothoids have unique morphological adaptations influenced by their site attachment on the host (gills, buccal, external surfaces or burrowed inside the flesh). Below are lists of characters that may be useful for genus and species diagnosis.

Body. – The dorsum ranges from weakly (e.g. *Livoneca* and *Mothocya* spp.) to strongly vaulted dorsum (e.g. *Ceratothoa* and *Smenispa* spp.), and is usually unornamented. Certain species exhibit bulbous ornamentation on the pereonite 1 (e.g. *Cymothoa hermani* Hadfield *et al.* 2011) and is more likely used as a species character rather than a genus. Some genera have asymmetrical or slightly twisted bodies (e.g. some species of *Mothocya* and *Joryma*).

Cephalon. – The shape of the posterior margin of a cephalon is diagnostic for some genera. For example, species of the genera *Nerocila* and *Plotor* have a trisinuate cephalon posterior margin (Bruce 1987c). The anterior margin shape of the cephalon may be useful as species diagnosis for certain genera (see species examples for *Cymothoa* in Chapter 5). Prior to this research for the genus *Ceratothoa* (see Chapter 3), specimens that had a distinct triangular cephalon were immediately identified as *C. trigonocephala*, but it became apparent that other characteristics did not associate with that species. The position of cephalon rostrum projection (e.g. without rostrum; ventrally directed and not folded posteriorly; or folded ventrally and posteriorly) and the presence of rostrum projection between the antennae can differ between genera.

Eyes. – The eyes are lateral when present (Poore 2002). The compound eyes are usually large and well pigmented in juveniles and are later reduced in size as the individual develops into adult male or female. The reduction of eye size is followed by loss of pigmentation and opaqueness of the cuticular covering (Brusca 1981).

Some species of *Cymothoa* have eyes that are not visible or vaguely distinct (see Chapter 5). The eye size is potentially a useful character for genus diagnosis.

Pleon. – *Pleonites* 1–5 are free for most cymothoids (Poore 2002). The flesh-burrowing genus *Ourozeuktes* has all pleonites fused with the pleotelson whereas in *Asotana splendida* (Leigh-Sharpe, 1937), the pleon is composed of four segments. Differences on a generic level can be observed in relative width of the pleon compared with pereonite 7, the degree of pereonite 7 extensions on the pereonites, and the progression of pleon width (decreasing or increasing from pereonites 1 to 5).

Pleotelson. – The pleotelson lacks setae. The shape of pleotelson margin are relatively uniform for some genera (e.g. *Agarna*, *Asotana*, *Joryma*) but vary for others and are used in species identification (e.g. *Ceratothoa*, *Cymothoa*, *Paracymothoa*).

Antennae. – Important generic characters are the relative lengths of both antennulaand antenna and the degree of separation between antennula bases. Most adult cymothoid species from different genera have either setae present or absent at the terminal end of the antennae.

Mouthparts. – These are largely uniform for most marine genera, but maxilla and mandible may offer supportive characters.

Coxae. –Coxae are more useful at the generic rather than species level. *Plotor* is diagnosed as having coxae 5–7 ventrally directed whereas *Amblycephalon* and *Creniola* have coxae 5–7 ventrally directed (Bruce 1987c). *Nerocila* has coxae 5–7 as long as or longer than the respective pereonite whereas *Anilocra*, *Renocila* and *Pleopodias* have coxae 5–7 shorter than the respective pereonite (Bruce 1987c). Other useful characters are coxae visibility in dorsal view, the projection of the posterolateral margins of pereonites 5–7 and the posterior margin shape of coxae 5–7 (Bruce 1987a, c). In this thesis, width size of coxae 2–7 posteroventral margins versus that of anteroventral margins is used for species identification of *Ceratothoa* (Chapter 3) and *Glossobius* (Chapter 4). Coxae 5–7 visibility and posteroventral margin shapes are used for species identification in *Cymothoa* (Chapter 5).

Pereopods. – All pereopods are prehensile (Poore 2002). The inferior distal margin of some cymothoid mancae species bear robust setae that continue to be present in some female species (e.g. species of *Nerocila*, see Bruce 1987c). The dactyli are generally strongly curved and are able to firmly grasp onto the fish's flesh. Dactyli of pereopods 4 to 7 are also generally more slender and longer than pereopods 1 to 3. The basis and carina of pereopods 6 and 7 are used for species identification in some genera (see examples for *Ceratothoa* in Chapter 3).

Brood pouch – Bruce (1987c) described the two types of Cymothoidae brood pouch. The 'Anilocrinae' have brood pouches formed by paired oostegite plates arising from coxae 1–4 and 6, with the largest arising from coxae 6 and forming a marsupium to contain developing embryos (Bruce 1987c). Other marine genera (particularly the gill- and buccal-attaching genera) have paired oostegites arising from coxae 1–4 and 6, 1–5 or 2–5, with all oostegites forming the marsupium.

Pleopods. – The pleopods are usually without marginal setae. The pleopods generally decrease in size from pleopods 1 to 5, with pleopod 1 overlapping the rest of the pleopods in most genera. The appendix masculina is present on pleopod 2 for mancae and juvenile males, without setae, denticles or grooves and may suggest that the structure may no longer function in copulation (Brusca 1981). Though most cymothoid species have unilaminate pleopods, others (e.g. species *Braga*) have multilaminate pleopods. Fleshy folds are apparent in some species, and can be used as a generic character (e.g. species of *Cymothoa*, see Chapter 5). Most gill- and buccal-attaching genera (e.g. *Mothocya*, *Cymothoa* and *Ceratothoa*) have laminar pleopods, a contrast to species of the genera *Anilocra*, *Nerocila* and *Creniola*, which have lobes on the posterior surface of the pleopod endopods 3–5 (Bruce 1987c).

Uropods. – Cymothoid_uropods are biramous, freely articulating, and situated at the ventrolateral angles of pleotelson (Brandt & Poore 2003). Adult cymothoids generally lack setae on the uropods whereas the mancae of some species have setae. The relative length of the uropod to the posterior margin of the pleotelson has been used as genus diagnosis (see Hadfield 2012). In *Mothocya* (as well as other cymothoid genera), the uropod shape, relative length of rami and shape of peduncle have also been used for species.

In conclusion, the general morphological characters used in descriptions of cymothoid isopods include: length and width of the cephalon, pereonites and pleotelson; shape of the cephalon and pleotelson; size and position of the eyes; number of articles in the antennula and antennae; shape and size of the coxal plates (and the dorsal visibility); configuration of the posterolateral angles of the pereonites, relative length of the uropod rami; and presence or absence of carinae on the pereopod basis (Brusca 1981; Hadfield *et al.* 2010). However, most of the listed characteristics should be approached with caution as these characteristics may show some degree of polymorphism.

2.7.1 Buccal-attaching cymothoids

Cymothoid genera found inhabiting the host's mouth are generally elongated, bilaterally symmetrical and dorsally vaulted, almost filling the entire buccal cavity, depending on the host species (Hadfield 2012). Buccal-attaching cymothoids have less pigmentation compared with the darker coloration of external-attaching cymothoids. The darker colouration of the external cymothoids likely aids in camouflage and blends well with its host or the water column (Williams & Williams 1985c). Some buccal-attaching species show heavy pigmentation on the anterior portion of the body, gradually becoming lighter at the posterior end of the body (Bruce & Bowman 1989; Colorni *et al.* 1997).

Most buccal-attaching cymothoids are positioned with the cephalon facing towards the host mouth entrance (Bunkley-Williams & Williams 1998a), either attached to the floor of the mouth or adhering to the host's tongue. Other forms of adult female cymothoids orientations occur within the host's buccal cavity. *Smenispa convexa* (see Menzies *et al.* 1955) and *Asotana magnifica* (see Thatcher 1988) face towards the throat; *Elthusa splendida* switch positions from facing the anterior to the posterior of the host's mouth (Sadowsky & Soares Moreira 1981); *Ceratothoa parallela* (see Trilles 1968, 1972b; Trilles & Hipeau-Jacquotte 2012), *Cinusa tetrodontis* (Hadfield 2012) and *Olencira praegustator* (see Turner & Roe 1967; Williams & Bunkley-Williams 1999) attach to the roof of the oral cavity; and *Catoessa ambassae* Bruce, 1990 attaches to the lateral internal face of the host's buccal cavity, with the cymothoid dorsal surface medial (Bruce 1990).

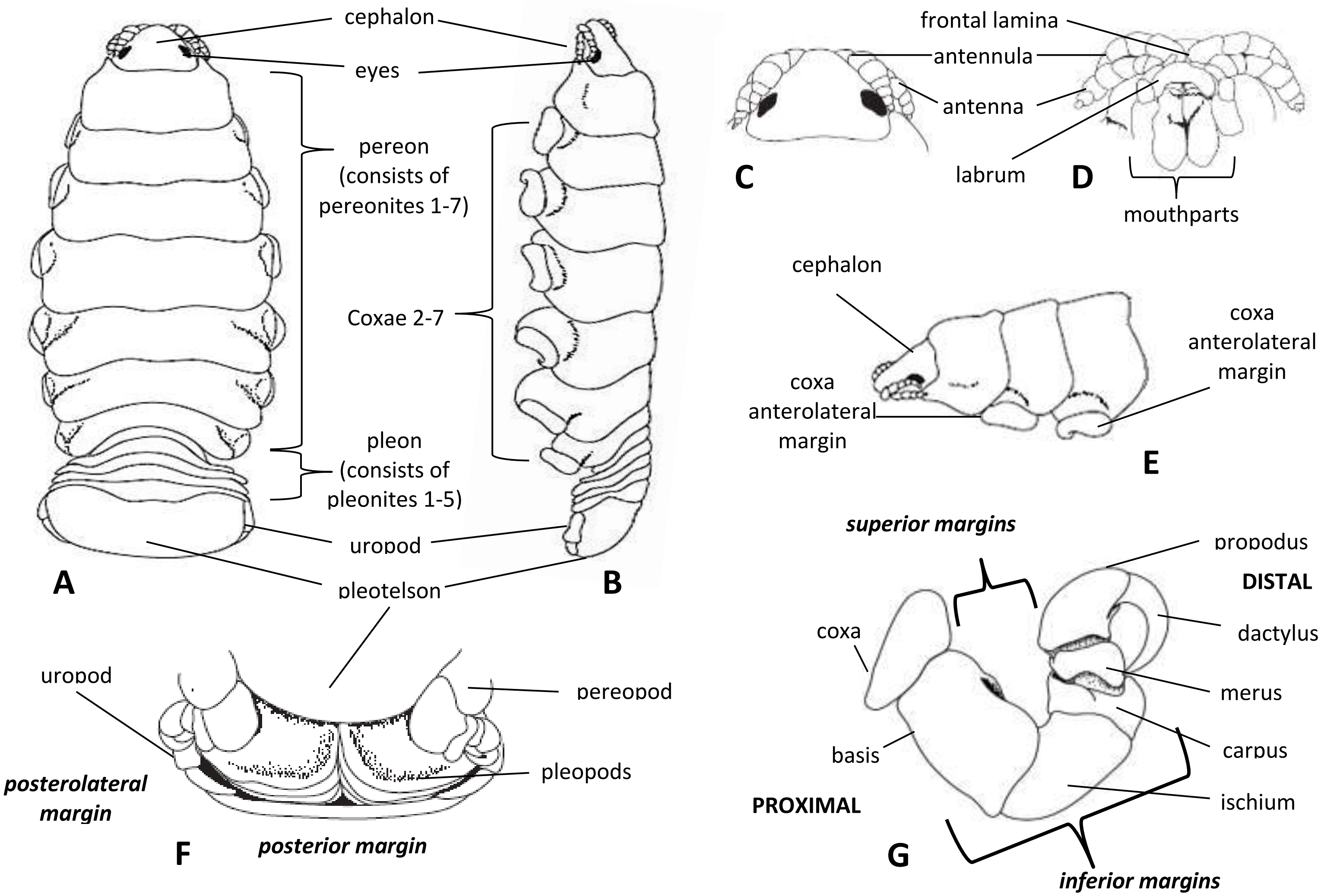

Figure 2.6 Terms and positions used in descriptions: A, body dorsal view; B, body lateral view; C, cephalon dorsal view; D, cephalon ventral view; E, lateral view of cephalon with pereonites 1–3; F, ventral view of pleotelson; G, pereopod segments.

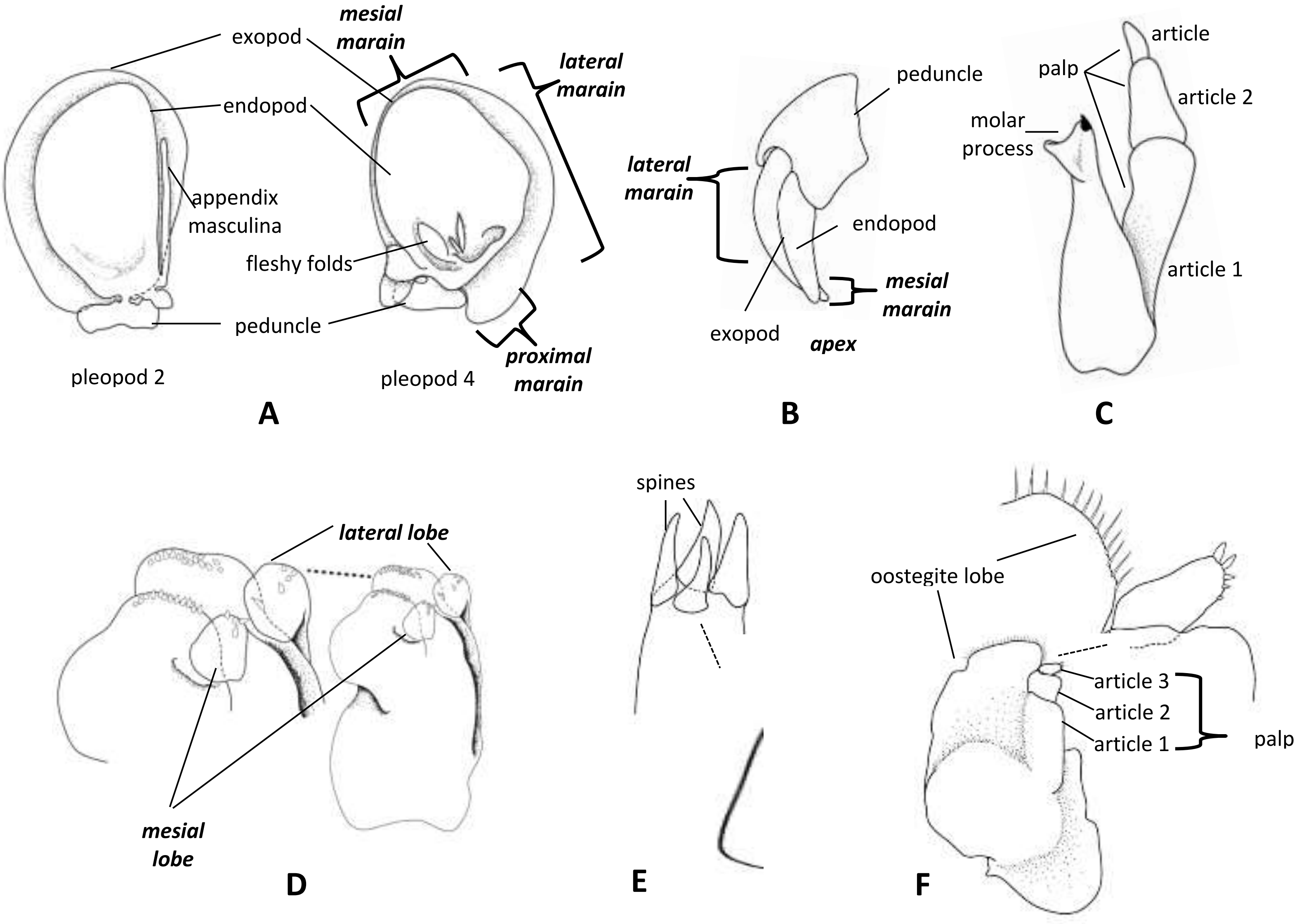

Figure 2.7 Terms and positions used in descriptions: A, pleopods; B, uropod; C, mandible; D, maxilla; E, maxillule; F, maxilliped.

2.8 PHYLOGENY OF THE CYMOTHOIDAE, WITH EMPHASIS ON THE BUCCAL-ATTACHING CYMOTHOIDS

Phylogenetic systematics is a method of reconstructing evolutionary relationships among taxa (Hennig 1966). Traits exhibited by any taxon with the combination of plesiomorphic and apomorphic characters are indicators of a phylogenetic relationship if the homologous traits are shared by two or more taxa. Of the shared homologous traits, synapomorphies (=shared derived homologous characters) represent characters from the most recent common ancestor as compared with symplesiomorphies (= shared ancestral homologous characters) (Kitching *et al*. 1998).

Phylogenetic analysis mainly comprises the selection of homologous characters and taxa, character argumentation, and determination of cladograms that define the relationship among the studied taxa (Kitching *et al*. 1998). Character argumentation separates characters that are plesiomorphic from apomorphic via transformation series (Wiley *et al*. 1991) using outgroup comparison and ontogenetic criterion (Kitching *et al*. 1998). The outgroup comparison defines a plesiomorphic character as trait found in at least one member of the ingroup (= the group investigated) to also occur in the outgroup (= taxa sister to the investigated group). Most computer algorithms that estimate morphologically-based phylogenies commonly use parsimony methods (Futuyma 1998), which estimate the least number of character transformations. The resultant tree topology is treated as a hypothesis to establish a system of classification for the taxa of interest. Hennig's (1966) method can be applied constructively to evaluate the evolutionary relationships of the Cymothoidae.

Hadfield (2012) presented the first morphological cladistics of the Cymothoidae based on 23 genera and 40 multistate characters. Hadfield (2012) conducted two analyses from her morphological dataset: the 50% majority rule consensus tree (first analysis, Fig. 7.4) and the strict consensus tree without the pleopod characters (second analysis, Fig 7.5). Hadfield's (2012) analysis (Fig. 7.4) of the 50% majority rule tree showed that the "*Anilocra*-type" group (clade 13) formed a clade with the gill-attaching *Livoneca* Leach, 1818 and the buccal-attaching *Smenispa* Özdikem, 2009 and *Paracymothoa* Lemos de Castro, 1955; the characters supporting this clade include posterior pocket present, antennula expanded, maxilla medial lobe partly fused, pereopods 5–7 basis with a weak carina and uropod rami longer than the length of the pleotelson. Sister to the "*Anilocra*-type" clade is the buccal-attaching clade (clade 9) and the remaining genera were all basally unresolved. The buccal-attaching "*Ceratothoa*-type clade" (clade 9, Fig. 7.4) formed a cohesive clade, where *Cymothoa* Fabricius, 1787 is sister to the *Cinusa* Schiöedte & Meinert, 1884+*Lobothorax* Bleeker, 1857 and *Ceratothoa* Dana, 1852+*Glossobius* Schiöedte & Meinert, 1883 clade; and is upheld by pereonite 1 anterolateral margins encompassing the cephalon (developed into lobes in *Glossobius*), pereopods 5–7 basis with large blade-like carina (except *Lobothorax* which has no carina), and maxilla medial lobe partly fused (Hadfield 2012).

In a second analysis to investigate the resulting tree topology with the removal of pleopod data, the topology showed a loss in the unity of both major clades, although there was a basal division. The *Anilocra*-type is still upheld, with the exception of *Norileca*. The buccal-attaching *Cinusa*+*Lobothorax* are now sister to the remaining *Ceratothoa*-type clade. Interestingly, *Catoessa* Schiöedte & Meinert, 1884 which is a buccal-attaching taxon, is sister to the gill-attaching *Mothocya* Hope, 1851 in both analyses, proving that the morphology of *Catoessa* does not belong to the *Ceratothoa*-type clade. In both of Hadfield's (2012) analyses, the buccal-attaching clade showed the apomorphic character of the pereonite 1 anterolateral margins encompassing the cephalon and pereopods 5–7 basis with large blade-like carina. *Anphira* Thatcher, 1993, *Cterissa* Schioedte & Meinert, 1884 and *Cuna* Williams & Williams, 1985 which were ancestral genera in the first analysis, are now derived in the second analysis.

Hadfield's (2012) morphological data matrix showed that most clades were not well supported due to the change of characters, particularly for some of the buccal-attaching genera. As her data matrix was fairly small with few characters and genera from the family, I aim to improve this data matrix with the combination of molecular data matrix for the family, which is discussed in detail in Chapter 7.

CHAPTER 3

Part I

Redescription of *Ceratothoa carinata* (Bianconi, 1869) and *Ceratothoa oxyrrhynchaena* Koelbel, 1878 (Crustacea: Isopoda: Cymothoidae), buccal-attaching fish parasites new to Australia

KEYWORDS

Crustacea, Isopoda, Cymothoidae, taxonomy, *Ceratothoa*, *Selar crumenophthalmus*, *Dentex spariformis*, fish parasites, Australia

This paper has been published in Zootaxa 3683 (4): 395–410

CHAPTER 3

Part II

Review of the fish-parasitic genus *Ceratothoa* Dana, 1852 (Crustacea: Isopoda: Cymothoidae) from Australia, with description of two new species

KEYWORDS

Isopoda, Cymothoidae, *Ceratothoa*, *Mothocya*, fish parasites, Australia

This paper has been published in Zootaxa 3963 (3): 251–294

CHAPTER 4

Review of the buccal-attaching fish parasite genus *Glossobius* Schioedte & Meinert, 1883 (Crustacea: Isopoda: Cymothoidae)

KEYWORDS

Cymothoidae, *Glossobius*, southern Africa, Australia, fish parasite, Beloniformes

This paper has been published in Zootaxa 3973 (2): 337–350

CHAPTER 5

Review of the fish-parasitic genus *Cymothoa* Fabricius, 1793 (Crustacea: Isopoda: Cymothoidae) from Australia

KEYWORDS

Cymothoidae, taxonomy, *Cymothoa*, fish buccal-attaching parasites, Australia

This paper has been submitted to Zootaxa

CHAPTER 6

Smenispa irregularis (Bleeker, 1857) (Crustacea: Isopoda: Cymothoidae), a buccal-attaching fish parasite from Australia

KEYWORDS

Crustacea, Isopoda, Cymothoidae, *Smenispa irregularis*, taxonomy, Western Australia, *Acanthopagrus latus*

This paper has been published in Records of the Australian Museum 66 (4): 233–240

CHAPTER 7

Morphological and molecular phylogenetics of the Cymothoidae (Crustacea: Isopoda)

7.1 INTRODUCTION

The Cymothoidae is recognised as a well-unified family, nested within the polyphyletic Cymothooidea Leach, 1814 (Fig. 7.1) a taxon of carnivorous, commensal, micropredatory and parasitic families. The widely accepted phylogeny of the Isopoda by Brandt & Poore (2003) showed that the family Aegidae White, 1850 was sister to the Cymothoidae and former Epicaridea (now Bopyroidea Rafinesque, 1815 and Cryptoniscoidea Kosmann, 1880). The aegids are well-known associates of fishes and rarely invertebrates, but unlike cymothoids, they are micropredators and attach temporarily to the external surfaces (Bruce 2009). The Cymothoidae and Bopyridae formed a well-supported clade based on strong molecular (Dreyer & Wägele 2001, 2002) and morphological (Brandt & Poore 2003) evidence. Dreyer & Wägele (2001) hypothesised that parasitism of other crustaceans by Bopyridae evolved from their parasitism of fishes (cymothoid-like ancestors).

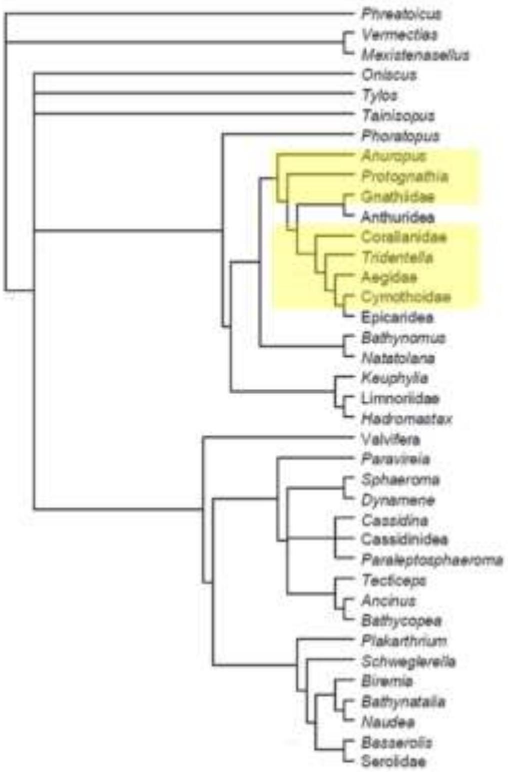

Figure 7.1 Strict consensus of 12 trees, reweighted morphological data from Brandt & Poore (2003). Yellow boxes highlight taxon of the superfamily Cymothooidea.

Brusca (1981) noted that the Cymothoidae is taxonomically poorly understood in comparison to other isopod families due to the complex synonymies and inconsistent diagnostic and descriptive characteristic application of both genera and species. There has been considerable taxonomic activity in the last 40 years (see history of discovery in Smit *et al.* 2014) and more recent attention for the buccal-attaching genera (see Hadfield *et al.* 2010, 2011, 2013, 2014a, b; Martin *et al.* 2013, 2014a, b, 2015a, b). To date, most of the cymothoid literature is alpha-taxonomic, with few studies dealing with phylogeny or biogeography.

Early classification within the Cymothoidae dates from the works of Schioedte & Meinert (1879, 1881, 1883, 1884) who at the time proposed the classification of Cymothoarum (=superfamily Cymothoidea); which included Aegidae, Anilocridae, Saophridae, and Cymothoidae; and the three subfamilies (formerly tribes) Ceratothoinae, Cymothoinae and Livonecinae. The Aegidae and Cymothoidae are now recognized as two separate families, whereas the Anilocridae, Saophridae and all three subfamilies are part of the Cymothoidae (Bruce 1987c).

It was perceived that the relationships within the Cymothoidae were influenced by their site attachment on the host (gills, buccal, external surfaces or burrowed inside the flesh). Schioedte & Meinert (1879, 1881, 1883, 1884) recognised the differences in body shape based on site attachment and proposed the classification of the subfamily Anilocridae (external-attaching), Saophridae and Cymothoidae (the latter two were regarded as the buccal and gill-attaching subfamily respectively). Brusca (1981) maintained this interpretation in his phylogenetic analysis for the family, identifying three evolutionary lineages: the external attaching clade (e.g. *Anilocra*, *Nerocila* and *Renocila*), the buccal+gill attaching clade (e.g. *Ceratothoa*, *Cymothoa* and *Elthusa* (as *Livoneca*)), and the less distinct polyphyletic clade (which constitutes nine South American freshwater cymothoid genera and all flesh-burrowing taxa). Brusca (1981) drew two conclusions from his analysis: (1) that cymothoids evolved from an external-attaching taxon (termed *Nerocila*-like ancestral cymothoid) and further advanced into the more derived gill/buccal-attaching clade and (2) that host specificity was expected to be higher in the more derived genera.

Bruce (1987c) retained Schioedte & Meinert's (1881) subfamily Anilocrinae (genera included *Nerocila*, *Plotor*, *Amblycephalon Creniola*, *Renocila*, *Anilocra*, *Pleopodias*) at the time and provided a diagnosis based on brood pouch, pleon and pleopod morphology. Bruce (1990) later withdrew the above view while revising the gill-attaching cymothoids from Australia. It became apparent that some species and genera of the external-attaching "Anilocrinae" also occurred on the gills (e.g. *Livoneca* and *Norileca*) and buccal cavity (e.g. *Smenispa*). Bruce (1990) concluded that it was best to avoid the use of Anilocrinae and subfamily names Cymothoinae, Ceratothoinae and Livonecinae due to inadequate data on morphological and ecological adaptations. Bruce (1990) suggested that more morphological data need to be made available for more genera to establish a comprehensive Cymothoidae classification.

Brusca's (1981) phylogeny has here been re-evaluated on both morphological and molecular approaches. The molecular analyses of Jones *et al.* (2008) using partial 16S rRNA gene and Ketmaier *et al.* (2008) using two mitochondrial genes 16S rRNA and cytochrome oxidase I (COI) rejected Brusca's (1981) hypothesis of the three lineages based on site-attachment. Jones *et al.* (2008) tree topology (Fig. 2) showed that *Nerocila* Leach, 1818 did not form a monophyletic clade with *Anilocra* and *Renocila*, but was sister to the buccal-attaching *Cymothoa indica* Schioedte & Meinert, 1884 and *Olencira praegustator* (Latrobe, 1802). Ketmaier's *et al.* (2008) analysis (Fig. 1) showed *Nerocila* as sister to *Ceratothoa*. Also shown in Ketmaier's *et al.* (2008) is the buccal-attaching *Olencira* and gill-attaching *Elthusa* are not monophyletic with *Ceratothoa*. Both these molecular analyses did not resolve the relationships between the cymothoid genera, though it was emphasized that their results were based on small datasets of 11 and 6 species (excluding outgroup) respectively, and were not regarded to be conclusive.

Hadfield's (2012) morphological analysis of the Cymothoidae was based on 23 genera and 40 multistate characters. Hadfield's (2012) 50% majority rule tree (Fig. 7.4) showed that the external attaching *Anilocra* group (clade 13) formed a morphologically well-supported clade that also included the gill-attaching *Livoneca* Leach, 1818, the buccal-attaching *Smenispa* and *Paracymothoa* Lemos de Castro, 1955, the characters supporting this clade included the posterior pocket present, antennula expanded (written as antennula), maxilla medial lobe partly fused, pereopods 5–7 basis with a weak carina and uropod rami longer than the length of the pleotelson. Sister to the *Anilocra* clade was the buccal-attaching *Ceratothoa* clade which includes *Cymothoa*, sister to the *Cinusa* +*Lobothorax* and *Ceratothoa*+*Glossobius* (Hadfield 2012). This buccal-attaching clade was upheld by pereonite 1 anterolateral margins encompassing the cephalon (developed into lobes in *Glossobius*), pereopods 5–7 basis with large blade-like carina (except *Lobothorax* which has no carina), and maxilla medial lobe partly fused. A second analysis by Hadfield (2012) was to test the resulting tree topology with the removal of pleopod characters. The second analysis resulted in the *Anilocra* clade still upheld whereas *Cinusa*+*Lobothorax* are now sister to the remaining *Ceratothoa* clade.

This chapter aims to reappraise the Cymothoidae relationships by incorporating both morphological and molecular analyses to investigate the monophyly of the buccal-attaching, external-attaching and gill-attaching clade. In this chapter, the morphological dataset has been expanded with seven additional genera and 31 new characters since Hadfield (2012). The molecular phylogeny based on 16S rRNA and CO1 (using maximum parsimony and maximum likelihood analyses) explored different DNA extracting methods from museum specimens of unknown preservation history as well as fresh material and incorporate our new sequences with that from Jones's *et al.* (2008) and Ketmaier's *et al.* (2008) study.

7.2 MATERIALS AND METHODS

7.2.1 Morphological data

7.2.1.1 Ingroup taxa

Thirty out of 43 genera from the family Cymothoidae are represented in the morphological analysis (Table 7.1). Data for the analysis were obtained from specimens held at the Museum of Tropical Queensland (Townsville), the Australian Museum (Sydney), the South Australian Museum (Adelaide) and Tasmanian Museum and Art Gallery (Hobart) and publications with well described genus and species diagnosis (e.g. works from Niel Bruce, Lucy Bunkley-Williams, Ernest Williams, Vernon Thatcher, Kerry Hadfield and results from this research). Genera that are excluded from this study (e.g. *Lathraena* Schioedte & Meinert, 1881; *Rhiothra* Schioedte & Meinert, 1884, etc.) are those which the type have not been examined, or without modern taxonomic review due to scanty generic characters and illustrations.

7.2.1.2 Outgroup taxon

The genus *Rocinela* Leach, 1818 from the family Aegidae is selected as the outgroup for the morphological cladistics of this study (Table 7.1). Wägele (1989) regarded the *Rocinela*-group as most similar to Cymothoidae and Bopyridae on the basis of the maxillipedal palp articles having only two articles. Brandt and Poore (2003) identified Aegidae as sister to the families Cymothoidae and 'Epicaridea'. These families possess a maxillipedal palp with terminal articles set obliquely, and with hooked robust setae, as well as a strongly curved pereopod 1 dactylus. *Rocinela* was selected from this family as it allowed for coding the maxilliped character which genera such as *Aega* Leach, 1815 or *Aegapheles* Bruce, 2009 did not (due to the different morphology and loss of maxillipedal palp articles in Cymothoidae).

Table 7.1 List of cymothoid genera and an aegid of the genus *Rocinela* Leach, 1818 (outgroup) selected for the morphological cladistic analysis. This table includes references for genus diagnosis which is used to create the character list and the different states. Parasitic habits are identified as follows: E (external-attaching cymothoid), B (buccal-attaching cymothoid), G (gill-attaching cymothoid), F (flesh burrowing cymothoid), M (Micropredator).

No	Genus	Authority	Site-attachment	References
1	*Agarna*	Schioedte & Meinert, 1884	G	Tiwari 1952; Pillai 1964
2	*Anilocra*	Leach, 1818	E	Bruce 1987a
3	*Anphira*	Thatcher, 1993	G	Thatcher 1993b; de Araujo & Thatcher 2003
4	*Artystone*	Schioedte, 1866	F	Thatcher & Carvalho 1988; Thatcher & Schindler 1999
5	*Asotana*	Schioedte & Meinert, 1881	B	Thatcher 1988
6	*Braga*	Schioedte & Meinert, 1881	B, G	Thatcher 1996; Thatcher *et al.* 2009
7	*Catoessa*	Schioedte & Meinert, 1884	B, G	Bruce 1990, Bowman & Tareen 1983
8	*Ceratothoa*	Dana, 1852	B	Hadfield *et al.* 2014a,b; Martin *et al.* 2015a
9	*Cinusa*	Schioedte & Meinert, 1884	B	Hadfield *et al.* 2010
10	*Creniola*	Bruce, 1987	E	Bruce 1987c
11	*Cterissa*	Schioedte & Meinert, 1884	G	Bunkley-Williams & Williams, 1986
12	*Cymothoa*	Fabricius, 1787	B	Hadfield *et al.* 2013
13	*Elthusa*	Schioedte & Meinert, 1884	G	Bruce 1990
14	*Glossobius*	Schioedte & Meinert, 1883	B	Bruce & Bowman 1989; Martin *et al.* 2015b
15	*Ichthyoxenus*	Herklots, 1870	B, G or F	Bruce 1990
16	*Joryma*	Bowman & Tareen, 1983	G	Bowman & Tareen 1983
17	*Kuna*	Williams & Williams, 1985	G	Williams & Williams, 1985a
18	*Livoneca*	Leach, 1818	G	Bruce 1990
19	*Lobothorax*	Bleeker, 1857	B	Yu & Bruce 2006
20	*Mothocya*	Hope, 1851	G	Bruce 1986
21	*Nerocila*	Leach, 1818	E	Bruce 1987c
22	*Norileca*	Bruce, 1990	G	Bruce 1990
23	*Ourozeuktes*	Milne-Edwards, 1840	F	Hale 1926
24	*Paracymothoa*	Lemos de Castro, 1955	B	Bowman 1986
25	*Pleopodias*	Richardson, 1910	E	Bruce 1987a
26	*Renocila*	Miers, 1880	E	Bruce 1987b
27	*Riggia*	Szidat, 1948	F	Bastos & Thatcher 1997
28	*Ryukyua*	Williams & Bunkley-Williams, 1994	G	Williams & Bunkley-Williams, 1994
29	*Smenispa*	Özdikem, 2009	B	Bruce 1990; Martin *et al.* 2014a
30	*Telotha*	Schioedte & Meinert, 1884	B	Taberner 1993
31	*Rocinela*	Leach, 1818	M	Bruce 2009

7.2.1.3 Character list and character states

Morphological characters were selected to assess potential monophyletic clades within the genera. Character states considered for all cymothoid genera for this study were sourced from literature listed in Table 7.1 or collections. A total of 71 morphological characters based on adult females were employed in the analysis. Male characters were excluded because females better express generic characters than do the males. Although the early perception of relationships within the family was strongly driven by site of attachment (see Brusca 1981) site attachment is not included as a character state to test if the phylogeny did result in clades based on that character.

1. *Pereon (lateral margins)*: 1. ovate; 2. weakly ovate; 3. subparallel.

2. *Body pereon widest at*: 1. pereonite 5; 2. pereonite 6; 3. pereonite 4; 4. pereonite 3.

3. *Cephalon frontal margin (ventral view)*: 1. straight and smooth; 2. ventrally directed, not folded posteriorly; 3. folded ventrally and posteriorly.

4. *Cephalon frontal margin (to antennula bases)*: 1. not projecting between antennula bases; 2. partially projecting between antennula bases; 3. projecting between antennula bases.

5. *Frontal margin shape (dorsal anterior margin)*: 1. triangular; 2. truncate; 3. rounded.

6. *Cephalon rostrum*: 1. with rostrum; 2. without rostrum.

7. *Cephalon (dorsal view to pereonite 1)*: 1. not immersed; 2. partially immersed; 3. 3/4 immersed.

8. *Antennula (bases)*: 1. narrowly separated; 2. widely separated; 3. bases partially in contact; 4. bases in contact.

9. *Antennula (to antenna)*: 1. shorter than antenna; 2. subequal to antenna; 3. longer than antenna.

10. *Antennula terminal article extension (to posterior margin of cephalon)*: 1. long, extending beyond cephalon posterior margin; 2. short, nearly reaching or reaching cephalon posterior margin.

11. *Antenna terminal article extension (to anterior margin of pereonite 1)*: 1. long, extending beyond pereonite 1 anterior margin; 2. short, nearly reaching or reaching pereonite 1 anterior margin.

12. *Eyes (size)*: 1. large (more than 40% width of cephalon); 2. small (less than 40% width of cephalon); 3. partially visible; 4. absent.

13. *Mandible palp articles 1–3 fusion*: 1. distinct, articulated; 2. fused or suture present.

14. *Mandible palp (article 2 setae)*: 1. present; 2. absent.

15. *Mandible palp (article 3 setae)*: 1. present; 2. absent.

16. *Mandible palp (article 2 and 3):* 1. article 2 longer than article 3; 2. articles 2 and 3 subequal; 3. article 2 shorter than article 3.

17. *Mandible (molar process and incisor)*: 1. molar and incisor distinct; 2. molar and incisor partially fused; 3. molar and incisor fused.

18. *Maxilla medial lobe*: 1. distinct; 2. partially fused; 3. fused.

19. *Maxilla (setae numbers)*: 1. 1 on lateral and 2 on medial lobe; 2. more than 2 setae each on medial and lateral lobe; 3. 1 setae each on medial and lateral lobe; 4. 2 setae each on medial and lateral lobe; 5. without setae.

20. *Maxillule*: 1. 5 or more terminal spines; 2. 4 terminal spines; 3. 3 terminal spines.

21. *Maxilliped*: 1. with 1 oostegite lobe; 2. with 2 oostegite lobes; 3. without oostegite lobe.

22. *Maxilliped article 3 with (robust setae)*: 1. 2 recurved robust setae; 2. 3 recurved robust setae; 3. more than 3 recurved robust setae; 4. no robust setae.

23. *Pereonite 1 anterolateral margin*: 1. not produced; 2. produced.

24. *Pereonite 1 anterolateral margin (shape)*: 1. flat; 2. acute; 3. rounded and appearing to encompass cephalon; 4. lobed.

25. *Pereonites 1–4 posterolateral margins (shape)*: 1. smooth and straight; 2. convex; 3. deeply convex.

26. *Pereonites 1–4 posterolateral margins (size)*: 1. similar size or weakly increasing in size; 2. progressively increasing in size.

27. *Pereonites 5–6 posterolateral margins shape*: 1. smooth and straight; 2. narrowly convex; 3. convex; 4. deeply convex; 5. lobed.

28. *Pereonites 5–6 posterolateral margin size*: 1. progressively decreasing in size; 2. similar size; 3. progressively increasing in size.

29. *Pereonite 7 (length)*: 1. does not extend laterally past pleonite 1; 2. extends past pleonite 1.

30. *Pereonite 7 (posterior margin shape)*: 1. shallowly concave; 2. straight; 3. deeply concave; 4. bisinuate.

31. *Pleonites 3–5 (width)*: 1. subequal width to pleonite 1 and 2; 2. narrower than pleonites 1 and 2; 3. wider than pleonites 1 and 2.

32. *Pleonites 1–5 fusion*: 1. not fused; 2. fused.

33. *Pleon maximum width (to pereon)*: 1. narrower than pereon; 2. subequal to pereon; 3. wider than pereon.

34. *Pleonite 1 (visibility)*: 1. visible; 2. partially visible, laterally concealed by pereonite 7; 3. not visible, completely concealed by pereonite 7.

35. *Pleonite 1 width*: 1. as wide as other pleonites; 2. narrower than other pleonites; 3. wider than other pleonites.

36. *Pleonite 1 and 2 (lateral margin)*: 1. not produced; 2. produced; 3. strongly produced.

37. *Pleonite 5 (posterior margin)*: 1. straight; 2. convex; 3. bisinuate; 4. trisinuate; 5. irregular.

38. *Pleotelson (to pereonite 7)*: 1. narrower than pereonite 7; 2. similar width to pereonite 7; 3. wider than pereonite 7.

39. *Pleotelson dorsal surface*: 1. without submedian depression; 2. anterior with median depression; 3. anterior with 2 submedian depressions.

40. *Pleotelson (lateral margins)*: 1. straight; 2. concave; 3. convex; 4. sinuate.

41. *Coxae 2 and 3 (size)*: 1. similar to other coxae; 2. smaller than other coxae; 3. larger than other coxae.

42. *Coxae 2–4 size (lateral view)*: 1. as long as respective pereonite; 2. shorter than respective pereonite; 3. longer than respective pereonite.

43. *Coxae 5–7 (direction)*: 1. ventrally; 2. posteriorly.

44. *Coxae 5–7 (dorsal view)*: 1. visible; 2. not visible; 3. partially visible.

45. *Coxae 5–7 size (lateral view)*: 1. longer than pereonite; 2. as long as pereonite; 3. shorter than pereonite.

46. *Coxae 5–7 shape*: 1. prominent and acute; 2. narrowly rounded; 3. broadly rounded.

47. *Brood pouch*: 1. from coxae 1–4 and 6; 2. from coxae 2–5; 3. from coxae 1–5; 4. from coxae 2–4 and 6.

48. *Oostegite 6 (size)*: 1. similar to other oostegites, forming part of the marsupium; 2. large than other oostegites, forming most of the marsupium; 3. absent.

49. *Posterior pocket*: 1. absent; 2. present.

50. *Pereopod 1 propodus (setae)*: 1. present; 2. absent.

51. *Pereopods 5–7 basis (anterior superior margin)*: 1. without carina; 2. with carina; 3. with large blade-like carina.

52. *Pereopods 5–7 posterior inferior margin*: 1. without carina; 2. with weak carina; 3. with large blade-like carina.

53. *Pereopod 6 inferior distal margin (robust setae)*: 1. present; 2. absent.

54. *Pereopod 7 inferior distal margin (robust setae)*: 1. present; 2. absent.

55. *Pereopod 7 (setae)*: 1. with small setae; 2. with robust setae; 3. without robust setae.

56. *Pereopod 7 (size)*: 1. slightly larger or more than 1.5 times longer than pereopod 1; 2. subequal in length to pereopod 1; 3. 2 or more times longer than pereopod 1.

57. *Pleopods (dorsal view visibility)*: 1. not visible; 2. visible.

58. *Pleopods (endopod lobes)*: 1. without proximomedial lamellar lobe; 2. all pleopods with proximomedial lamellar lobe; 3. with proximomedial lamellar lobe on 3–5; 4. with proximomedial lamellar lobe on 5 only.

59. *Pleopods (multiple lamellar)*: 1. absent; 2. present.

60. *Pleopod exopods (alignment)*: 1. partially off-alignment, 1/3 or less of endopod exposed; 2. aligned with and completely overlapping endopod; 3. exopod off-set from endopod, 1/2 or more of endopod exposed.

61. *Pleopods peduncle lobes on lateral margin*: 1. absent; 2. present.

62. *Pleopod 1 operculate (ventral view to other pleopods)*: 1. exopod does not conceal other pleopods; 2. exopod conceals other pleopods.

63. *Pleopod 1 exopod (lateral margin)*: 1. weakly convex; 2. straight; 3. strongly convex.

64. *Pleopod 1 (mesial margin)*: 1. weakly convex; 2. straight; 3. strongly convex.

65. *Pleopod 1 exopod (distally)*: 1. narrowly rounded; 2. broadly rounded; 3. narrowly acute; 4. subtruncate; 5. mesial margin weakly to strongly oblique.

66. *Pleopods 3–5 (endopod pockets)*: 1. without pleats, smooth (lamellar); 2. with small pleats or pockets; 3. with large fleshy folds.

67. *Uropod peduncle (exopod length)*: 1. shorter than exopod length; 2. similar to exopod length; 3. longer than exopod length.

68. *Uropod rami (to pleotelson)*: 1. extending to pleotelson apex; 2. not extending beyond pleotelson apex; 3. extending beyond pleotelson apex.

69. *Uropod rami (length)*: 1. exopod shorter than endopod; 2. subequal; 3. exopod longer than endopod.

70. *Uropod exopod apex*: 1. narrowly rounded; 2. broadly rounded; 3. acute.

71. *Uropod endopod apex*: 1. narrowly rounded; 2. broadly rounded; 3. acute.

7.2.1.4 Character matrix

A data matrix was constructed in DELTA (Dallwitz *et al.* 1997; see Table 7.2) and a nexus file was generated for input into PAUP* 4.0b10 (beta-test version for 32-bit Microsoft Windows; Swofford 2001). All data (mentioned in 7.4.2.3) were treated as unordered and having equal weights (for the first analysis) to prevent predetermination of cladogram topology.

The outgroup comparison approach was used to polarize characters. The plesiomorphic state from the out group is represented by state 1 in all the characters. Characters scored as “2”, “3”, etc. signified apomorphic states; “–” were treated as inapplicable; “?” represented missing or unknown state and multistate characters were identified as polymorphisms.

Table 7.2 Character matrix of 31 taxa and 71 characters used in the cladistic analysis of Cymothoidae. Key: (#) refers to alternate states 1 or 2; (^) refers to alternate states 1 or 3; (+) refers to alternate states 2 or 3; (*) refers to alternate states *3 or 4; (z) refers to alternate states 2 or 4; (y) refers to alternate states 4 or 5; (x) refers to alternate states 1 or 5; (w) refers to alternate states 3 or 5; (v) refers to alternate states 1 or 4; (u) refers to alternate states 1, 2 or 3.

Taxon	1 2 3 4 5 6 7 8 9 **1**	1 2 3 4 5 6 7 8 9 **2**	1 2 3 4 5 6 7 8 9 **3**	1 2 3 4 5 6 7 8 9 **4**	1 2 3 4 5 6 7 8 9 **5**	1 2 3 4 5 6 7 8 9 **6**	1 2 3 4 5 6 7 8 9 **7**	1
Rocinela (Aegidae)	# 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 # 1	1 1 1 1 1 1 1 # 1 #	1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 – 1 1 1	1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1	1
Agarna	1 ^ 1 1 3 2 2 – 3 2	1 2 1 2 – 1 2 1 z 2	3 3 2 2 3 2 3 1 2 3	1 1 2 2 ? 1 1 2 3 3	1 1 1 1 2 1 – – – 2	2 1 – 2 3 1 1 – – –	– – – – – – 2 1 3 1	2
Anilocra	2 # 3 3 1 1 1 1 1 2	1 1 1 # 1 1 1 2 4 2	1 3 1 1 1 1 1 3 1 1	2 1 1 1 3 1 1 1 ^ 1	1 1 2 2 3 2 1 2 2 1	2 1 2 1 2 3 2 3 1 ^	1 1 1 2 1 3 1 3 2 ^	+
Anphira	1 3 1 1 3 2 3 – 3 2	2 2 1 2 2 1 3 2 3 1	1 1 1 1 3 1 3 # 1 1	1 1 1 1 1 1 2 1 1 3	1 1 2 1 2 3 – – – 2	1 1 2 2 3 1 1 1 1 2	1 1 2 2 1 1 1 2 3 3	3
Artystone	1 3 1 1 1 2 1 2 # 2	2 2 1 2 1 1 3 3 4 1	2 1 1 1 1 1 3 1 2 1	1 1 1 1 1 1 1 1 1 ^	1 2 2 2 2 2 1 1 1 1	1 1 1 1 1 1 1 3 1 2	1 1 2 2 3 1 3 2 3 1	1
Asotana	+ 3 2 1 3 1 1 2 1 2	2 2 1 2 1 1 3 2 5 1	2 1 1 1 1 1 3 1 2 1	1 1 1 3 2 1 1 1 1 3	1 3 2 2 3 2 3 3 – 2	1 1 1 1 1 1 1 – – –	– – – – – – 3 2 1 2	2
Braga	2 ^ 1 1 3 1 1 2 1 2	2 2 1 2 1 1 3 1 4 1	1 1 2 2 2 1 3 1 1 1	1 1 1 1 2 1 1 1 3 3	1 1 1 2 2 2 1 1 1 2	1 1 2 2 3 3 2 2 2 2	1 1 3 3 5 1 2 2 3 1	2
Catoessa	2 3 1 1 2 1 1 2 + 2	2 1 1 2 1 1 2 1 1 2	3 2 1 1 2 2 5 2 2 3	1 1 1 2 1 1 3 2 1 3	1 # 2 3 3 2 4 1 1 2	2 1 ? 2 3 1 1 3 1 1	1 – 3 3 2 1 1 2 3 2	?
Ceratothoa	+ 1 1 1 ^ 1 # 4 2 2	2 2 1 2 2 1 # 2 2 2	1 1 2 3 # # + 1 2 1	3 1 # 2 2 1 * # 1 3	1 1 2 3 3 3 1 1 1 2	3 1 2 2 3 1 1 3 1 2	1 2 1 1 2 2 2 1 2 1	1
Cinusa	1 3 2 1 1 1 1 3 1 2	2 2 1 1 1 1 1 2 2 2	1 2 1 1 3 2 5 1 2 1	3 1 1 1 1 1 1 2 3 3	1 1 1 1 3 2 2 3 1 2	3 2 2 2 3 2 1 3 1 2	1 1 3 1 5 2 2 2 2 2	2
Creniola	1 # 1 1 2 2 1 2 2 1	2 * 1 2 1 # 1 2 4 2	1 2 1 1 # # 4 3 2 3	1 1 1 # 1 1 1 1 1 1	2 1 1 2 3 1 1 2 2 2	3 2 2 2 2 1 1 3 1 2	2 2 3 1 2 3 1 2 3 2	2
Cterissa	1 1 2 3 1 1 2 2 1 2	1 2 1 2 1 1 2 – 4 1	– 1 2 3 3 # 3 # 2 3	3 1 3 2 2 1 4 3 1 4	– 3 2 1 1 3 – – – 2	2 1 – 1 3 1 1 1 1 –	1 – – – – – 2 2 + 2	2
Cymothoa	+ ^ # 2 2 2 3 2 2 2	2 * 1 2 2 1 2 2 2 2	1 3 2 3 # # 3 # 2 1	3 1 1 2 2 1 x 2 1 ^	1 1 2 3 3 2 4 1 1 2	3 1 2 2 3 1 1 3 1 2	2 2 3 3 5 3 # 2 2 1	1
Elthusa	# * 2 2 1 1 3 # 2 2	1 2 1 2 2 1 1 1 4 2	1 3 2 2 3 # 3 # 2 1	2 1 3 2 1 1 1 # 1 #	1 1 2 1 2 3 1 1 1 2	3 1 2 2 3 1 1 1 1 2	1 2 3 3 2 1 2 2 3 1	1
Glossobius	+ # # 1 1 1 1 4 2 2	2 2 1 2 2 1 1 1 2 1	2 1 2 4 # # 2 # 2 1	1 1 # # 1 1 3 + 1 3	1 # 1 1 3 3 1 1 1 2	3 1 2 2 3 1 1 3 1 2	1 2 3 3 2 2 # 2 + 1	1
Smenispa	2 ^ 1 1 2 2 2 2 1 2	2 3 1 2 1 2 1 1 4 2	3 1 2 3 3 2 w # 1 1	1 1 # 1 1 1 1 1 1 1	? 1 1 3 1 2 1 2 2 2	2 1 2 2 3 1 1 3 1 2	2 1 3 1 5 3 1 1 2 1	2
Ichthyoxenus	1 3 2 2 1 1 1 1 1 1	1 2 1 2 1 1 1 1 4 2	– 2 2 2 + 2 y 1 2 3	1 1 1 2 1 # 3 # 1 3	1 1 1 3 3 3 4 1 1 2	2 1 2 2 3 1 1 3 1 1	1 – 3 2 x 1 1 2 3 3	1
Joryma	# 3 – – # 1 3 – 2 1	2 2 2 – – – 1 2 4 2	3 3 2 4 3 2 5 # 2 1	1 1 2 2 # 1 5 1 1 3	3 3 2 3 3 2 – – – 2	1 1 2 2 2 1 1 4 1 1	1 – 1 2 3 1 1 2 3 3	3
Kuna	1 3 2 2 3 1 1 1 2 2	2 2 1 2 2 1 1 2 3 2	1 2 2 2 3 2 2 # 1 4	2 1 2 1 1 2 ? 1 1 3	1 1 1 3 2 2 – – 1 2	1 – – 2 3 2 1 1 1 3	1 1 1 1 2 1 3 2 3 2	2
Livoneca	# # 1 1 2 2 1 # 2 2	2 2 1 2 # # 1 2 4 2	1 2 1 2 1 # 4 1 1 1	2 1 1 2 ^ 1 5 1 1 u	2 1 1 3 3 3 1 2 2 2	2 1 2 2 3 3 1 3 1 2	2 1 3 1 2 3 1 3 2 2	2
Lobothorax	3 3 1 1 1 1 3 1 1 1	1 2 1 2 1 2 3 1 4 +	3 3 2 4 # # # 1 2 1	3 1 2 2 2 1 1 3 1 2	– 2 – 3 3 – 4 1 1 2	2 1 2 2 3 – 1 2 1 1	1 – 3 3 2 2 1 1 2 3	3
Mothocya	1 1 2 1 2 2 + 2 3 2	2 1 1 2 2 1 1 1 4 2	3 3 2 + + 2 5 # 2 1	1 1 2 2 2 1 1 + 1 3	2 3 2 1 1 3 4 1 1 2	1 1 2 2 1 1 1 3 1 ^	2 1 1 2 z 1 # 3 3 1	1
Nerocila	# # 1 1 2 2 1 # 2 2	2 2 1 2 # # 1 + 4 2	1 3 1 2 1 # 4 3 2 1	2 1 1 1 ^ 3 1 1 1 u	2 3 2 1 3 1 1 2 2 2	1 1 1 1 2 1 1 3 1 1	2 1 1 1 2 3 1 3 3 ^	^
Norileca	1 ^ 2 1 2 2 1 2 2 1	2 1 1 2 2 1 1 1 v 2	3 3 2 2 3 2 3 3 2 1	2 1 1 1 ^ 1 1 1 1 1	1 1 2 3 2 2 2 2 2 1	3 1 – 2 3 1 1 3 1 1	2 1 1 1 2 3 1 2 2 1	1
Ourozeuktes	1 * 1 1 3 2 2 2 1 2	2 2 1 2 2 1 1 2 3 2	2 4 2 2 + 2 5 1 2 3	1 2 1 – – 1 – 1 2 1	2 3 1 3 2 1 1 1 1 2	3 3 2 2 3 3 2 2 – –	– 2 2 2 4 2 3 2 3 3	3
Paracymothoa	2 1 1 – + 2 1 2 1 2	2 2 1 2 1 1 1 2 4 –	1 3 1 2 1 1 + # 2 1	1 1 1 2 3 1 2 + 1 3	? 1 2 1 1 3 – – – 2	2 2 2 2 3 3 1 – ? –	– – – – – – 2 1 2 2	2
Pleopodias	2 # 3 3 2 1 1 1 2 1	1 1 1 1 1 # 1 1 4 3	1 2 1 2 1 1 # # 1 1	2 1 1 1 3 ? + 1 1 1	1 1 2 2 3 2 1 2 2 2	1 1 1 1 2 3 2 3 1 3	1 1 1 1 1 2 1 3 2 ^	^
Renocila	1 # 1 2 2 2 2 2 3 2	2 2 1 2 2 # 1 2 4 1	1 3 1 1 1 1 4 3 2 3	1 1 1 2 3 1 3 1 1 2	2 1 2 3 3 1 1 2 2 2	3 2 1 2 2 1 1 3 1 +	1 2 1 1 2 + 1 2 3 1	#
Riggia	# 4 1 1 2 2 2 2 2 2	2 2 1 2 1 1 3 2 4 1	2 1 2 3 + 2 3 1 2 1	2 2 1 – – 1 – 1 1 1	3 1 2 3 3 3 – – – 2	1 1 2 2 3 1 1 3 1 2	2 – 1 2 1 1 2 1 3 3	3
Ryukyua	1 * 1 # 3 2 2 # 3 2	2 1 1 1 2 1 1 2 4 2	1 2 2 4 3 2 5 # 2 3	3 1 3 3 1 1 1 1 1 1	1 3 1 3 3 3 – – – 2	2 2 2 2 3 3 1 1 1 –	– 1 – – – 1 2 1 1 2	2
Telotha	1 3 – – 3 2 2 2 3 2	2 2 1 2 1 1 – 1 3 1	1 1 2 3 + 2 # # 1 2	1 1 1 1 1 1 1 1 1 1	1 3 2 2 2 2 – – – 2	2 1 2 2 3 1 1 2 1 2	2 – 3 1 4 1 2 3 3 1	1

7.2.1.5 Cladistic analysis

A heuristic search from the data matrix (Table 7.2) was initiated with tree-bisection-reconnection (TBR) branch swapping algorithm to avoid an exhaustive search of all available topologies. The current optimality criterion was set to parsimony. Starting trees were obtained via stepwise addition. The search was conducted with 1000 random addition sequence repetitions, where no more than three trees of one step greater or equal to the minimum length tree at each iteration were saved. The initial starting tree at each iteration was selected at random. The PAUP* command for the above method is as follow (hsearch start=stepwise addseq=random nreps=1000 savereps=yes nchuck=3 chuckscore=1 dstatus=none randomize=trees). Branches of these resultant trees were swapped in a second search, which retained all minimum length trees using the following PAUP* command (hsearch start=current nchuck=0 chuckscore=0). The topology of the saved trees was calculated with a strict consensus tree (Fig. 7.2) and a 50% majority rule consensus tree (Fig. 7.3) to access congruence of the phylogenetic hypothesis.

A second analysis to improve resolution was accomplished using the reweight option in PAUP* (reweight minforfit=range). The characters were reweighted by maximum value of rescaled consistency indices. This was achieved in a single reweight run using the search protocols above. Character transformation of a single tree was performed using the following PAUP* command (describetrees /apolist=yes) and were mapped on a successively weighted consensus tree (Fig. 7.4). To access the stability and relative support of the reweighted tree, a bootstrap analysis was conducted in PAUP* using a heuristic search with random addition sequence on 200 bootstrap replicate data sets (bootstrap nreps=200). Trees were drawn using Mesquite Version 2.75 (build 564) (© Maddison & Maddison 2011) and Adobe Illustrator CS6 (64 bit).

7.2.2 Molecular data

7.2.2.1 *Specimens*

Thirty two cymothoid specimens from 13 species used for molecular analysis were primarily obtained from the Museum of Tropical Queensland (MTQ). Specimens from MTQ were preserved in 90% ethanol, though the fixative history prior to this study is not known (e.g. formalin). Under the circumstance that the museum specimens were formalin-fixed, the extraction of nucleic acids may yield degraded DNA. Freshly collected specimens were preserved in 70–95% ethanol or RNA later. Specimen details are listed in Table 7.3.

7.2.2.2 *DNA extraction*

Cymothoid specimens were brushed and rinsed of debris with deionized water. Appendages (pereopods and pleopods) and females with eggs were dissected to form a small tissue pulp. DNA from cymothoid tissue replicates were extracted using three different protocols: 1) PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad CA, 2) Isolate II Genomic DNA Kit (Bioline, London, UK) and 3) standard phenol chloroform extraction. The first two methods were carried out according to the manufacturer’s protocol whereas the latter is explained in detail below:

7.2.2.2.1 *Standard phenol chloroform extraction of DNA*

Tissue samples (5ml) were transferred into a 10ml sterile centrifuge tube. Biomass was re-suspended in 10ml TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0) and incubated overnight while agitated. Tissue samples were then centrifuged at 5,000 g for 5 min and once again incubated overnight (repeat incubation and centrifuge step for at least three times). After the final incubation step, the tissue was resuspended in 500µl of TE and transferred to 2ml microcentrifuge tube. 50µl of 10% SDS and 25µl of proteinase K were added to the 2ml microcentrifuge tube and vortexed for 30 seconds. The biomass is later incubated overnight at 65 ºC while agitated. For the standard phenol chloroform extraction, 100µl of phenol (pH 8) were added to the sample, vortexed, and left to settle for 15 minutes. Later, 800µl of 24:1 chloroform: isoamyl alcohol is added to sample and vortexed.

The sample was centrifuged for 5 minutes (maximum setting at 16,000g), of which the top aqueous phase was recovered. The phenol and chloroform: isoamyl alcohol step was repeated for at least three times or until the interface was clear. Contaminating proteins were precipitated by adding 1 volume (800µl) of 7.5 ammonium acetate and incubated at room temperature for 30 min. The suspension was centrifuged at 16, 000g for 10 min, after which 400µl of supernatant were transferred to two 2ml sterile microcentrifuge tubes. 1.6ml of 100% ethanol were added to each tube and incubated at room temperature for a minimum of 2 hours to precipitate the nucleic acids (preferably overnight). The suspension was centrifuged at 16,000 g for 30 min ethanol discarded, and nucleic acid pellet was washed in 200µl of 70% ethanol via centrifugation and evaporation. Finally, pellet was re-suspended at 20µl of TE buffer (and stored in -20 ºC until required).

7.2.2.3 Polymerase Chain Reaction (PCR) amplification

The cytochrome c oxidase 1 mtDNA (COI) sequences were amplified using universal primers (LCO1490 and HCO2198, ~442 base pair) (Folmer *et al.* 1994) and cymothoid specific primer pair (16S-cym-for: 5'-AGCCCTGTTCAATGGGATTA-3'; 16s-cym-rev: 5'-TCCCTGGGGTAGTTTCATCTT-3' (Ketmaier *et al.* 2008) were used for the mitochondrial 16S rRNA fragment (~493 base pair).

The PCR (50µl) comprised of 31.5µl milipore H2O water, 10µl Bioline Buffer (Bioline Reagents Ltd., London, UK) 4µl forward and reverse primers (10 pmol), 0.25µl Bovine Serum Albumin (New England Biolabs, Ipswich, MA) (20mg/ml), 0.25µl My Taq Polymerase (Bioline Reagents Ltd., London, UK) (5 units µl-1) and 4µl DNA template, made up to 50µl reaction mixture.

All PCR reactions were performed in a BioRad (T100TM) thermocycler with an initial denaturing of 94°C at 10 min, followed 10 cycles of 30 sec at 94°C; 30 sec at 50–40°C; 30 sec at 72°C; then 25 cycles of 1 min at 95°C; 1 min at 40°C; 1 min at 72°C; and a final extension of 72°C at 7 min. PCR amplification products (5µL) were electrophoresed through SYBR® Safe DNA Gel Stain (Thermo Fisher Scientific Inc., USA) and quantified by visual comparison with a low DNA 100 bp mass ladder (5µl, Promega). Amplified DNA was purified using Bioline ISOLATE II PCR and Gel Kit (Bioline, UK) according to manufacturer's protocol.

7.2.2.4 Sequencing and alignment

Consensus sequences were assembled using Sequencher (version 5.3, Gene Codes Corporation©, Ann Arbor, MI, USA). Cymothoid 16S and COI sequences were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/) using a BLAST search and from previous literature (Wetzer 2001, 2002; Jones *et al.* 2008; Ketmaier *et al.* 2008; Thangaraj *et al.* 2014) (see Table 7.3). Sequences were aligned using Clustal W (Thompson *et al.* 1994) implemented in MEGA version 6.0 (Tamura *et al.* 2013). The alignment was corrected manually using the alignment editor of the software MEGA version 6.0.

7.2.2.5 Phylogenetic reconstruction

16S rRNA sequence of *Excirolana chiltoni* (Richardson, 1905) and COI sequence of *Cirolana rugicauda* were retrieved from GenBank: (http://www.ncbi.nlm.nih.gov/nuccore/) (accession numbers in Table 7.3). These were aligned to sequences obtained for this study and used as outgroups in this molecular phylogeny. These outgroup species are from Cirolanidae and were selected at increasing levels of taxonomic separation as previously demonstrated by previous studies (Brusca & Wilson 1991; Wetzer 2002; Brandt & Poore 2003); numerous cirolanid 16S rRNA and COI sequences are available in GenBank.

The alignments for 16S and COI sequences were analysed separately with maximum parsimony (MP) and maximum likelihood (ML) using MEGA version 6.0. Pairwise comparisons of uncorrected sequence divergence for all taxa were calculated with gaps treated as missing data.

Table 7.3 Cymothoid species, parasitic habit, host-association and voucher numbers used in this study. Cymothoid parasitic habits are identified as follows: E (external-attaching), B (buccal-attaching), G (gill-attaching), F (flesh burrowing). (*) indicates specimens sequences to be submitted to Genbank.

Species	Parasitic habit	Host	Museum voucher	GenBank Accession		Reference
				16S	COI	
Anilocra physodes(Linnaeus, 1758)	E	*Symphodus tinca*	-	EF455808.1	EF455818.1	Ketmaier *et al.* (2008)
			-	EF455809.1	EF455817.1	
Anilocra nemipteri (Bruce, 1987)	E	*Scolopsis bilineatus*	W28286	EF422806.1 EF422790.1		Jones *et al.* (2008)
Anilocra pomacentri (Bruce, 1987)	E	*Chromis nitida*	W28285	EF432778.1		Jones *et al.* (2008)
Anilocra apogonae (Bruce, 1987)	E	*Apogon fasciata*	W28283	EF422800.1		Jones *et al.* (2008)
Anilocra longicauda (Schioedte & Meinert, 1881)	E	*Diagramma labiosum*	W28284	EF422797.1 EF422789.1		Jones *et al.* (2008)
Ceratothoa barracuda (Martin, Bruce & Nowak, 2015)	B	*Sphyraena forsteri*	W28287	EF422802.1 *		Jones *et al.* (2008)/ Present study
Ceratothoa collaris (Schioedte & Meinert, 1883)	B	*Lithognathus mormyrus*;	-	EF455807.1	EF455816.1	Ketmaier *et al.* (2008)
Ceratothoa italic (Schioedte & Meinert, 1881)	B	*Lithognathus mormyrus*		EF455806.1	EF455815.1	Ketmaier *et al.* (2008)
				EF455805.1	EF455814.1	
				EF455804.1	EF455813.1	
Cymothoa indica (Schioedte & Meinert, 1884)	B	*Sillago ciliata*	W28288	EF422791.1 EF422801.1		Jones *et al.* (2008)
Elthusa vulgaris (Stimpson, 1857)	G	—		AF259546.1	AF255790.1	Wetzer (2002)
				EF455812.1	EF455821.1	
Joryma hilsae	G	—			KC 896398	Thangaraj *et al.* (2014)
Olencira praegustator (Latrobe, 1802)	B, G	—		AF259547.1		Wetzer (2001)
					AF255791.1	Wetzer (2002)
				EF455811.1		Ketmaier *et al.* (2008)
					EF455820.1	Ketmaier *et al.* (2008)
Nerocila bivittata (Risso, 1816)	E	*Sarpa salpa*			EF455819.1	Ketmaier *et al.* (2008)
Nerocila longispina (Miers, 1880)	E	—			KC896398	Thangaraj *et al.* (2014)
Nerocila monodi (Hale, 1940)	E	*Acanthopagrus australis*	W28290	EF422805.1		Jones *et al.* (2008)
Mothocya renardi (Bleeker, 1857)	E	*Tylorus* sp.	W28289	EF422803.1		Jones *et al.* (2008)
Renocila ovata (Miers, 1880)	E	*Blenniella chrysospilos*	W28291	EF422788.1		Jones *et al.* (2008)

PRESENT STUDY (table 7.3 continued)

Species	Parasitic habit	Host	Museum voucher	GenBank Accession		Reference
				16S	COI	
Ceratothoa banksii (Leach, 1818) No.1	B	*Trachurus* sp. (from Chile)	W34276	*		Martin *et al.* (2015a)
Ceratothoa banksii (Leach, 1818) No.2	B	Species of trevally (from Australia)	W21519	*		Martin *et al.* (2015a)
Ceratothoa banksii (Leach, 1818) No.3	B	*Salmo salar*	W14780	*		Martin *et al.* (2015a)
Ceratothoa banksii (Leach, 1818) No.4	B	*Selenotoca multifasciata*	W 7249	*		Martin *et al.* (2015a)
Ceratothoa imbricata (Fabricius, 1775)	B	*Trachurus declivis*	W34278	*		Martin *et al.* (2015a)
Cymothoa pulchrum (Lanchester, 1902)	B	*Tetraodon nigroviridis*	W34290		*	Martin *et al.* (submitted)
Cymothoa eremita (Brunnich, 1783)	B	*Pristipomoides multidens*	W30413		*	Martin *et al.* (submitted)
Cymothoa hermani (Hadfield, Bruce & Smit, 2011)	B	Species of parrotfish (from Australia)	W8961	*	*	Martin *et al.* (submitted)
Cymothoa rhina (Schioedte & Meinert, 1884)	B	*Lutjanus carponotatus*	ZRC 2014.0119		*	Bruce *et al.* (accepted)
Cymothoa curta (Schioedte & Meinert, 1884)	B	*Anableps anableps*	W34284	*	*	Martin *et al.* (submitted)
Nerocila orbignyi (Guérin-Méneville, 1832)	E	*Callorhinchus milii*	unregistered		*	
Nerocila poruvae	E	—	unregistered		*	
Nerocila monodi (Hale, 1940)	E	—	unregistered		*	
Nerocila sundaica (Bleeker, 1857)	E	—	unregistered		*	
Ourozeuktes bopyroides (Lesueur, 1814)	F	*Acanthaluteres vittiger*	unregistered		*	
Smenispa irregularis (Bleeker, 1857)	B	*Acanthopagrus latus*	unregistered		*	

OUTGROUP

Cirolana rugicauda Heller, 1861		—			AF255788	Wetzer (2002)
Excirolana chiltoni (Richardson, 1905)		—		AF260849.1		Wetzer (2001)

7.3 RESULTS

7.3.1 Morphological results

7.3.1.1 Morphological summary

The initial heuristic search of the first analysis using unweighted characters had 68 characters which were parsimony informative (the remainder were parsimony-uninformative). This analysis produced 2091 equally parsimonious trees while the second search of the resulted trees retained 11 minimum length trees of length 490 steps; total rearrangements of 99, 896; consistency index (*CI*) 0.2796 (0.2737 excluding uninformative characters); homoplasy index (*HI*) of 0.7204 (0.7263 excluding uninformative characters) retention index (*RI*) 0.4361 and rescaled consistency index (*RC*) 0.1219.

The strict consensus tree (Fig. 7.2) and 50% majority rule consensus tree (Fig. 7.3) of the 11 trees showed two major clades, with better resolution in the majority rule consensus tree. The basal clade (*Anilocra* + *Pleopodias*) were consistent in both consensus and showed high level of stability in the 50% majority rule tree (100%, Fig. 7.3).

The second analysis using reweight method in PAUP* produced 18 equally parsimonious trees of length, with tree length 63.18421 steps, consistency index (*CI*) 0.3860 (0.3445 excluding uninformative characters); homoplasy index (*HI*) of 0.6140 (0.6555 excluding uninformative characters) retention index (*RI*) 0.5591 and rescaled consistency index (*RC*) 0.2158. The second run resulted in a single resolved tree (Fig. 7.4), of which the discussion on the clades is based upon this tree topology.

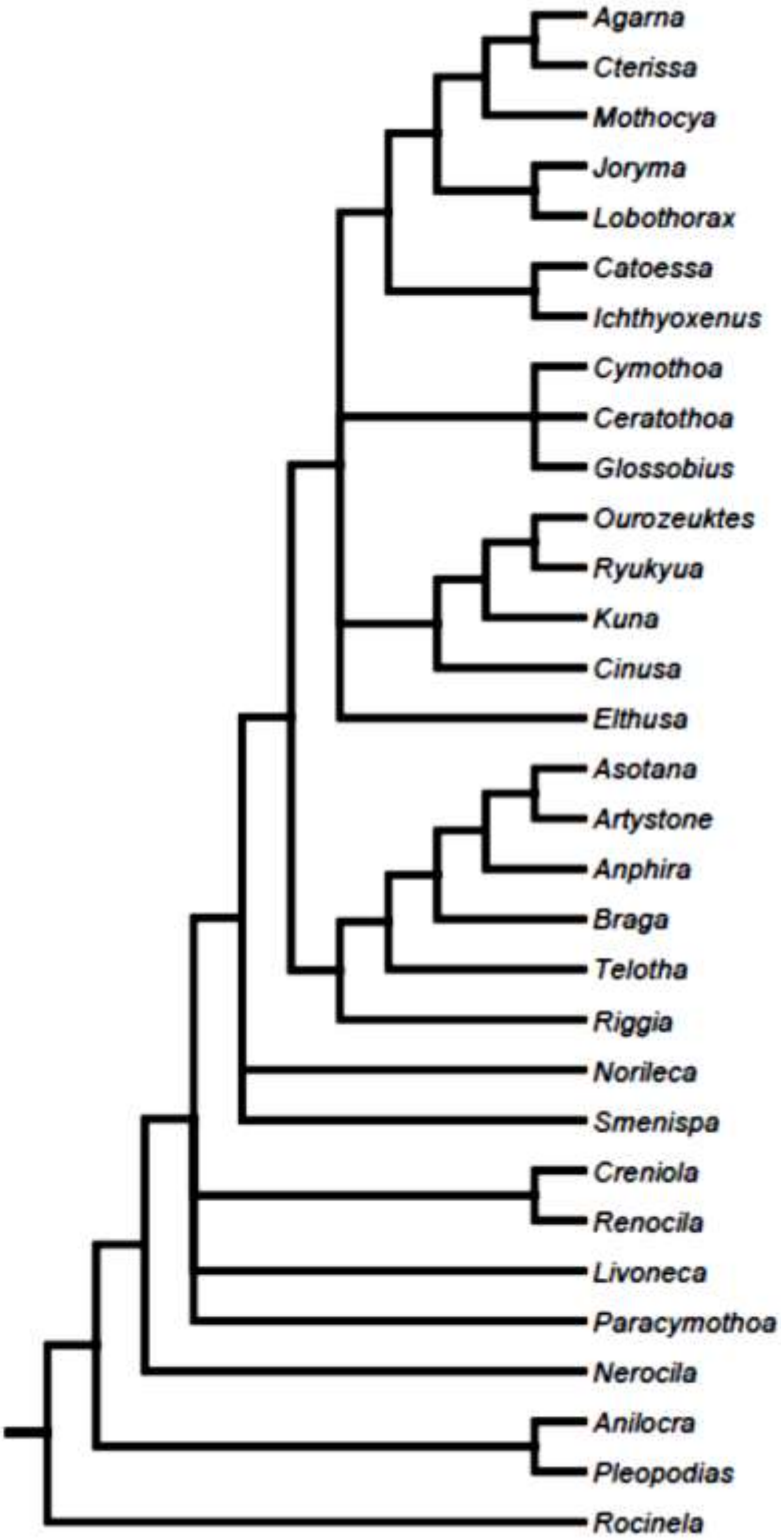

Figure 7.2 Clades in the family Cymothoidae: Strict consensus of 11 trees, unweighted data

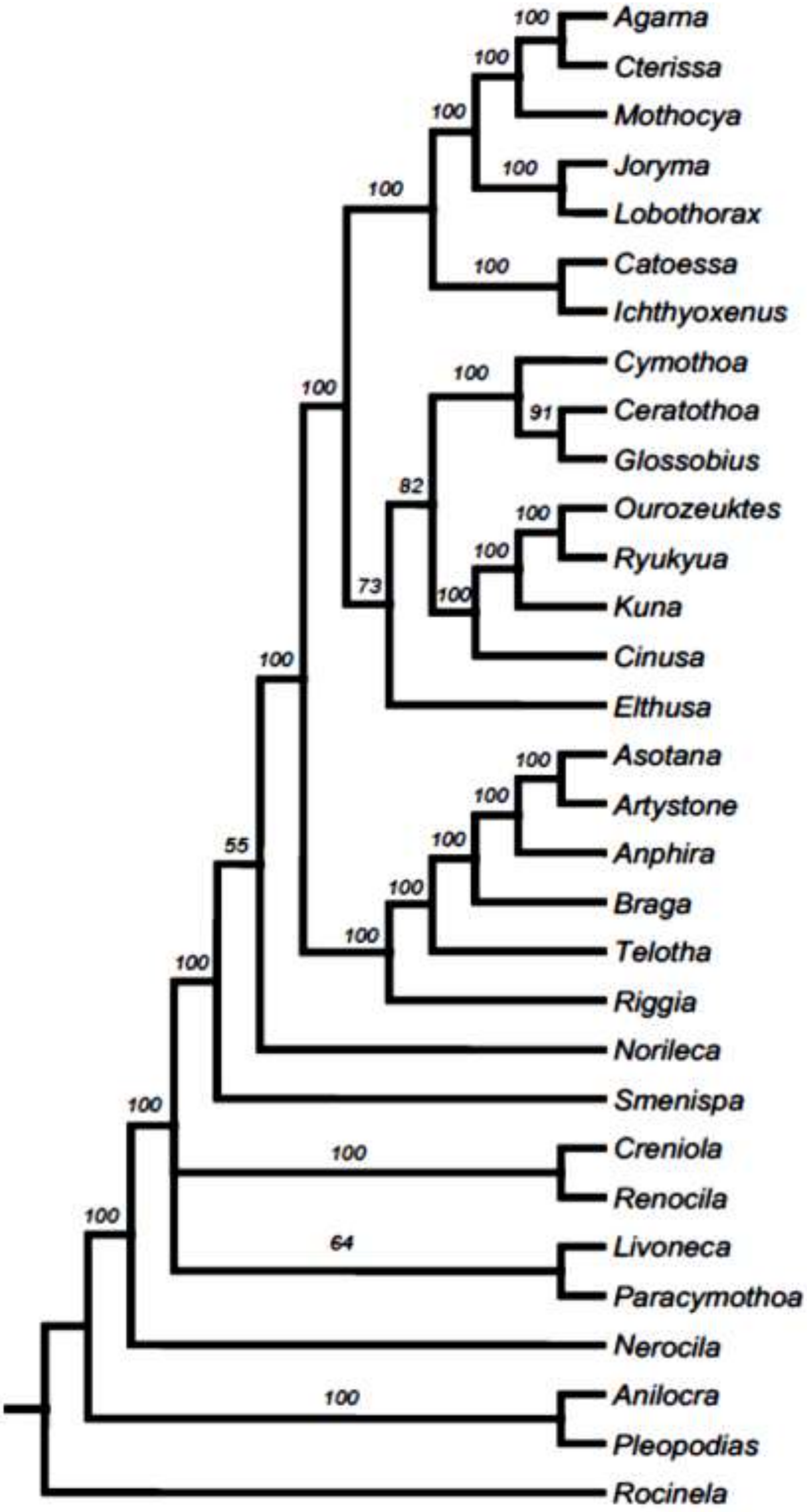

Figure 7.3 Clades in the family Cymothoidae: 50% majority rule consensus of 11 trees, unweighted data

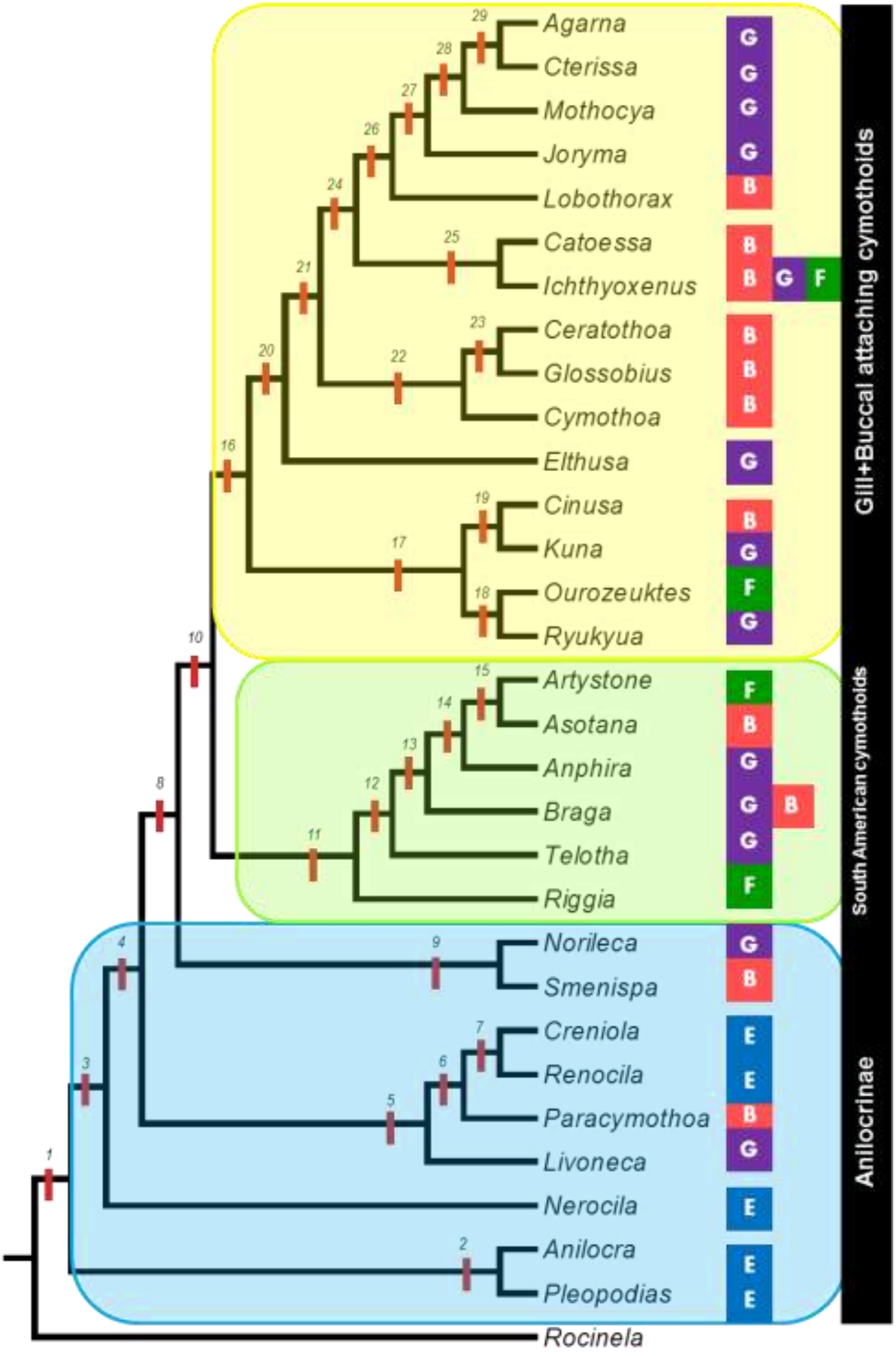

Figure 7.4 Clades in the family Cymothoidae: successively weighted tree. Site-attachment: E (external-attaching), B (buccal-attaching), G (gill-attaching), F (flesh burrowing).

7.3.1.2 Discussion of clades

In the analysis of the successively reweighted tree (Fig.7.4), the genera of the ‘Anilocrinae’ are all basal to the rest of the cymothoid genera but are not monophyletic. It is here represented in three separate clades (Clade 2, 5, 9). There is a monophylectic clade (clade 11) of South American endemic genera with the exception of *Paracymothoa*, with *Riggia* sister to the remaining genera. The terminal clade is a major clade which includes all buccal and gill-attaching cymothoids and a single flesh burrowing *Ourozeuktes* (Clade 16).

Clade 2 comprises *Anilocra* and *Pleopodias* and is sister to the rest of the family. The clade is defined by: pereon lateral margins weakly ovate (Ch 1.2); head frontal margin folded ventrally and posteriorly (Ch 3.3) and projecting between antennula bases (Ch 4.3); pleonite 1 wider than other pleonites (Ch 35.3); coxae 5–7 dorsal view not visible (Ch 44.2) and narrowly rounded (Ch 46.2); pereopod 7 2.0 or more times longer than pereopod 1 (Ch 56.3) pleopods dorsal view visible (Ch 57.2) with exopods off-set from endopod (1/2 or more of endopod exposed) (Ch 60.3); and uropod endopod apex acute (Ch 71.3). Of all the other Anilocrinae, the frontal margin folded ventrally and posteriorly is synapomorphic to clade 2. The frontal margin projecting between antennula bases is a character state unique to the *Anilocra*+*Pleopodias* clade compared to the rest of the Anilocrinae, with *Cterissa* the only other genus to share this state. As for Character 57.2, *Anilocra*, *Pleopodias*, *Braga* and *Ourozeuktes* are the only genera to have dorsally visible pleopods, this being most prominent in *Anilocra* and *Pleopodias*.

Clade 5 includes *Creniola*, *Renocila*, *Paracymothoa* and *Livoneca*. The clade is defined by pleonite 1 partially visible (Ch 34.2); pleonite 1 wider than other pleonites (Ch 35.3); pereopod 7 2.0 or more times longer than pereopod 1 (Ch 56.3); and uropod exopod apex broadly rounded (Ch 70.2). *Livoneca* is sister to the rest of this clade based on three apomorphies: maxilliped article 3 with 3 recurved robust setae (Ch 22.2); pleonite 5 posterior margin irregular (Ch 37. 5); and uropod rami extending beyond pleotelson apex (Ch 68.3). Apomorphic characters that differentiate *Paracymothoa* within clade 5 are: pereon lateral margins weakly ovate (Ch 1.2);

pleonite 5 posterior margin convex (Ch 37.2); pleotelson lateral margins convex (Ch 40.3) and uropod peduncle similar to exopod length (Ch 67.2). Clade 7 (*Creniola*+*Renocila*) is nested within clade 5 and is upheld by the following apomorphic states: increase in size from pereonites 5–6 posterolateral margin (Ch 28.3); and uropod exopod longer than endopod (Ch 69.3). Clade 7 is also supported by: pereonite 7 posterior margin deeply concave (Ch 30.3) which is found in clades 18, 25 and 29; pereopods 5–7 anterior superior margin basis with large blade-like carina (Ch 51.3) which is found in clade 22; and pereopod 7 with robust setae (Ch 55.2), similarly found in clade 2, *Nerocila* and *Joryma*.

Clade 9 comprise two sister taxa, *Norileca* and *Smenispa*. This clade is defined by the following apomorphic states: the maxilliped without oostegite lobe (Ch 21.3) and coxae 5–7 are narrowly rounded (Ch 46.2). Apomorphic characters for *Smenispa* are eyes size partially visible (Ch 12.3); mandible palp articles 2 and 3 subequal (Ch 16.2); pereonite 1 anterolateral margin rounded and appearing to encompass cephalon (Ch 24.3) and pleopod 1 exopod mesial margin weakly to strongly oblique (Ch 65.5). *Norileca* is recognised by the frontal margin ventrally directed and not folded posteriorly (Ch 3.2); mandible palp article 3 setae absent (Ch 15.2); pereonites 5–6 posterolateral margin size increase (Ch 28.3) and brood pouch arising from coxae 2–5 (Ch 47.2).

Clade 11 consists of *Riggia*, *Telotha*, *Braga*, *Anphira*, and *Asotana*+*Artystone*. The six South American freshwater genera (with the exclusion of *Paracymothoa*) are upheld by the mandible molar process and incisor fused (Ch 17.3); maxillule with 5 or more terminal spines (Ch 20.1) and maxilliped article 3 with 2 recurved robust setae (Ch 22.1). *Riggia* is sister to the rest of the genera in this clade and is supported by body pereon widest at pereonite 3 (Ch 2.4); frontal margin (dorsal anterior margin) truncate (Ch 5.2); pleopod 1 exopod lateral margin weakly convex (Ch 63.1) and uropod rami extending to pleotelson apex (Ch 68.1). *Riggia* is also defined by the fused pleonites 1–5 (Ch 32.2) with one homoplasious occurrence in *Ourozeuktes*; and coxae 2 and 3 are larger than other coxae (Ch 41.3) this character is also found in *Joryma*. *Telotha* differs from the other genera by the pereopods 5–7 basis anterior superior margin with carina (Ch 51.2); pleopod 1 mesial margin weakly convex (Ch 64.1); pleopod 1 exopod distally subtruncate (Ch 65.4); uropod rami to pleotelson extending beyond pleotelson apex (Ch 68.3) and the autapomorphic character of pereonite 7 posterior margin straight (Ch 30.2). *Braga* is defined in this clade by pereonite 1 anterolateral margin acute (Ch 24.2); pleotelson dorsal surface with 2 submedian depressions (Ch 39.3); coxae 5–7 ventrally directed (Ch 43.1); pereopod 7 2.0 or more times longer than pereopod 1 (Ch 56.3); pleopods dorsally visible (Ch 57.2); pleopod 1 mesial margin strongly convex (Ch 64.3), and the autapomorphic state of pleopods 1–5 with multiple lamellar (Ch 59.2).

Clade 14 is nested within clade 11, with *Anphira* sister to the *Asotana*+*Artystone* group (clade 15). *Anphira* is separate from clade 11 and 15 by cephalon 3/4 immersed in pereonite 1 from dorsal view (Ch 7.3); antennula longer than antenna (Ch 9.3); pleonite 5 posterior margin convex (Ch 37.2); and coxae 5–7 are visible from dorsal view (Ch 44.1). The *Asotana*+*Artystone* are supported by the smooth and straight posterolateral margins on pereonites 1–4 (Ch 25.1), the robust setae on the inferior distal margins of pereopod 6 and 7 (Ch 53.1, Ch 54.1) and the uropod peduncle longer than exopod length (Ch 67.3).

Clade 16 includes all marine gill and buccal-attaching genera, which are supported by homoplasious states (Ch 15.2 and Ch 31.3). Clade 17 is sister to the gill+buccal clade, which is further split into clades 18 (*Ourozeuktes*+*Ryukyua*) and 19 (*Cinusa*+*Kuna*). Clade 18 is upheld by six apomorphic states: cephalon without rostrum (Ch 6.2); pereonite 7 posterior margin deeply concave (Ch 30.3); coxae 2–4 longer than respective pereonites from the lateral view (Ch 42.3); pereopod 7 2.0 or more times longer than pereopod 1 (Ch 56.3); pleopod 1 exopod lateral margin straight and distally subtruncate (Ch 63.2, Ch 65.4). Clade 19 is strongly supported by the frontal margin ventrally directed (Ch 3.2); pleotelson lateral margins convex (Ch 40.3) and coxae 5-7 shape narrowly rounded (Ch 46.2).

Clade 22 contains only three buccal-attaching genera: *Ceratothoa*, *Cymothoa* and *Glossobius*. This clade is upheld by the pereon lateral margins weakly ovate (Ch 1.2) and maxilla with more than 2 setae each on both medial and lateral lobe (Ch 19.2). *Cymothoa* is sister to the *Ceratothoa*+*Glossobius* group (clade 23) and is defined by the frontal margin partially projecting between antennula bases (Ch 4.2, ventral view) and anterior margin truncate from dorsal view (Ch 5.2); cephalon without rostrum (Ch 6.2); mandible molar and incisor partially fused (Ch 17.2); coxae 5–7 posteriorly narrowly rounded (Ch 46.2); pleopods peduncle with lobes on lateral margin (Ch 61.2); pleopod 1 exopod mesial margin weakly to strongly oblique (Ch 65.5) and pleopods 3–5 endopod with large fleshy folds (Ch 66.3). *Ceratothoa*+*Glossobius* are upheld by the synapomorphic state of antennula bases in contact (Ch 8.4). Other supporting characters for clade 23 are maxilliped article 3 with 2 recurved robust setae (Ch 22.1) and brood pouch from arising from coxae 1–4 and 6 (Ch 47.1).

Clade 24 is further split into clades 25 and 26. Clade 25 comprises two genera: *Catoessa* (which comprise of gill and buccal-attaching species) and *Ichthyoxenus*, which includes flesh burrowers and gill+buccal attaching species. The *Catoessa*+ *Ichthyoxenus* are supported by the apomorphic character states maxilliped article 3 with 3 recurved robust setae (Ch 22.2); pleonite 1 as wide as other pleonites (Ch 35.1); pleonite 5 posterior margin bisinuate (Ch 37.3).

Clade 26 comprise of four gill-attaching taxa (*Agarna*, *Cterissa*, *Mothocya*, *Joryma*) and the single buccal-attaching *Lobothorax*, which is sister to the rest of gill-attaching genera. *Lobothorax* is separated from the rest if the gill-attaching clade by the antennula bases being narrowly separated (Ch 8.1); mandible palp articles 2 and 3 subequal (Ch 16.2); mandible molar and incisor fused (Ch 17.3); pleotelson lateral margins concave (Ch 40.2); all pleopods endopod lobes with proximomedial lamellar lobe (Ch 58. 2); and pleopods 3–5 endopod pockets with small pleats or pockets (Ch 66.2). *Agarna*+*Cterissa* (clade 29) is nested within the gill-attaching clade and is upheld by the mandible molar process and incisor partially fused (Ch 17.2); pereonites 5-6 posterolateral margins convex (Ch 27.3); pereonite 7 posterior margin deeply concave (Ch 30.3) and uropod endopod apex broadly rounded (Ch 71.2). *Mothocya* is sister to the *Agarna*+*Cterissa* group and separated by the large eyes (more than 40% width of cephalon) (Ch 12.1); *coxae 2 and 3* smaller than other coxae (Ch 41.2); pereopod 7 with small setae (Ch 55.1); pleopods peduncle with lobes present on lateral margins (Ch 61.2) and uropod rami extending beyond pleotelson apex (Ch 68.3).

7.3.2 Molecular results

The tree topologies for 16S rRNA (Fig. 7.5) and CO1 (Fig. 7.7) were inferred using the Maximum Parsimony (MP) method. Tree #1 out of 2 most parsimonious trees with length = 235 is shown in (Fig. 7.5) for 16S rRNA whereas the most parsimonious tree with length = 955 is shown in (Fig. 7.7) for CO1. The tree topology for 16S rRNA showed the consistency index is 0.600000, the retention index is 0.852327, and the composite index is 0.518650 (0.511396) for all sites and parsimony-informative sites (in parentheses). For CO1 (Fig. 7.7), the consistency index is 0.405435, the retention index is 0.534468, and the composite index is 0.228338 (0.216692) for all sites and parsimony-informative sites (in parentheses). The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm (Nei & Kumar 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The analysis involved 27 nucleotide sequences for 16S rRNA and 22 nucleotide sequences for CO1. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 128 positions for 16S rRNA and 412 positions for CO1 in the final dataset. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985).

The tree topologies for 16S rRNA (Fig. 7.6) and CO1 (Fig. 7.8) were inferred by using the Maximum Likelihood (ML) method based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (-4224.3004) and (-6072.3923) is shown in for 16S rRNA (Fig. 7.6) and CO1 (Fig. 7.8) respectively. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+*G*, parameter = 1.2677 for 16S rRNA and 0.7387 for CO1)]. The analysis involved 27 nucleotide sequences for 16S rRNA and 25 nucleotide sequences for CO1. There were a total of 540 positions for 16S rRNA and 624 positions for CO1 in the final dataset.

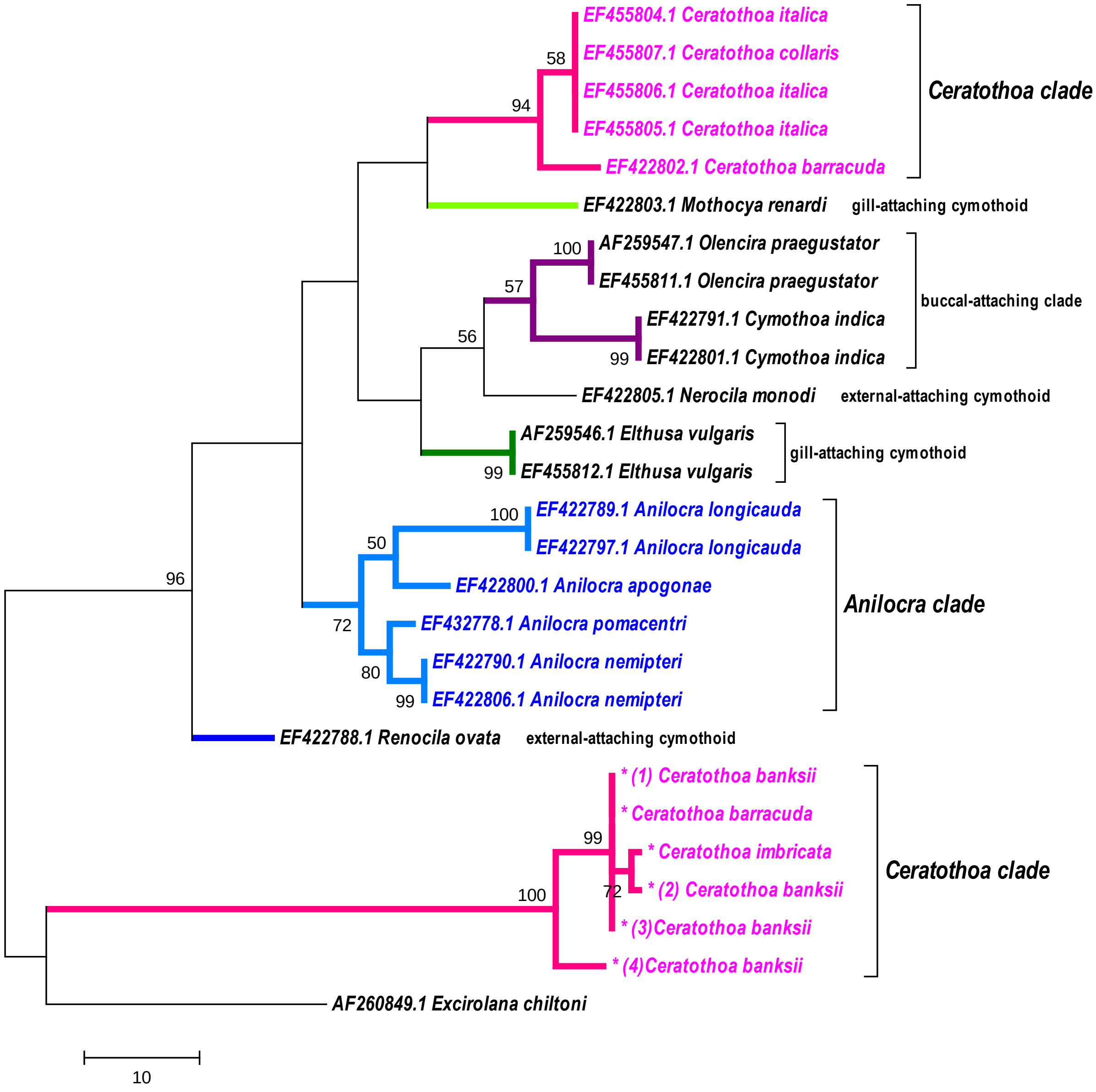

Figure 7.5 Maximum parsimony analysis of Cymothoidae taxa based on 16S rRNA sequences. Posterior probabilities <50% are not shown. (*) refers to sequences obtained from this research. Pink text indicates species of the genus *Ceratothoa*, blue text indicates species of the genus *Anilocra*, and coloured branches indicate different cymothoids and the relationships based on site attachment.

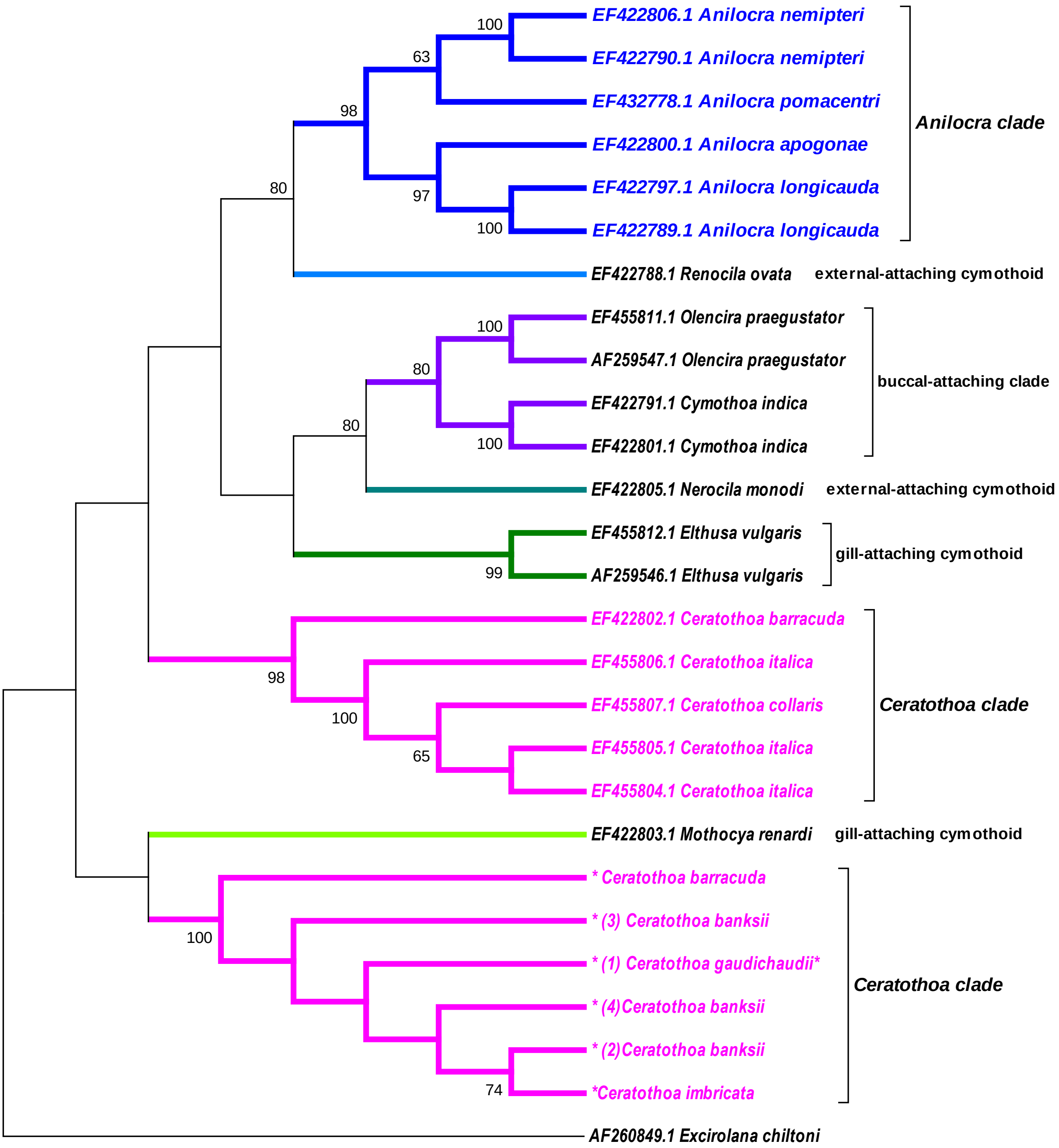

Figure 7.6 Maximum likelihood analysis of Cymothoidae taxa based on 16S rRNA sequences. Posterior probabilities <50% are not shown. (*) refers to sequences obtained from this research. Pink text indicates species of the genus *Ceratothoa*, blue text indicates species of the genus *Anilocra*, and coloured branches indicate different cymothoids and the relationships based on site attachment.

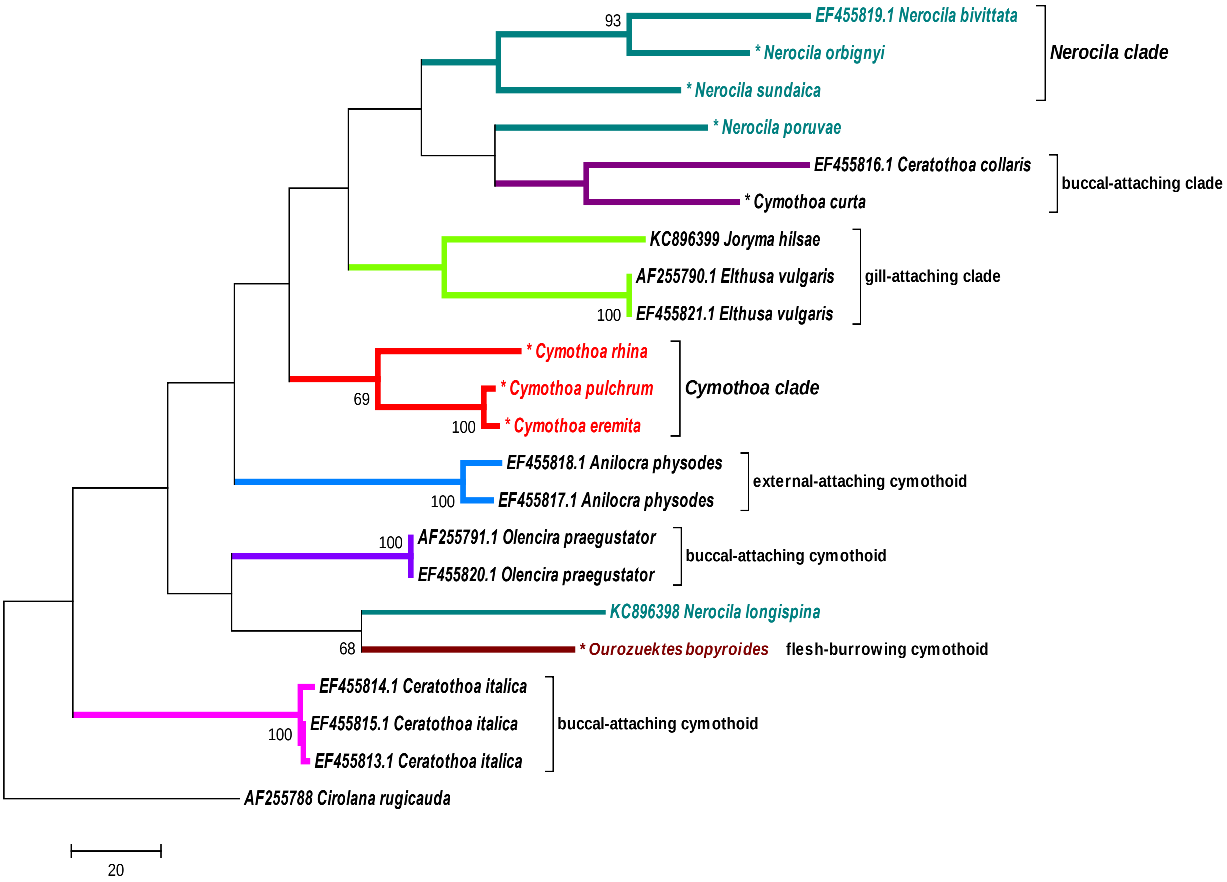

Figure 7.7 Maximum parsimony analysis of Cymothoidae taxa based on CO1 sequences. Posterior probabilities <50% are not shown. (*) refers to sequences obtained from this research. Red text indicates species of the genus *Cymothoa*, cyan text indicates species of the genus *Nerocila*, and coloured branches indicate different cymothoids and the relationships based on site attachment.

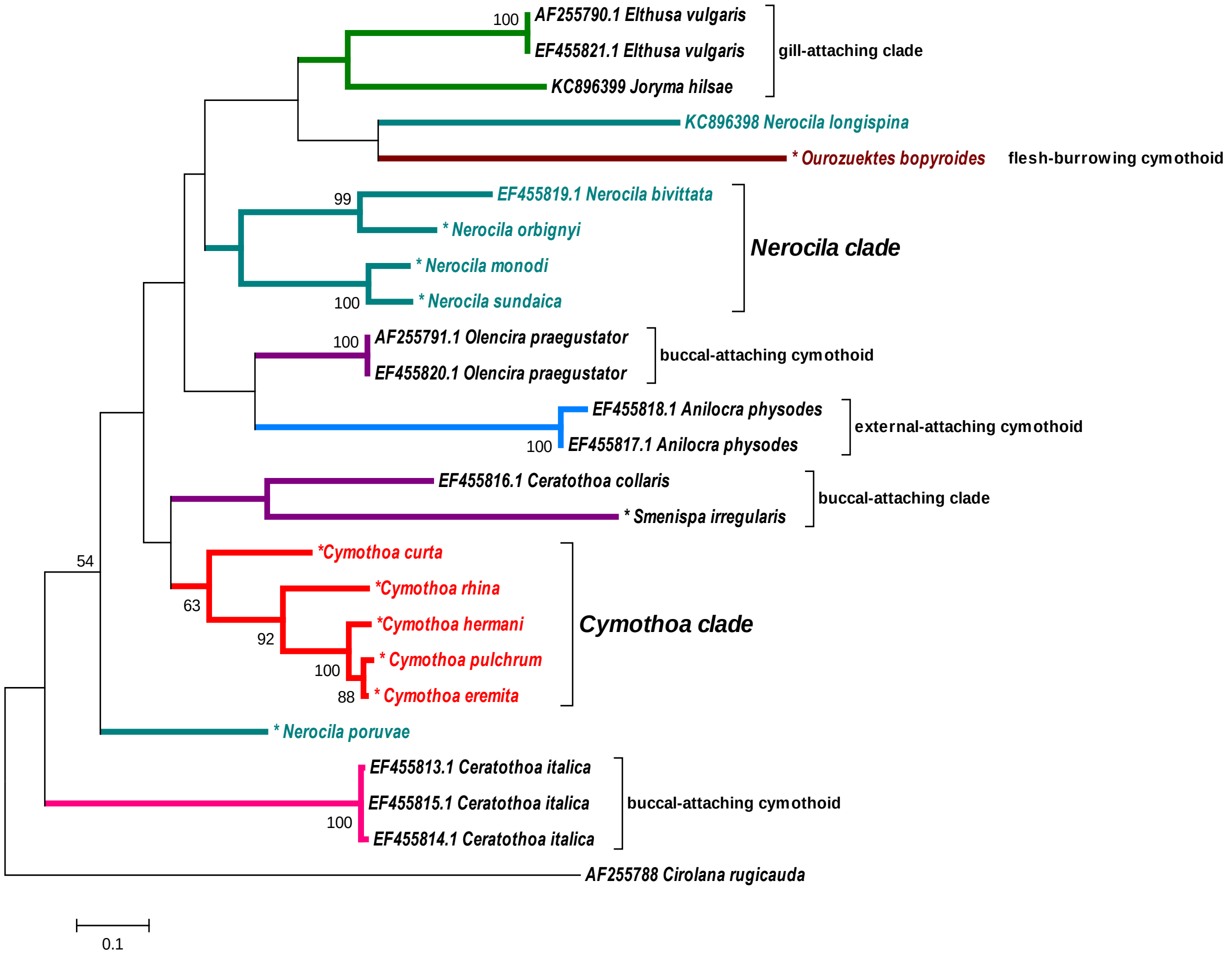

Figure 7.8 Maximum likelihood analysis of Cymothoidae taxa based on CO1 sequences. Posterior probabilities <50% are not shown. (*) refers to sequences obtained from this research. Red text indicates species of the genus *Cymothoa*, cyan text indicates species of the genus *Nerocila*, and coloured branches indicate different cymothoids and the relationships based on site attachment.

7.4 DISCUSSION

It is still not possible to identify a potential ancestor for the Cymothoidae. Smit *et al*. (2014) mentioned the ancestor should likely be the most related to either the Corallanidae or Aegidae. It was suggested that *Rocinela* is similar to the Cymothoidae on the basis of the mouthpart morphology (Dreyer & Wägele 2001) and the dorsally flattened body is similar to the gill-attaching cymothoids (Smit *et al.* 2014). Other studies recognised cirolanids to be a more distant ancestor to the cymothoids (Dreyer & Wägele 2001; Brandt & Poore 2003) and that family has been used as the outgroup for molecular studies (Jones *et al.* 2008; Ketmaier *et al.* 2008).

According to the present morphological analysis, *Anilocra* and *Pleopodias* are basal in both the first (unweighted) and second (reweighted) morphological analysis. Compared to the other genera in the study, *Anilocra* and *Pleopodias* share more than 40 plesiomorphic characteristics with *Rocinela*, pertaining to characters such as cephalon rostrum shape (Ch 6–8); antennula bases (Ch 9); antenna extension to pereonite 1 ratio (Ch 11); mouthparts shape (Ch 12–18); pleon shape (Ch 32–34); pleotelson shape (Ch 38–40); coxae size (Ch 41–42); pereopods basis shape and setae (Ch 52, 54); pleopods peduncle lobes and ventral view (Ch 61–66) and uropod shapes (Ch 67–71). These results differ from Hadfield’s (2012) first analysis (Fig. 7.4; Hadfield 2012) which placed *Anphira* (gill-attaching) as the most basal genus whereas in the second analysis without pleopod characteristics (Fig.7.5) the buccal-attaching genus *Braga* as sister to the rest of the family. Current molecular results for both 16S and CO1 differ from the morphological analyses, with *Ceratothoa* basal to the rest of the family in both MP and ML analyses (see Figs. 7.5 to 7.8).

Brusca (1981) hypothesized that the externally attaching cymothoids are more primitive than the derived buccal and gill-dwellers. Results from this study (Fig. 7.4 to 7.8) show that site attachment is weakly linked to the major clades. No flesh-burrowing clade was upheld despite the incorporation of two other flesh burrowing genera (*Ourozeuktes*, *Artystone*) in addition to the other previously included flesh burrowers in Hadfield’s (2012) morphological analysis (*Riggia*, *Ichthyoxenus*). Interestingly, *Ourozeuktes* was always sister to the gill-attaching *Ryukyua* in the morphological phylogeny but sister to *Nerocila longispina* in both MP and ML analysis based on CO1 sequences. In another example, the South American freshwater clade (with the exception of *Paracymothoa*) consisted of gill+buccal attaching and flesh-burrowing cymothoids (Fig. 7.4). The monophyly of the South American clade is significant, suggesting that a common ancestor entered South American freshwaters and which radiated independently to all other cymothoids, into gill and buccal attaching forms. These results are evidence that the evolution of gill and buccal-attachment has occurred repeatedly within the family. As for the major buccal+gill attaching group of the morphological analysis, only *Ceratothoa*+*Glossobius* and *Cymothoa* formed a consistent buccal-clade. Interestingly though, *Ceratothoa* and *Cymothoa* were not monophyletic in the molecular phylogeny for both 16S and CO1, though results from 16S sequences formed fairly congruent generic clades compared to molecular clades from CO1 sequences. Therefore, Brusca's (1981) 'linear lineage' of primitive external attaching cymothoids to derived buccal+gill attaching cymothoids is not supported.

Mapping site-attachment against the trees (Fig 7.4) it is evident that there has been several independent developments of buccal-attachment as those genera occurs in all three major clades. In Fig. 7.4, Clade 16 contains most of the gill and buccal-attaching genera, and further divides in clades 24 and 22 that are primarily gill and buccal-attaching respectively. Not all buccal-attaching genera attach in the same manner. The genera *Ceratothoa*, *Cymothoa* and *Glossobius* are closely related to form a clade, but others such as *Lobothorax*, *Cinusa* and *Catoessa* clearly do not share a common ancestor. Further, there are genera that retain the plesiomorphic characters of the externally-attaching taxa are gill-attaching (*Norileca*) and buccal-attaching (*Smenispa*).

Hadfield (2012) initially showed that the South American genera were weakly upheld in the first analysis (Fig. 7.4) but was better resolved in the second analysis (Fig. 7.5) and grouped as a sister clade to the *Ceratothoa* clade upon the removal of the pleopod characteristics. In the present analysis, the South American cymothoids formed a strong clade supported by the mandible molar process and incisor fused (Ch 17.3); maxillule with 5 or more terminal spines (Ch 20.1) and maxilliped article 3 with 2 recurved robust setae (Ch 22.1). Thatcher (1997) proposed establishing the subfamily Artystoninae Thatcher, 1997 to contain all the freshwater cymothoids from

South America, mainly based on their mouthparts (of which current results supports this view). Brusca (1981) suggested that the South American fauna were the result of interrelated series of various biogeographic events thought to be the subsequent separation of South America from Africa in the Late Cretaceous to early Paleocene era. It would be worth further investigating (on both molecular and morphological level) if South American cymothoids (both freshwater and marine) are monophyletic and its true relationship with the rest of the Cymothoidae (basal or terminal). It would be interesting to include other representatives from this group (e.g. *Isonebula*, *Vanamea*, *Philostomella*) for added clarification on this grouping.

Bruce (1990) suggested that the "Anilocrinae" included the external attaching *Anilocra*, *Nerocila*, *Renocila*, *Creniola* and *Pleopodias*; the buccal-attaching *Smenispa* and the gill-attaching *Livoneca* and *Norileca* were also included on the basis of similar brood pouch structure, oostegite 6 and posterior pocket morphology and pleopod characteristics. Despite the similar coding for the morphology of the characters mentioned above, no supporting apomorphies are apparent for the monophyly of the external attaching genera. Firstly, the morphological study showed *Anilocra* and *Pleopodias* as sister to *Nerocila* and to clade 5 (which includes *Renocila*) but formed an *Anilocra*+*Renocila* assemblage in 16S. On the other hand, the molecular phylogeny of both 16S and CO1 excludes *Nerocila* with *Anilocra* in both MP and ML, similar to Jones's *et al*. (2008) and Ketmaier's *et al.* (2008) research. Despite *Nerocila* appearing morphologically similar to *Anilocra* and *Renocila*, both Jones *et al*. (2008) and Ketmaier *et al.* (2008) suggested the most plausible explanation for this is due to convergent evolution driven by selection pressures common to living on a fish's exterior.

Similar to the Anilocrinae, the buccal+gill clade from the morphological phylogenetic tree did not conform to the molecular study. It is important to note that there are more genera representing the terminal buccal+gill clade in the morphological study whereas more species are present within fewer genera in the molecular study. Thus, due to the lack of full generic and species level representation, this may fall short of resolving generic relationships within the family. For the molecular study of 16S, three consistent buccal+gill clades were upheld, which were 1) *Elthusa vulgaris* always sister to the *Olencira praegustator*+*Cymothoa indica* clade and two separate

Ceratothoa clades. The CO1 analysis yielded poor support for the buccal-clade or gill clade, although it is observed that *Cymothoa* in both MP and ML formed a clade. Though it is still not clear why such inconsistencies occur for all analyses, this may likely reflect the complex nature of the habit and the high morphological variation that exists within genus as well as the inter- and intra-species level variability. Such is evident in the genus *Ceratothoa*, and Ketmaier *et al.* (2008) demonstrated the difficulties in recovering *Ceratothoa collaris* and *Ceratothoa italica* in a single clade. For example, the body of *Ceratothoa collaris* is morphologically more heavily built than other species of *Ceratothoa* (Ketmaier *et al.* 2008) and was divided into three forms by Monod (1924a, b) based on this variability. It is also interesting to note that despite *Ceratothoa banksii* occurring within the eastern Australian fauna, genetic heterogeneity exist within the species. This may be a result of different host usage, as explained by Jones *et al.* (2008) for *Anilocra apogonae*.

Many authors agree that cymothoids are taxonomically difficult to resolve due to the nature of their habit and ecology and are highly variable or polymorphic (Brusca 1981; Horton 2000; Ketmaier *et al.* 2008). Bruce (1990) suggested caution to be taken because cymothoid taxonomic arrangements (though in some ways are based on the position on the host) were likely to reflect convergence due to similar life-styles rather than true phylogenetic affinities. It is interesting to note that 16S sequences data are able to better resolve species relationship at the genus level (e.g. *Anilocra* and *Ceratothoa*) compared to CO1 (e.g. *Nerocila* and *Cymothoa*). These results suggest complex history based on the parasitic diversification strategies, host-specificity, pathogenicity and life history (Brusca 1981; Ketmaier *et al*. 2008).

7.4.1 Limitations

Cymothoid sampling can be particularly challenging since the ecology of most cymothoids are poorly known (Ketmaier *et al*. 2008). Smit *et al.* (2014) stated that it is not possible to obtain a broad range of cymothoid species within a single catch or location. The combination of fish trawls, fish sampling (opportunistic, research or commercial) and examining by-catch may provide material for morphological systematics and also provide the opportunity for molecular analysis if immediately frozen and then preserved in molecular grade ethanol or other fixatives (e.g. RNAlater) and kept in conducive temperature (-80 to -20˚C).

Museums and certain research institutes hold important archival material with rare species from difficult to access locations. Recent studies by Martin *et al.* (2013, 2014a, b 2015 a, b, submitted) on buccal-attaching cymothoids have increased current knowledge of host-specificity and geographical distribution of most species using museum collections. However, due to the morphological similarity of many species, more molecular data will be essential to resolve the identity and the monophyly of some of the genera. The drawback to working with archival material is the difficulty to extract high quality DNA and very few accessible cymothoid sequences from the Genbank website (http://www.ncbi.nlm.nih.gov/nuccore/?term=cymothoid). In this research, out of 13 species from my collection, only three species could be sequenced for 16S and 10 species for CO1. A BLAST search for both 16S rRNA and cytochrome oxidase I sequences confirmed that there was no host contamination. Thus phylogenetic comparison between CO1 and 16S are biased since not all species or genera are fully represented in both topologies. To understand the evolution of gene family, it is essential to sample as widely as possible between different genera and species within genus.

7.5 CONCLUSION

Present evidence (both molecular and morphological phylogeny) shows that the Cymothoidae is monophyletic. In the morphological analysis there are two major clades: the South American freshwater genera and the terminal gill+buccal clade. The external-attaching ‘Anilocrinae’ is not monophyletic in both 16S molecular (*Nerocila* forms a separate clade *Anilocra* and *Renocila*) and morphological phylogeny (Clades 2, 5, 9).

Brusca’s (1981) hypothesis that 1) cymothoid evolution is directly linked to site attachment and 2) linear evolutionary pathway starts with the basal external-attaching forms (Anilocrinae) and ends up with gill/mouth dwellers (Livonecinae and Cymothoinae) is partially supported here partially supported. The morphological analysis in this study (Fig. 7.4) agrees with Brusca’s (1981) hypothesis where most of the external attaching genera are sister to the rest of gill/mouth dwellers, but is independent of site attachment. The morphological analyses clearly show the multiple evolutions of gill and buccal-attaching taxa in several clades. Molecular phylogeny of 16S and CO1 from this research proves otherwise, where all analyses show the buccal-attaching *Ceratothoa* is sister to the rest of the cymothoids.

In light of new morphological and molecular data for the cymothoid genera, it is still early to comprehensively present a new classification for the family or retain the subfamily names and tribe previously proposed for the family (see Schioedte & Meinert 1881, 1883, 1884; Bruce 1987c; Trilles 1994; Thatcher 1997). This is clearly showed in the “Anilocrinae” that does not solely include all external attaching cymothoid in both molecular and morphological analyses. The molecular dataset still requires more species from different genera, to better represent species and generic cladding within the family for 16S and CO1, which would then enable robust comparison between the two genes. At this stage, 16S is able to form species clades for *Anilocra* and *Ceratothoa* whereas CO1 forms species clades for *Cymothoa* and *Nerocila*. Future work to resolve the relationships could be elucidated by exploring complete mtDNA genomes from well preserved specimens suitable for molecular analysis and using next generation sequencing approaches that has been utilised for other parasitic taxa (Jex *et al.* 2010; Besnard *et al.* 2014; Bonnet *et al.* 2014). The morphological dataset should include species-level phylogeny since it is known that certain species site-attachment is uncommon with other species within the same genus. For example, *Mothocya lineata* (see Chapter 3 part II) and *Elthusa vulgaris* are the only know buccal-attaching species within the gill-attaching genera. This is important as it would further support the hypothesis of multiple parasitic evolution within the family.

Brusca (1981) hypothesized that host-specificity is expected to be higher in the more derived gill and buccal genera. Based on current taxonomic review of the buccal-attaching cymothoids (see Chapters 3 to 6), it is apparent that some cymothoids show low host specificity (e.g. *Ceratothoa banksii*, *Cymothoa indica*, *Cymothoa eremita*, *Smenispa irregularis*). As for the external attaching cymothoids, Bruce (1987a, b, c) demonstrates how some *Anilocra*, *Renocila* and *Creniola* sp. have narrow host use that is more linked to latitude and associated sea temperature, with high-latitude cold-water cymothoids using a wide array of hosts and low-latitude warm-water cymothoids using a narrow range of hosts.

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CHAPTER 8

Summary

8.1 THE TAXONOMY OF BUCCAL-ATTACHING CYMOTHOIDS FROM AUSTRALIA

The initial objective of this thesis in an aquaculture perspective was to identify if the species *Ceratothoa imbricata* was conspecific on two hosts, (both important commercial fish species) in Tasmania: *Salmo salar* and *Latris lineata*. Within the first year, it became apparent that it was impossible to resolve the conspecificity of *C. imbricata* because of the lack of identity resolution in relation to two other species: *Ceratothoa banksii* and *Ceratothoa trigonocephala*. The reasons for this taxonomic dilemma, and common for most species within the Cymothoidae are: species polymorphism; inadequate available material; lack of researchers for taxonomic studies; and/or lack of comprehensive literature with type material, species descriptions and diagnosis, and detailed figures or illustrations (see Bruce 1986, 1987a, b, c, 1990; Brusca 1981; Trilles 1994; Hadfield *et al.* 2010).

Bruce (1987b, 1990) and Bruce *et al.* (2002) also acknowledged the buccal-attaching genera (particularly the genera *Ceratothoa* and *Cymothoa*) remain unrevised at the time within Australia. The first complete review of the buccal-attaching genera in Australian waters was Hale's work (1926, 1924, 1929, 1940). Hale's (1926) keys to the Australian Cymothoidae included only two buccal-attaching genera (three *Cymothoa* species and a single *Ceratothoa* species) are now updated. Hale's works were later followed by Avdeev's (1975a, b, c, 1978a, b, 1979a, b) and partially Trilles's (1972b, 1975) research in the 1970s. The 1980s to 1990s saw a landmark in a number of new records and new species from Bruce's (1986, 1987a, b, c, 1990, 1991) research. Bruce's contribution resolved complex synonymies, descriptions, diagnosis and ecology for species and genera of the external and gill-attaching cymothoids, but to a lesser extent for the buccal-attaching genera (see Bruce & Bowman 1989; Bruce 1990). This thesis aimed to pull together all these previous literature, together with new examined material and to thorough review the buccal-attaching genera.

The marine buccal-attaching cymothoids are represented in Australia by six of the nine buccal-attaching genera: *Ceratothoa*, *Cymothoa*, *Glossobius*, *Smenispa*, *Catoessa* and *Tetragonocephalon*. The Zoological Catalogue of Australia listed six out of 31 *Ceratothoa* species, 13 out of 49 *Cymothoa* species, one out of nine *Glossobius* species, one out of two *Smenispa* species, one of four *Catoessa* species, and the single *Tetragonocephalon* species (Bruce *et al*. 2002), bringing a total of 23 species from Australia. The taxonomy of the first four genera has been thoroughly covered in this thesis (Chapters 3 to 6) whereas the latter two still requires attention (see section 8.4.1 for comments on future studies).

The availability of ample museum collections, borrowed type material, collections from the Australian Maritime College flagship training vessel MV Bluefin and donations from researchers of different institutions (see ‘acknowledgement’ in chapters 3 to 6) allowed for a complete review for the four mentioned buccal-attaching cymothoid genera. This was essential because the comparison of ample specimens from different locations and host-associations enabled 1) better resolution for species descriptions by noting implicit (not always clearly observed or may be insignificant, but still worthy of mention) and explicit (clearly observed and constantly present) characters, 2) decrease assumptions and confusions of inaccurate identifications by noting species which are polymorphic, 3) listing of multistate characters and identifying apomorphic (particularly synapomorphic) and plesiomorphic characters for cladistic analysis, 4) better understanding of cymothoid host-association and geographical distribution and 5) better understanding of commonly occurring cymothoids (particularly if sampling is done at random).

8.2 PHYLOGENETICS

The reappraisal of both morphological and molecular phylogeny of the Cymothoidae reveals that the family is monophyletic as found in previous studies (Bruce 1981; Trilles 1991; Roman & Dalens 1999).

The current phylogeny shows that Brusca’s (1981) linear evolutionary pathway (with external-attaching cymothoids sister to derived buccal and gill attaching genera) cannot be supported, demonstrating that convergent evolution site attachment occurred repeatedly within the Cymothoidae. This then implies that the subfamily classification of Anilocrinae, Ceratothoinae and Livonecinae should not be used since these taxa are not monophyletic in the current phylogeny. A similar scenario occurs for the parasitic isopods of the superfamily Bopyroidea and Cryptoniscoidea. The families Bopyridae, Entoniscidae and Dajidae were thought to be monophyletic based on the reproductive biology, life cycles, and the morphology of the males. Current phylogeny (both molecular and morphological) by Williams & Bokyo (2012) showed evolutionary pattern which differ from previous studies, and may be linked to multiple occurrences of host switching over time. It is even suggested that the family Bopyridae and superfamily Bopyroidea are non-monophyletic taxa (Williams & Bokyo 2012), which would later require revision of family level classification once the phylogeny is resolved.

The phylogenetic unrelated cymothoid linages based on site attachment from this study may be linked to the evolution of parasitism. Poulin & Randhawa (2015) mentioned that convergent evolution of parasites based on similar morphological characteristics may not be reflected genetically. This may be due to developmental constrains that limit the number of possible phenotypes (Orr 2005). Parasites would also have to face pressures associated with host- to host transmission, invasion of and survival of the host and sustainable exploitation of the host (Poulin & Randhawa 2015).

Aegids are most similar to cymothoids then there rest of the cymothooideans from the acute hook like dactylus of pereopods 1 to 3 and the smaller pars incisiva as compared to cirolanids (Dreyer & Wägele 2001). Brandt & Poore's (2003) morphological phylogeny also upholds the taxon Aegidae, Cymothoidae and the former Epicaridea from the possession of maxillipedal palp with terminal articles set obliquely and with hooks, and a strongly curved pereopod 1. Apart from the similar morphological body shape between aegids and the external attaching cymothoids, the mode of life is fairly similar. Aegids are micropredators, with temporary attachment on the fish host. Cymothoid mancae are also temporary in attachment, but the adult cymothoids later become obligate parasites to its host. Dreyer & Wägele (2001) assumed that the derived Cymothoidae+Bopyridae share a common ancestor that was parasitic on fishes and later developed modified mouthparts to suck the hemolymph of crustaceans to give rise to the current Bopyridae.

Although information on current molecular data is insufficient to resolve the phylogeny of the Cymothoidae, there is some useful information that can be derived from the present 16S rRNA and CO1 sequences alignment. To some degree, sequences of both mitochondrial genes are able to form congruent clades on a species level (*Anilocra* and *Ceratothoa* clades for 16S rRNA and *Nerocila* and *Cymothoa* clades for CO1). Like the morphological phylogeny, the external attaching "Anilocrinae" cannot be upheld since *Nerocila* is not monophyletic with *Anilocra* in CO1 phylogeny, whereas *Renocila* is sister to *Anilocra* in the 16S rRNA phylogeny. Jones *et al*. (2008) assumed that the external attaching cymothoids would have evolved from the gill and buccal-attaching ancestors multiple times due to similar selection pressure of living externally. Conclusive results can only be drawn once more sequences from different species and genera are available and can be readily compared with a more robust morphological phylogenetic tress.

8.3 GENERAL REMARKS

8.3.1 Host-cymothoid relationship

The 25 buccal-attaching species from Australian waters have been recorded (from museum specimens and previous literature) from 79 host species from 37 families (Table 8.1). A host-isopod association is here reported from 11 host orders: Perciformes (44 species, 19 families); Beloniformes (11 species, three families); Tetraodontiformes (seven species, two families); Mugiliformes (three species, one family); Syngnathiformes (three species, one family); Aulopiformes (two species, two families); Scorpaeniformes (two species, two families); Pleuronectiformes (two species, one family);Salmoniformes (one species, one family) and Clupeiformes (one species, one family).

The highest number of isopod species recorded within the order Perciformes mainly belonged to the families Carangidae (13 species), Sparidae (7 species), Lutjanidae (4 species) and Sphyraenidae (3 species), whereas the remaining families either have a single or two reported isopod species. *Pseudocaranx dentex* has the highest number of reports within the Carangidae, acting as host for three *Ceratothoa* species (*Ceratothoa banksii*, *Ceratothoa carinata* and *Ceratothoa usacarangis*). Cymothoid association with the sparids are here reported from the genera *Dentex*, *Boops*, *Spicara*, *Pagellus* and *Acanthopagrus*, and are host for the cymothoids *Ceratothoa oxyrrhynchaena*, *Cymothoa indica* and *Smenispa irregularis*. The family Lutjanidae are host to the species *Ceratothoa carinata*, *Cymothoa epimerica*, *Cymothoa eremita* and *Tetragonocephalon lutianus*. All records from the family Sphyraenidae are from the genus *Sphyraena* and are host to *Ceratothoa barracuda*, *Cymothoa indica* and *Cymothoa eremita*.

Most of all buccal-attaching cymothoids from this study show high but varying degree of host specificity. On a generic level, *Ceratothoa barracuda* is strongly associated with the host genus *Sphyraena*, *Ceratothoa guttata* with *Parexocoetus*, *Ceratothoa imbricata* with *Trachurus*, *Ceratothoa retusa* with *Hemiramphus* and *Catoessa ambassae* with *Ambassis jacksoniensis*. On a family level, *Ceratothoa carinata* are mainly found on Carangidae (with a single species from Lutjanidae); *Ceratothoa oxyrrhynchaena* are mostly from Sparidae (and a single species reported from Acropomatidae); *Cymothoa bychowskyi* from Fistulariidae; *Cymothoa parupenei* from Mullidae; *Glossobius impressus* from Exocoetidae and *Glossobius anctus* from Hemiramphidae. As for the host order, *Cymothoa carangi* and *Cymothoa epimerica* are associated fishes from the order Perciformes and *Cymothoa pulchrum* with Tetraodontiformes (families Diodontidae and Tetraodontidae).

Of the 25 buccal-attaching cymothoid species, five are here listed as having low host preference. *Ceratothoa banksii* is here listed from 12 host species (10 families in five host orders); *Cymothoa eremita* from 11 host species (10 families in five host orders); *Cymothoa indica* from 11 host species (nine families in five host orders), *Cymothoa vicina* from two host species (two families in two host orders) and *Smenispa irregularis* in three host species (three families in two host orders). Though the five mentioned cymothoids are morphologically variable, there are no explicit morphological characteristics that would otherwise differentiate the cymothoid species associated with the different host families. To date, there is no known host characteristic (morphology or behaviour) that influences cymothoid host preference (Smit *et al.* 2014).

Table 8.1 The buccal-attaching cymothoids from Australia and the known host-associations

Cymothoid	Order	Family	Host species
Ceratothoa banksii	Perciformes	Carangidae	*Pseudocaranx dentex*
			Gnathanodon speciosus
		Scatophagidae	*Selenotoca multifasicata*
			Scatophagus argus
		Kyphosidae	*Girella tricuspidata*
		Latridae	*Latris lineata*
		Pomatomidae	*Pomatomus saltatrix*
		Scombridae	*Gasterochisma melampus*
	Beloniformes	Hemiramphidae	‘Garfish’
	Mugiliformes	Mugilidae	*Paramugil georgii*
	Salmoniformes	Salmonidae	*Salmo salar*
	Scorpaeniformes	Platycephalidae	*Platycephalus* sp.
Ceratothoa barracuda	Perciformes	Sphyraenidae	*Sphyraena forsteri*
Ceratothoa carinata	Perciformes	Carangidae	*Selar crumenophthalmus*
			Decapterus muroadsi
			Pseudocaranx dentex
		Lutjanidae	*Lutjanus adetii*
Ceratothoa guttata	Beloniformes	Exocoetidae	*Parexocoetus brachypterus*
Ceratothoa imbricata	Perciformes	Carangidae	*Trachurus declivis*
			Trachurus novaezelandiae

Table 8.1 (continued)

Cymothoid	Order	Family	Host species
Ceratothoa oxyrrhynchaena	Perciformes	Sparidae	*Dentex spariformis*
			Boops boops
			Spicara maena
			Spicara smaris
		Acropomatidae	*Doederleinia berycoides*
Ceratothoa retusa	Beloniformes	Hemiramphidae	*Hemirhamphus far*
			Hemirhamphus robustus
Ceratothoa usacarangis	Perciformes	Carangidae	*Pseudocaranx dentex*
Cymothoa bychowskyi	Syngnathiformes	Fistulariidae	*Fistularia petimba*
			Fistularia villosa
			Fistularia commersonii
Cymothoa carangi	Perciformes	Carangidae	*Caranx* sp.
Cymothoa epimerica	Perciformes	Serranidae	*Epinephelus coioides*
		Lutjanidae	*Lutjanus malabaricus*
Cymothoa eremita	Perciformes	Stromateidae	*Pampusargenteus*
			Peprilusparu sp.
		Carangidae	*Parastromateus niger*
		Haemulidae	*Plectorhinchusgibbosus*
		Lutjanidae	*Pristipomoides multidens*
		Siganidae	*Siganus canaliculatus*
		Sphyraenidae	*Sphyraena obtusata*

Table 8.1 (continued)

Cymothoid	Order	Family	Host species
Cymothoa eremita (continued)	Aulopiformes	Aulopidae	*Himeformosana*
	Mugiliformes	Mugilidae	*Ellochelon vaigiensis*
	Pleuronectiformes	Psettodidae	*Psettodeserumei*
	Tetraodontiformes	Tetraodontidae	*Arothron leopardus*
Cymothoa frontalis	Scorpaeniformes	Cyclopteridae	*Cyclopterus* sp.
Cymothoa hermani	Perciformes	Scaridae	*Leptoscarus vaigiensis*
Cymothoa indica	Perciformes	Gobiidae	*Oxyurichthys macrolepis*
			Glossogobius giuris
		Sphyraenidae	*Sphyraena chrysotaenia*
			Sphyraena obtusata
		Latidae	*Lates calcarifer*
		Siganidae	*Siganus javus*
		Sparidae	*Pagellus erythrinus*
	Aulopiformes	Synodontidae	*Synodus myops*
	Beloniformes	Belonidae	*Strongylura strongylura*
	Clupeiformes	Clupeidae	*Nematolosa nasus*
	Siluriformes	Bagridae	*Mystus gulio*
Cymothoa parupenei	Perciformes	Mullidae	*Parupeneus spilurus*
			Upeneus sulphureus
Cymothoa propria	Perciformes	Carangidae	*Selaroides leptolepis*

Table 8.1 (continued)

Cymothoid	**Order**	**Family**	**Host species**
Cymothoa pulchrum	Tetraodontiformes	Diodontidae	*Diodon holocanthus*
			Diodon hystrix
			Diodon liturosus
			Chilomycterus reticulatus
		Tetraodontidae	*Arothron stellatus*
			Arothron meleagris
	Perciformes	Carangidae	*Caranx sp.*
Cymothoa rotunda	Siluriformes	Plotosidae	*Euristhmus lepturus*
Cymothoa vicina			*Cnidoglanis macrocephalus*
	Mugiliformes	Mugilidae	‘Mullet”
Glossobius impressus	Beloniformes	Exocoetidae	*Cheilopogonexsiliens*
			Cypselurus sp.
	Beloniformes	Exocoetidae	*Exocoetus* sp.
			Hirundichthysspeculiger
			Hirundichthysaffinis
Glossobius anctus	Beloniformes	Hemiramphidae	*Euleptorhampusviridis*
Smenispa irregularis	Perciformes	Carangidae	*Caranx carangus*
		Sparidae	*Acanthopagruslatus*
	Pleuronectiformes	Psettodidae	*Psettodeserumei*
Catoessa ambassae	Perciformes	Ambassidae	*Ambassis jacksoniensis*
Tetragonocephalon lutianus		Lutjanidae	*Lutjanus sebae*

It is increasingly evident that cymothoids generally show high host specificity, thus further studies are needed to understand the complexities of cymothoids with low host-specificity. Brusca (1978, 1981) suggested that some cymothoids may not choose its host based on taxonomic preference but are inclined to select host with ecological similarities (e.g. demersal, schooling, etc.). The species *Ceratothoa banksii* is here found to associate with benthopelagic schooling fishes (e.g. *Girella tricuspidata*, *Selenotoca multifasciata*), reef-associated (e.g. *Pseudocaranx dentex*, *Gnathanodon speciosus*), and pelagic (e.g. *Pomatomus saltrix*). The priority is to ensure that the species-level taxonomy is resolved before proceeding to study host-association as it impacts our understanding of host specificity. For example, *Ceratothoa imbricata* was previously considered to parasitize more than 10 host species, but taxonomic revision using more and new material together with re-identification of previous records resulted in two species being brought out of synonymy with a consequent narrowing of host use, with *C. imbricata* host specific to the genus *Trachurus*.

It is also essential to accurately record cymothoid site-attachment on the host during sample collection to prevent future confusion. Recent donations to MTQ (W34301–34308) recorded *Ceratothoa imbricata* from the gut of *Arripis trutta*. Further correspondence with the collector mentioned it is possible that *Ceratothoa imbricata* came from the original host *Trachurus* sp. as the preys were taken from the gut contents of *Arripis trutta* (which had the remains of *Trachurus* sp.). This study also highlights how the limited kinds of host data collected impacts our understanding of cymothoid diversity. It is evident that most host species collected are pelagic schooling fishes (see Table 8.1). There are currently few records of available for reef-associated fishes, and amateur and professional photographs of fishes with isopods may suggest a greater diversity of cymothoids (Smit *et al.* 2014), but animal ethics and mass fish collections poses its own challenges.

8.3.2 Diversity and distribution

The overall map records for *Ceratothoa* (Fig. 8.1) and *Cymothoa* (Fig 8.2) in Australian waters generally shows a higher species diversity within the tropical rather than temperate waters. For the lower latitudes (≤30˚S), eight *Ceratothoa* and 12 *Cymothoa* species are recorded from the tropical regions, as compared to three *Ceratothoa* and two *Cymothoa* species in the temperate region. The world map for the distribution of *Glossobius* species (Fig. 8.3) also indicates high species records and diversity are found in the tropical regions. Despite the poor records for certain species (e.g *Cymothoa rotunda*, *Ceratothoa usacarangis* etc.), evidence indicates that most marine cymothoids are not highly endemic to a particular region, as compared to the South American freshwater cymothoids. Brusca (1981) suggested that the relatively high diversity of cymothoids occurring in the tropics has a typical Tethyan distribution and the South American cymothoid fauna are a result of (direct quote) "correlated series of vicariant biogeographic events, presumably subsequent to the splitting of South America from Africa" (Brusca 1981).

Brusca (1981) suggested that cymothoid distribution may be strongly influenced by the nature of the host distribution. Some cymothoid species from this research demonstrated extensive geographical distributions (e.g. *Glossobius auritus*, *Glossobius impressus*). This likely holds true as some schooling fish are known for extensive migrations. For example, *Ceratothoa carinata* is associated with the carangids *Decapterus muroadsi*, *Pseudocaranx dentex* and *Selar crumenophthalmus*, all hosts known to have a global distribution (except for the polar regions). The distribution of *Ceratothoa carinata* matches that of its host distribution and is here recorded from the Western Indian Ocean and the western Pacific Ocean (see chapter 3). Similar cymothoid-host distributions are recorded in this research (e.g. *Ceratothoa oxyrrhynchaena*, *Ceratothoa imbricata*).

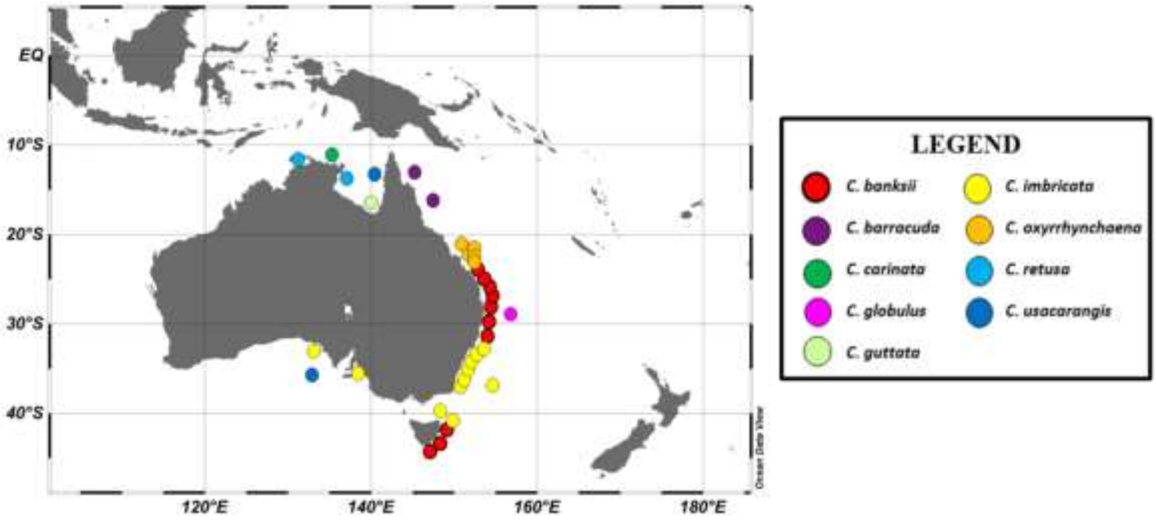

Figure 8.1 Map of the distribution of *Ceratothoa* species from Australia and adjacent Indo-Malayan region.

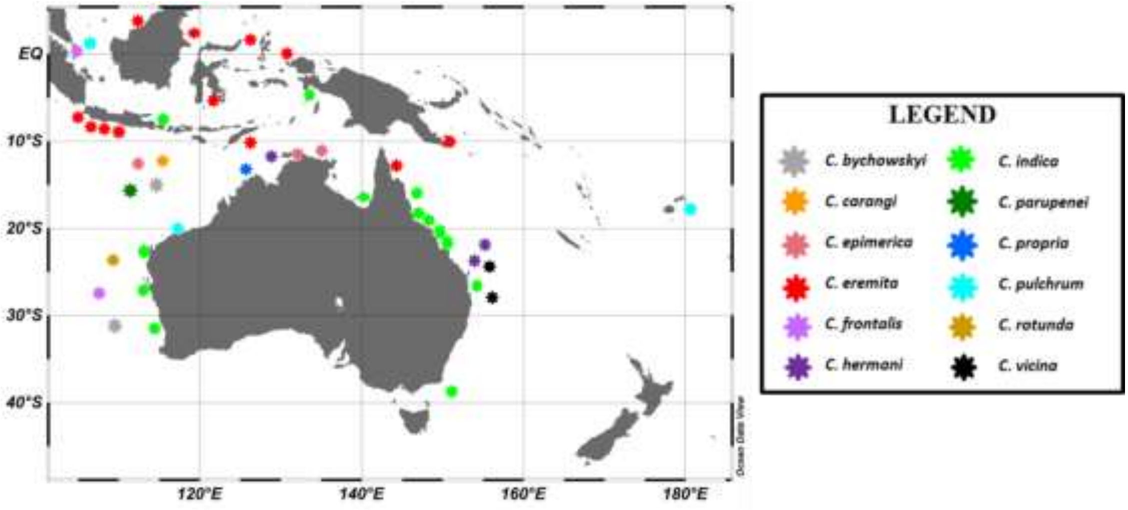

Figure 8.2 Map of the distribution of *Cymothoa* species from Australia and adjacent Indo-Malayan region.

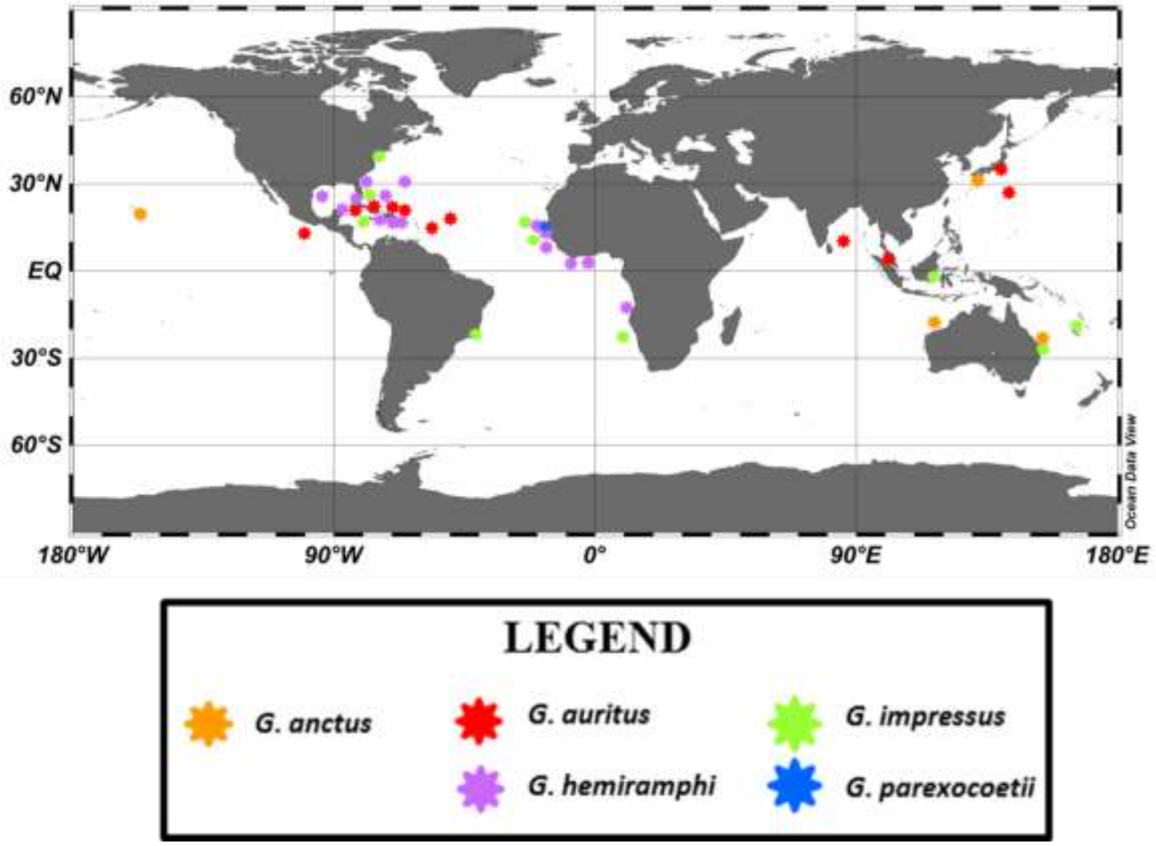

Figure 8.3 World map of the distribution of *Glossobius* species

The distribution of different *Glossobius* species (Fig. 8.3) is not the same as *Ceratothoa carinata* or *Ceratothoa oxyrrhynchaena*, since most *Glossobius* species are on pelagic hosts and therefore trans-oceanic currents can affect their distribution. *Glossobius impressus* has a multi-ocean distribution and its apparent absence for the Pacific may just be due to lack of collecting.

Some cymothoid species may show strong relationship between geographical distribution of the isopod and the number of hosts (Brusca 1981). The eastern Pacific cymothoids (Brusca's 1981; see Table 1) showed that the greater the geographical range of the cymothoids, the more host species it was likely to parasitize. However, this should be approached with caution, since my recent review of *Ceratothoa* (see Chapter 3 part II) showed that the East Pacific *Ceratothoa gaudichaudii* is probably more than one species. In another approach, Bruce (1987b) proposed that host specificity increases with decreasing latitude. For example, the temperate species of *Anilocra physodes* uses more host taxa than tropical *Anilocra* species (Bruce 1987b).

It is interesting to note that the number of new cymothoid records was higher than that of new species (4 new records vs 2 new species). One such record, *Ceratothoa oxyrrhynchaena*, has great geographical range, reported from the western Pacific Ocean to the north-eastern Atlantic and Mediterranean regions. One reason may be changes in environmental conditions (particularly global warming) resulting in the wide range expansion of certain species. Horton & Okamura (2002) reported a similar distribution for *Ceratothoa steindachneri,* a species uncommon in temperate waters prior to their study. The spread of *Ceratothoa steindachneri* into south-western British waters, despite the western end of the English Channel forming a boarder for warmer and Arctic/boreal species, is strongly suggestive due to climate change (Horton & Okamura 2002) and it is anticipated that similar cymothoid taxa in Australia could have disjunct distribution (two or more groups of a similar taxon that are extensively separated geographically).

8.4 FUTURE STUDIES

8.4.1 Taxonomic studies of the buccal-attaching cymothoids from Australia

Bruce (1990) has provided a provisional but in-depth diagnosis for *Catoessa* and species identification for *C. ambassae* can be done with a great degree of confidence. Although, few species occur in the genus *Catoessa*, it is important to provide a review for the genera which would include the following: 1) genus diagnosis, 2) description of all known species (and new available species), 3) key to the species of *Catoessa*, 4) description based on females and male specimens, 5) host preference, 6) known distribution, and 7) attachment site. Of the two recent records by Bowman & Tareen (1983) and Bruce (1990) of *Catoessa*, species of this genus are known mainly from host of the order Perciformes. The Australian species *Catoessa ambassae* is known only from a single host species (*Ambassis jacksoniensis* Macleay, 1881) and only known from New South Wales, whereas *Catoessa gruneri* Bowman & Tareen, 1983 is known from four different host species and found in the Persian Gulf. It is interesting to note that *Catoessa ambassae* is known to attach to the inner lateral surface of the buccal region (Bruce 1990) of its host whereas *Catoessa gruneri* attaches to the gills of its host (Bowman & Tareen 1983).

Tetragonocephalon lutianus Avdeev, 1975 is another species which requires further investigation. Avdeev (1975c) described the species based on a single female and male specimen from the Arafura Sea on the host *Lutjanus sebae* and the species has never been reported since then. While analysing explicit morphological characters to generate a key for all marine buccal-attaching genera, it became evident that illustrations of *Tetragonocephalon lutianus* are most similar to the specimen of *Smenispa irregularis* recently reviewed in this research (Chapter 6). The illustrations of *Tetragonocephalon lutianus* (see Avdeev 1975c) show the females are similar to *Smenispa irregularis* (Chapter 6) in: the cephalon embedded in pereonite 1, pereonite 1 anterior margin trilobed; lateral margins of pereonites subparallel, pereonites 1 and 2 arching anteriorly, lateral margins of pleonites subparallel and arching posteriorly, pleotelson subtruncate and endopod larger than exopod. The only difference is the more produced anterolateral margins of pereonite 1 in *Tetragonocephalon lutianus* and only known from the host of family Lutjanidae. The male specimen illustrated in

Avdeev (1975c) is also similar to that illustrated in Chapter 6 in body, pleopod and pereopod morphology, the only differences seem to be that male specimen eyes are absent in the Avdeev's (1975c) illustration. If both specimens are morphologically in agreement, a future review would include: 1) synonymizing *Tetragonocephalon lutianus* with *Smenispa irregularis*, 2) providing updated species description and diagnosis to prevent future confusion and 3) establishing host preference and species distribution. If this holds true, the revised *Smenispa irregularis* is likely to have an Indo-Pacific distribution with low host specificity.

8.4.2 Prevalence and abundance

The prevalence of cymothoids on wild or aquaculture fish population is unpredictable and can therefore pose a challenge for sampling. In a study of the prevalence of *Anilocra nemipteri* on the host *Scolopsis bilineatus* in the Great Barrier Reef, Roche *et al.* (2012) reported a relatively high prevalence of 4.3% on average from 12 different reefs. The highest prevalence of infected fish was at Bird Islets (28%) and Bird Lagoon (23%). Roche *et al.* (2012) also demonstrated that cymothoid prevalence on juvenile host was 9.8% compared to 4.3% on adult host. Their results are contrary to previous prevalence reports, with Grutter (1994) reporting a single *Anilocra nemipteri* parasitizing *Scolopsis bilineatus* and no reports on any cymothoid by Lester & Sewell (1989), despite examining 122 fish specimens from 39 families. In India, a series of recent studies showed high prevalence of 93.18% of *Mothocya renardi* on host *Strongylura leiura* (Aneesh *et al.* 2013a) and 74.42% of *Cymothoa frontalis* on host *Strongylura strongylura* (Aneesh *et al.* 2014) whereas Rameshkumar *et al.* (2013) reported low prevalence of 7.9% of *Cymothoa bychowskyi* (3 of 38 hosts) from host *Fistularia petimba*, suggesting low cymothoid occurrence. Thus it is not known why different cymothoid species would show low or high host occurrence, although one reason may suggest seasonal prevalence may influence cymothoid occurrence (see examples in Maxwell 1982; Inostroza *et al.* 1993; Andrews *et al.* 2013; Aneesh *et al.* 2013b). Though the degree of prevalence in different habitats is underestimated and variable, there is a likelihood that large bays and lagoons could have higher rates of infestation as compared to oceanic open waters (for examples of high prevalence *Ceratothoa* spp. in bays and lagoons see Bragoni *et al*. 1984; Charfi-Cheikhrouha *et al*. 2000; Horton & Okamura 2002).

8.4.3 Aquaculture

It is well known that cymothoids parasitize a range of host species including commercially important fishes (see Chapter 2 under section aquaculture) and can cause adverse effects on the host (see Chapter 2 under section Cymothoidae host effects). From this study, cymothoids with low host specificity in Australian waters include *Ceratothoa banksii*, *Cymothoa eremita*, *Cymothoa indica* and *Smenispa irregularis* (see table 8.1). It would be worth investigating these cymothoid species further by 1) understanding what other possible reservoirs (particularly wild fish species) as these cymothoids have low host specificity, 2) monitoring and reconfirming wild-fish encounters with aquaculture fishes, and most interesting, 3) studying the life cycle of the cymothoids and comparing their host use. Sievers *et al.* (1996) study found that *Ceratothoa gaudichaudii* parasitizing farmed Atlantic salmon in Chile reached the adult female stage, but were never ovigerous. A similar observation was made for *Ceratothoa banksii* for farmed *Salmo salar* and *Latris lineata* in Tasmania, where female cymothoids were big and bloated, but never ovigerous (personal observation, see materials examined in Chapter 3 Part II).

8.4.4 Morphometrics to aid identification

Morphology still plays a crucial role in the evaluation of patterns of diversification despite the advancements in molecular analysis (Kamilari & Sfenthourakis 2009), especially since it is the basis of taxonomic classification. However, rather than only emphasizing phenotypic characters, applying qualitative measures to describe and compare shapes of whole organisms or parts of it would be a more useful attempt. Rohlf & Marcus (1993) mentioned the use of morphometrics to resolve species variation, local geographic populations, developmental stages, and genetic and/or environmental effects. The applications of morphometrics have been studied in depth in many crustaceans (Grandjean *et al.* 1997; Sampedro *et al.* 1999; Muino *et al.* 1999; Fernandez-Vergaz *et al.* 2000).

Quantitative analysis of morphological features is currently not available for cymothoids, but will be beneficial for field use, especially for biologist and aquaculturists. The taxonomic review of the buccal-attaching cymothoids was essential because it provided better resolution of important morphological characters, which can then be applied in morphometrics. My preliminary study for morphometrics on *Ceratothoa banksii* and *Ceratothoa imbricata* did show implicit differences using ovigerous females, but was unable to complete it as a chapter due to time constraints. Further study should be conducted to provide better insight not only for ovigerous females, but other life stages of the cymothoid (e.g. adult males, males, and mancae).

8.4.5 DNA barcoding and molecular & morphological phylogeny for the Cymothoidae

There is a need for more molecular analyses of a wider range of cymothoid genera. Furthermore, most specimens used were from museum collections, and thus the preservation history is unknown and could have degraded the DNA quality of the cymothoids. DNA barcoding will not only provide easy access for species identification online, but potentially resolve cryptic species and also provide species tagging. More freshly preserved cymothoid specimens (preferably fixed in RNAlater) from different cymothoid genera will essentially provide a better resolution of the phylogenetic relationship of the family and be complimented with better resolved morphological tree (essentially with better character states for the genera). The mitochondrial genes of both 16S rRNA and cytochrome oxidase I also seem to result in slightly different trees (see Chapter 7). Therefore it is essential to choose the best molecular marker for various studies such as interspecific and intraspecific variations (particularly species which have high morphological variations such as *Ceratothoa banksii*, *Cymothoa indica* and *Cymothoa eremita*) and phylogenetic relationships based on host association and geographic distribution. It would be interesting to study the evolutionary history of cymothoid association with the different host and to evaluate Brusca's (1981) hypothesis of the historically distribution of cymothoids in the Tethys Sea.

Future studies should develop the morphological cladistics of the Cymothoidae by 1) revising morphological characters and providing better defined morphological states, and 2) include all generic taxon based on type material or reliable literature. This would eventually resolve the monophyly of the different taxa and can later be compared with molecular phylogeny to further establish the relationship within the family.

8.5 CONCLUSION

Prior to this research, the taxonomy of buccal-attaching genera from Australian waters was unresolved, with some species of unclear identity. This thesis has contributed to the knowledge on the buccal-attaching *Ceratothoa*, *Cymothoa*, *Glossobius* and *Smenispa* by increasing species numbers and new species records, revising complex species synonymies and providing better resolution for species and generic diagnoses. The review of all four genera has also provided better insight into host-cymothoid relationships and cymothoid distribution and diversity. This thesis also highlights the complexities of the Cymothoidae on both molecular and morphological phylogenetics. Using the morphological dataset which Hadfield (2012) had worked on and updated morphological dataset within this research, this was compared with the molecular phylogeny using COI and 16S rRNA cymothoid sequences for this research and sequences available from GenBank. The preliminary results suggest complex history within the family, and further resolution of data based on parasitic diversification strategies, host-cymothoid association, pathogenicity and life history would help better understand the phylogenetic relationship of the Cymothiodae. The taxonomic and phylogenetic output from this study is summarized below:

Genus *Ceratothoa* (Chapter 3)

1) The discovery and description of two new *Ceratothoa* species: *Ceratothoa barracuda* **sp. nov.** from Cairns and *Ceratothoa globulus* **sp. nov.** from Lord Howe Island
2) The discovery and description of two new records: *Ceratothoa carinata* and *Ceratothoa oxyrrhynchaena*
3) The subsequent designation of *Cymothoa parallela* as type species for *Ceratothoa*
4) The redescription and illustration of *Ceratothoa imbricata* based on Australian material from host *Trachurus* sp. and comparison with the type published by Hadfield *et al.* (2014a)
5) The validation of *Ceratothoa banksii* and bringing it out of synonymy with *Ceratothoa imbricata*
6) The transfer of *Ceratothoa lineata* to the genus *Mothocya*
7) The synonymization of
    a. *Ceratothoa curvicauda* with *Ceratothoa carinata*.
    b. *Ceratothoa trillesi* and *Ceratothoa huttoni* with *Ceratothoa imbricata*
    c. *Mothocya ihi* with *Mothocya lineata* (Miers, 1876) **comb. nov.**
8) The exclusion of *Ceratothoa trigonocephala* and *Mothocya lineata* (Miers, 1876) **comb. nov.** from Australian waters
9) The consideration of *Ceratothoa contracta*, the East Pacific *Ceratothoa gaudichaudii*, and the New Zealand *Ceratothoa novaezelandiae* Filhol, as *species inquirenda*
10) A key to the Australian species of *Ceratothoa*

Genus *Glossobius* (Chapter 4)

1) The discovery and description of a new record *Glossobius impressus* from Australia and southern Africa
2) The synonymization of *Glossobius ogasawarensis* with *Glossobius auritus*
3) The removal of *Glossobius crassa* from synonymy with *Glossobius auritus* and placed into *nomen dubium*
4) The transfer of *Glossobius arimae* to the genus *Ceratothoa*
5) The re-diagnosis of the genus and bringing the total number of *Glossobius* species from nine to five
6) A key to the species of *Glossobius*

Genus *Cymothoa* (Chapter 5)

1) The discovery and description of a new record *Cymothoa hermani* from Australian waters.
2) The redescription of holotype/paratype material: *Cymothoa carangi*; *Cymothoa curta*; *Cymothoa parupenei*; *Cymothoa plebeia*; *Cymothoa propria*; and *Cymothoa rotunda*
3) The exclusion of *Cymothoa curta* and *Cymothoa plebeia* from the Australian fauna
4) The placement of *Cymothoa limbata* into junior synonymy with *Cymothoa eremita*
5) *Cymothoa eremita* and *Cymothoa indica* are known to have high morphological variability and display low host specificity
6) A key to the Australian species of *Cymothoa*

Genus *Smenispa* (Chapter 6)

1) The nomenclatural change from *Enispa* to *Smenispa*
2) Re-diagnosis of the genus
3) The redescription of *Smenispa irregularis* from female and male specimens

Morphological and molecular phylogenetics (Chapter 7)

1) The family Cymothoidae is monophyletic
2) The phylogeny of Cymothoidae is independent of site attachment, thus does not specifically support the evolution from external-attaching taxon and advanced into the more derived gill and buccal-attaching genera
3) Brusca's (1981) hypothesis of host specificity is higher in the more derived gill and buccal genera is not supported
4) Two clades are upheld in the morphological phylogeny:
    a. A predominantly gill and buccal-attaching clade
    b. A South American freshwater cymothoid clade
5) The phylogeny of 16S rRNA cymothoid sequences (both maximum likelihood and maximum parsimony analyses) supported an *Anilocra* and two *Ceratothoa* clade
6) The phylogeny of CO1 cymothoid sequences (both maximum likelihood and maximum parsimony analyses) supported a *Cymothoa* and *Nerocila* clade
7) Both morphological and molecular phylogeny show convergent evolution of gill, buccal and external cymothoids within the family

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APPENDICES

APPENDIX 1: ASSOCIATED RESEARCH PUBLICATIONS

- Bruce, N. L., **Martin, M.,** Hadfield, K. & Nowak, B. (**accepted** in *Singapore Raffles Bulletin of Zoology*) Redescription of the fish parasitic “Tongue Biter” *Cymothoa Rhina* Schioedte & Meinert, 1884 (Crustacea: Isopoda: Cymothoidae) from Singapore.

- **Martin, M.,** Bruce, N. & Nowak, B. (**2014**) Redescription of *Ichthyoxenus puhi* (Bowman, 1962) (Crustacea: Isopoda: Cymothoidae), parasite of moray eel from Mauritius. *Crustaceana* 87 (6): 654-665.

- Andrews, M., Cobcroft, J. M., Battaglene, S. C., Valdenegro, V., **Martin, M. B.** & Nowak, B. F. (**2013**) Parasitic crustaceans infecting cultured striped trumpeter *Latris lineata*. *Aquaculture* 416–417: 280–288.

APPENDIX 2: CONFERENCE PROCEEDINGS

- **Martin, M.,** Shahsavari, E., Bruce, N., Bott, N. & Nowak, B. (**30th June- 3rd July 2014**) Morphological and molecular phylogenetics of fish parasitic cymothoids (Crustacea: Isopoda). ***Australian Society for Parasitology 50th Anniversary Conference***, Canberra (Oral & Poster presentation).
- **Martin, M.,** Bruce, N., & Nowak, B. (**25-29 August 2013**) Review of the fish tongue biter *Cymothoa* Fabricius, 1793 (Crustacea: Isopoda: Cymothoidae) in Australian waters. ***World Association for the Advancement of Veterinary Parasitology and Australian Society for Parasitology***, Perth (Oral presentation).
- **Martin, M.,** Bruce, N., & Nowak, B. (**8-12 July 2013**) Review of the fish-parasitic cymothoid crustacean 'tongue biter' genus *Ceratothoa* (Fabricius, 1775) in Australian waters. ***Second FRDC Australasian Scientific Conference on Aquatic Animal Health***, Cairns (Oral presentation).
- **Martin, M.,** Leef, M., Adlard, R., Bruce, N., & Nowak, B. (**8-12 July 2013**) The identity and occurrence of fish parasitic isopod *Ceratothoa* cf. *imbricata* (Fabricius, 1775) in southeastern Australia. ***Australian Society for Parasitology***, Hobart (Oral & poster presentation).