Phylogeny of five species of *Anilocra* Leach 1818 (Isopoda: Cymothoidae) from Puerto Rico and the Virgin Islands

by

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ABSTRACT

Members of the genus *Anilocra* Leach 1818 are large external isopod parasites of a variety of coral reef associated fishes. In the Caribbean Sea, this genus is represented by nine species whose descriptions are based solely on their morphology. Their disjunct geographic distributions and host specificity suggests varying degrees of incipient speciation. This study used mitochondrial cytochrome c oxidase subunit 1 gene sequences to elucidate phylogenetic relationships of five species of *Anilocra* from Puerto Rico and the Virgin Islands through parsimony, maximum likelihood, and Bayesian inference. The results show that the Caribbean *Anilocra* species form a monophyletic group and are not closely related to *A. physodes*, the type species of the genus. Parsimony and Bayesian inference analyses recovered three clades: clade A (*A. chromis*), clade B (*A. holocentri*), and clade C (*A. acanthuri, A. chaetodonti, A. haemuli*), while maximum likelihood analyses only recovered clade A and C. These analyses depict *A. chromis* as the basal species and *A. chaetodontis* as the most recently evolved species of the Caribbean *Anilocra.* Phylogenetic reconstructions show population structure based on host for *A. haemuli* and based on geographical location for *A. chaetodontis*, which suggest that each of these species might represent cryptic species with morphological stasis.

RESUMEN

Los miembros del género *Anilocra* Leach 1818 son isópodos parásitos externos de una variedad de peces asociados a arrecifes de coral. En el Mar Caribe este género está representado por nueve especies cuyas descripciones están basadas solamente en su morfología. Su distribucion geográfica disjunta y la especificidad de hospederos sugieren varios grados de especiación incipiente. Este estudio utilizó secuencias del gen mitocondrial citocromo c oxidasa subunidad 1 para elucidar las relaciones filogenéticas de cinco especies de *Anilocra* de Puerto Rico y de las Islas Vírgenes por medio de análisis de parsimonia, máxima verosimilitud e inferencia bayesiana. Los resultados muestran que las especies de *Anilocra* del Caribe forman un grupo monofilético y no están relacionadas a *A. physodes*, la especie tipo del género. Los análisis de parsimonia e inferencia bayesiana recuperaron tres clados: clado A (*A. chromis*), clado B (*A. holocentri*) y clado C (*A. acanthuri, A. chaetodonti, A. haemuli*), mientras que los análisis de máxima verosimilitud sólo recuperaron el clado A y C. Estos análisis muestran a *A. chromis* como la especie basal y a *A. chaetodontis* como la especie que ha evolucionado más reciente dentro de *Anilocra* del Caribe. Las reconstrucciones filogenéticas muestran una estructura poblacional basada en hospedero para *A. haemuli* y una estructura poblacional basada en localidad geográfica para *A. chaetodontis,* lo cual sugiere que estas especies pueden ser especies crípticas con estasis morfológico.

DEDICATION

To all those who contributed in my formation as a biologist.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

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Isopods are thought to have originated in marine water and later invaded freshwater and land environments. Only one group, the suborder Oniscidea Latreille 1802, has successfully invaded the terrestrial environment by evolving a hard, calcareous exoskeleton and respiring through modified pleopods, i.e. leaf-like appendages on the ventral side of their pleon. Meanwhile, freshwater isopods comprise several families in various suborders (e.g. suborders Asellota Latreille 1803, Phreatoicidea Stebbing 1893, and Valvifera Sars 1882) which can be found in rivers, lakes, hot springs, and subterranean groundwater. Some freshwater isopods have very restricted distributions, such as the suborder Calabozoidea Van Lieshout 1983 which has one described species in Venezuela and another in Brazil (Schotte, 2006). Isopods found in marine environments show the greatest diversity in morphology and size. They can range from a 0.5 mm amorphous sac, as in the parasitic forms in the superfamily Cryptoniscoidea Kosmann 1880, to 0.5 m predators of the genus *Bathynomus* Milne-Edwards 1879. Marine isopods include detrivores, omnivores, carnivores, predators, and host-associated forms which can be found living in sponges, algae, sea grasses, coral reefs, mangrove roots, coral rubble, sediments, and associated with fishes and crustaceans.

There are more the 10,000 described species of isopods (Schotte et al., 1995 onwards; Dreyer and Wägele, 2002; Wetzer, 2002), the majority being free-living organisms with only a few groups having parasitic species on fishes and crustaceans. Isopod parasites of crustaceans are found exclusively in the superfamilies Bopyroidea

Rafinesque 1815 and Cryptonoscoidea, while those that are fish parasites are found in the superfamily Cymothooidea Leach 1814. Of the latter superfamily, only the family Cymothoidae Leach 1814 are permanent parasites of fishes (i.e. they do not change or leave host during a single life cycle stage), and do not have free-living species.

The isopods of the family Cymothoidae are all external parasites with the majority found in tropical or subtropical regions worldwide. In the Caribbean Sea, the family is represented by 10 genera: *Agarna* Schiöedte and Meinert 1883*, Anilocra* Leach 1818*, Ceratothoa* Dana 1852*, Cymothoa* Fabricius 1793*, Glossobius* Schiöedte and Meinert 1883*, Kuna* Williams and Williams 1986*, Livoneca* Leach 1818*, Mothocya* Costa 1851*, Nerocila* Leach 1818 and *Renocila* Miers 1880 (Kensley and Schotte, 1989). In the Caribbean, the genus *Anilocra* was thought to be represented by a single species, *Anilocra laticauda* Milne Edwards 1840. For many years the taxonomic longevity of the *Anilocra laticauda* complex was ensured by the combination of characters included in redescriptions based on multiple specimens of *Anilocra,* which were actually morphologically diverse. Williams and Williams (1981) reevaluated the *A. laticauda* complex, declared it a *nomen dubium*, and reclassified the complex into nine species: *A. abudefdufi, A. acanthuri, A. chaetodontis, A. chromis, A. haemuli, A. holacanthi, A. holocentri, A. myrispristis,* and *A. partiti.* Williams (1984) noted the geographic distribution of these nine new species and discovered that several showed disjunct distributions and host switching from one geographic area to the next even though all host species occur at all localities throughout the Caribbean Sea. The species range of host specificity and geographic distribution suggested varying degrees of incipient speciation, therefore, Williams (1984) hypothesized that there are species complexes

comprised of two or three species (i.e. *A. chromis, A. acanthuri, A. haemuli*) which were too similar to be distinguished morphologically.

Therefore, the objective of this work was to establish the phylogenetic relationships of the species of *Anilocra* from the Caribbean Sea and determine gene variations, if any, between populations. This research characterized individuals of *A. acanthuri, A. chaetodontis, A. chromis, A. haemuli,* and *A. holocentri* from Puerto Rico and the Virgin Islands using the mitochondrial cytochrome c oxidase subunit I (COI) gene.

LITERATURE REVIEW

Phylogenetic overview of the order Isopoda Latreille 1817

Isopods are an arthropod group classified in the suborder Peracarida Calman 1904 of the class Malacostraca Latreille 1802. Peracarids are distinguished as a group by the presence of a brood pouch and the direct development of their young. Within the Peracarida, the phylogenetic position of the Isopoda has remained uncertain because earlier authors (see Schultz, 1969) consider it to be the most derived order but recent studies (Spears et al., 2005; Wilson, 2009) hypothesize a basal position. Wilson (2009) also addressed the issue of whether Amphipoda Latreille 1816 or Tanaidacea Dana 1849 is the sister group of the Isopoda. The results, based on morphological and molecular data, rejected the Amphipoda-Isopoda clade hypothesis and supported the Tanaidacea as the closest relative to the Isopoda.

The order Isopoda is a monophyletic group characterized by the following synapomorphies (see Fig. 1):

- biphasic molting,
- carapace reduced or absent,
- eyes lack the expression of the eye stalks or lobes,
- antennular lateral flagellum is never fully expressed,
- palp is absent in maxilla 1,
- heart is located posteriorly,
- gut tube is entirely ectodermally derived,
- branchial structures abdominal,

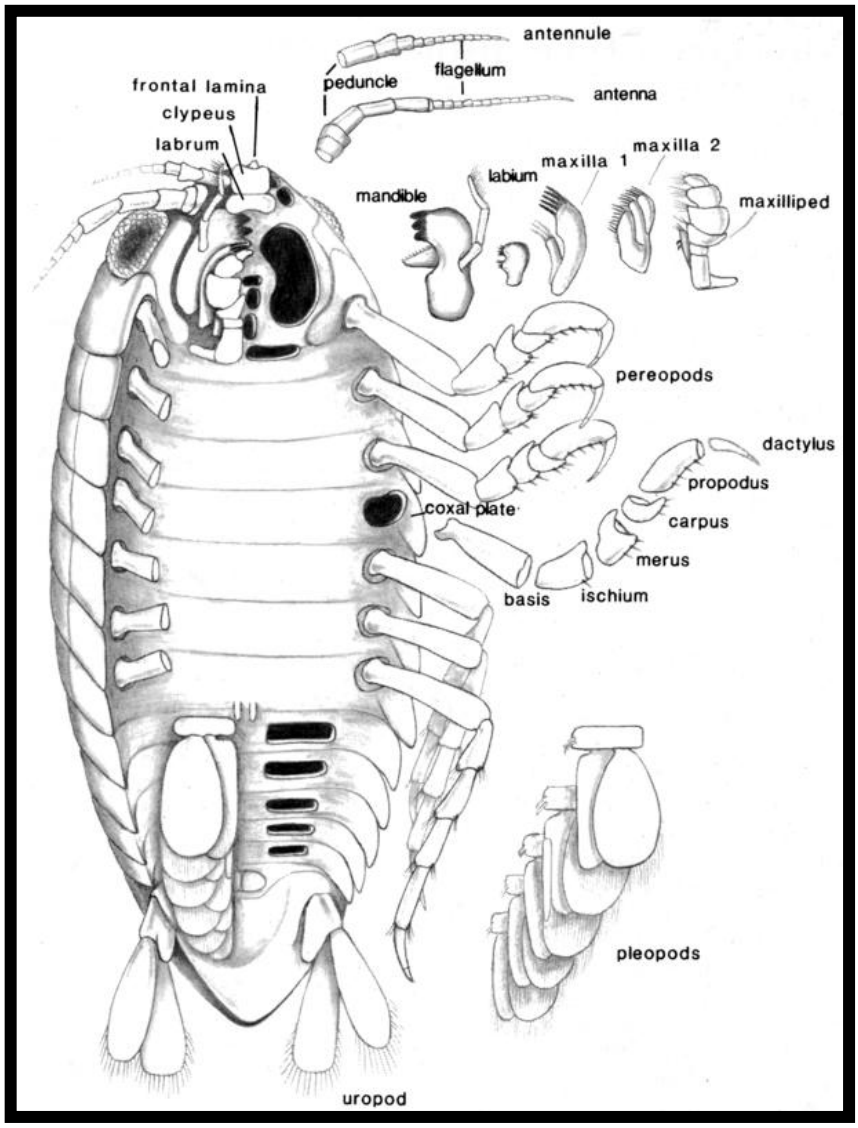

Figure 1. Isopod anatomy. (from Kensley and Schotte, 1989)

- internal fertilization and associated copulatory organ (appendix masculinum),
- pereopodal exopods absent,
- pereopods II-VII with small plate on the distal part of the propodus,
- pereopods II-VII ischium elongate with a major basis-ischium flexure,
- pleon segment VI fused with telson forming a pleotelson (Brusca and Wilson, 1991; Wilson, 2009).

A recurring issue in isopod phylogeny (to which a definitive answer has not been forthcoming) regards which group occupies the basal position. Brusca and Wilson's (1991) morphological analyses depicted the Phreatoicidea as the earliest derived group of living isopods followed by the Asellota. This scenario is also supported by Wetzer's (2002) combined morphological and molecular data analysis. Meanwhile, other studies (Dreyer and Wägele, 2001, 2002; Wilson, 2009), based on morphological, molecular, or combined data, showed the Asellota to be the basal group followed by the Phreatoicidea. Notwithstanding, Wilson's (2009) combined data analysis resulted in a new hypothesis where the parasitic groups of the suborder Cymothoida Wägele 1989 comprised the basal branch. This result is contradictory to previous studies (Brusca, 1981; Brusca and Wilson, 1991; Dreyer and Wägele, 2001, 2002; Wetzer, 2002) since parasitic isopods are considered to be the more derived group which evolved from a cirolanid-like ancestor.

Dreyer and Wägele (2001) illustrated an evolutionary scenario (see Fig. 2) where necrophagous and predatory cirolanid-like isopods specialized to feed on fishes eventually evolved to temporary and later permanent parasites of fishes (modern day family Cymothoidae). Using molecular and morphological data they showed that the suborder Epicaridea Latreille 1831 evolved from a cymothoid-like ancestor, from which one line of descendants must have specialized to suck the hemolymph of crustaceans. With this new information, they proposed to lower the group to family level, Bopyridae Rafinesque 1815, and to discard the suborder name Epicaridea. Bopyridae would then be of equal rank as their sister group, the Cymothoidae.

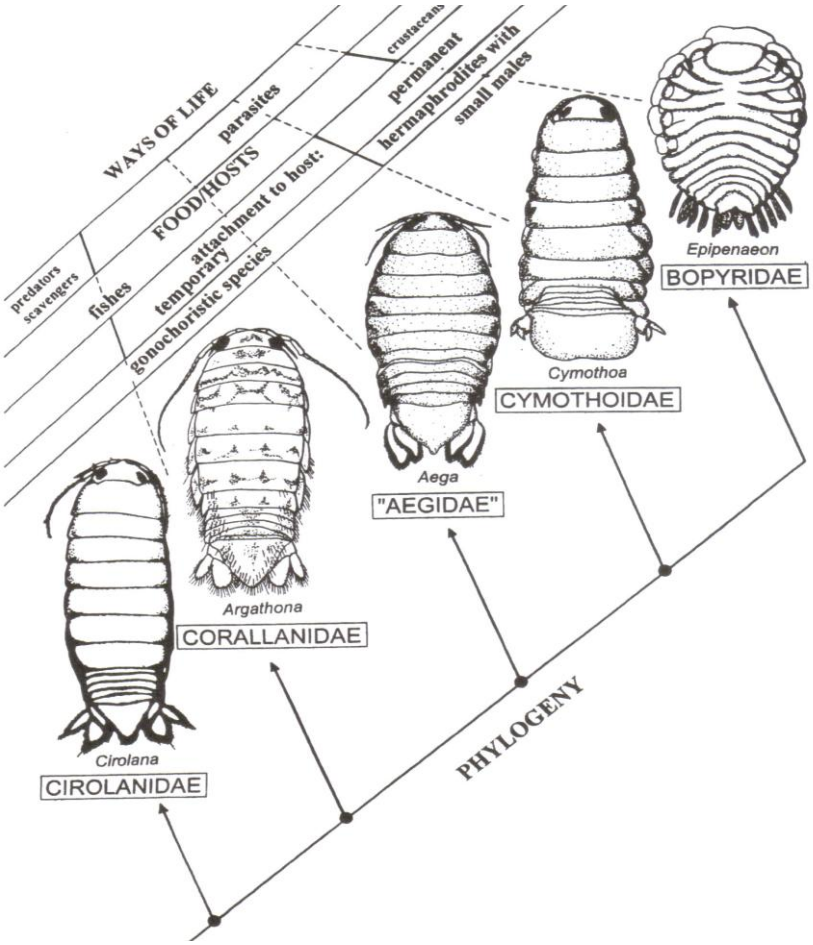

Figure 2. Evolutionary scenario of the ways of life of host-associated isopods. (from Dreyer and Wägele, 2001)

Another classificatory issue was the suborder in which these parasitic families, and other free-living and parasitic families, were placed. Various authors (e.g. Brusca and Wilson, 1991; Martin and Davis, 2001) have agreed that the suborder Flabellifera Sars 1882 is not a natural, i.e. monophyletic group, yet none proposed a new classification. The Flabellifera, as revised by Martin and Davis (2001), is comprised of the families: Aegidae, Ancinidae, Anuropidae, Bathynataliidae, Cirolanidae, Corallanidae, Cymothoidae, Gnathiidae, Hadromastacidae, Keuphyliidae, Limnoriidae, Phoratopodidae, Plakarthriidae, Protognathiidae, Serolidae, Sphaeromatidae,

Tecticepitidae and Tridentellidae (see Table 1 for authors). Dreyer and Wägele (2002) did not use the suborder Flabellifera but rather adopted the use of other suborders (Cymothoida and Sphaeromatidea Wägele 1989) suggested before Martin and Davis' (2001) revision.

Table 1. Isopod suborders, superfamilies and families of the Scutocoxifera. (from Brandt and Poore, 2003)

Suborder	Superfamily	Family
Oniscidea Latreille 1802	See Martin and Davis, 2001	See Martin and Davis, 2001
Tainisopidea Brandt and Poore 2003		Tainisopidae Wilson 2003
Phoratopidea Brandt and Poore 2003		Phoratopodidae Hale 1925
Cymothoida Wägele 1989	'Cymothooidea' Leach 1814	Aegidae White 1850
		Anuporidae Stebbing 1893
		Corallanidae Hansen 1890
		Cymothoidae Leach 1814
		Gnathiidae Leach 1814
		Protognathiidae Wägele and Brandt 1988
		Tridentellidae Bruce 1984
	Bopyroidea Rafinesque 1815	Bopyridae Rafinesque 1815
		Dajidae Giard and Bonnier 1887
		Entoniscidae Kosmann 1881
	Cryptoniscoidea Kosmann 1880	Asconiscidae Bonnier 1900
		Cabiropidae Giard and Bonnier 1887
		Cryptoniscidae Bonnier 1900
		Podasconidae Bonnier 1900
	Anthuroidea Leach 1914	Anthuridae Leach 1814
		Antheluridae Poore and Lew Ton 1988
		Expanathuridae Poore 2001
		Hyssuridae Wägele 1981
		Leptanthuridae Poore 2001
		Paranthuridae Menzies and Glynn 1968
	Cirolanoidea Dana 1852	Cirolanidae Dana 1852
Limnoriidea Brandt and Poore 2002	Limnorioidea White 1850	Hadromastacidae Bruce and Müller 1991
		Keuphyliidae Bruce 1980
		Limnoriidae White 1850
Valvifera Sars 1882		Antarcturidae Poore 2001
		Arcturidae Dana 1849
		Arcturididae Poore 2001
		Austrarcturellidae Poore and Bardsley 1992

Table 1. (continued)

Suborder	Superfamily	Family
		Chaetiliidae Dana 1849
		Holidoteidae Wägele 1989
		Holognathidae Thomson 1904
		Idoteidae Samouelle 1819
		Pseaudidotheidae Ohlin 1901
		Rectarcturidae Poore 2001
		Xenarcturellidae Sheppard 1957
Sphaeromatidea Wägele 1989	Sphaeromatoidea Latreille 1825	'Sphaeromatidae' Latreille 1825
		Tecticipidae Iverson 1982
		Ancinidae Dana 1852
		Paravireia Chilton 1925 *incerta sedis*
	Seroloidea Dana 1852	Basserolidae Brandt and Poore 2003
		Plakarthriidae Hansen 1905
		Schweglerellidae Brandt et al. 1999
		Serolidae Dana 1852
		Bathynataliidae Kensley 1978

Meanwhile, Dreyer and Wägele (2002) proposed a new taxon, the Scutocoxifera, as seen on tree partitions of morphological (also seen in Brusca and Wilson, 2001) and molecular phylogenies where the more basal isopods, Phreatoicidea and Asellota, are separated from the rest. This new monophyletic taxon comprises the suborders Anthuridea Monod 1922, Cymothoida, Oniscidea, Sphaeromatidea, and Valvifera. Furthermore, Brandt and Poore's (2003) revision of isopod classification accepted the Scutocoxifera taxon, but with a few changes as presented in Table 1. The changes included the creation of two new suborders (Tainisopidea and Phoratopidea Brandt and Poore 2003), the lowering of the suborder Anthuridea to subfamily rank, the inclusion of the suborder Limnoriidea Brandt and Poore 2002, among other things. Also, they dispensed with the suborder Flabellifera for which a synapomorphy could not be found.

On another level, several studies have focused on cryptic speciation (e.g. Held, 2003; Held and Wägele, 2005; Raupach and Wägele, 2006) and phylogeography of

free-living isopods (e.g. Ketmaier et al., 2003; McGaughran et al., 2006; Teske et al., 2006). These studies used mitochondrial genes (COI or 16s rRNA) as molecular markers to distinguish haplotypes and cryptic species. Meanwhile, for parasitic species molecular markers have been used to characterize juvenile and adult life cycle stages in order to match them (Grutter et al., 2000; Jones et al., 2008). Subsequently, the stages are typed morphologically in order to aid in field identification and avoid having to complete the life cycle to identify the species, since most species identification is based solely on adult morphology.

Life cycle and biology of the family Cymothoidae Leach 1814

Cymothoids develop in brood pouches formed by overlapped oostegites. Oostegites are medially directed thin-plated structures arising from the coxa (which sometimes are expanded into coxal plates) in the female (Kensley and Schotte, 1989). Within the brood pouch, eggs develop through four stages (egg, prehatch I stage, prehatch II stage, manca) during an average of 44 days (Adlard and Lester, 1995). Prehatch I stage is characterized by the presence of the egg membrane, eye pigment, and 6 pairs of pereopods, while the prehatch II stage has lost the membrane and has well developed eyes and somatic pigmentation (Fig. 3a-b). Manca differ from the prehatch II stage by the presence of setae and a smaller pleotelson in relation to body length (Fig. 3c). The number of mancae produced in each brood correlates positively with the length of the female isopod (e.g. 37 mancae for a female 10.6 mm in length, 182 mancae for a female of 17.2 mm), therefore larger isopods produce more offspring (Brusca, 1981; Adlard and Lester, 1995; Fogelman and Grutter, 2008).

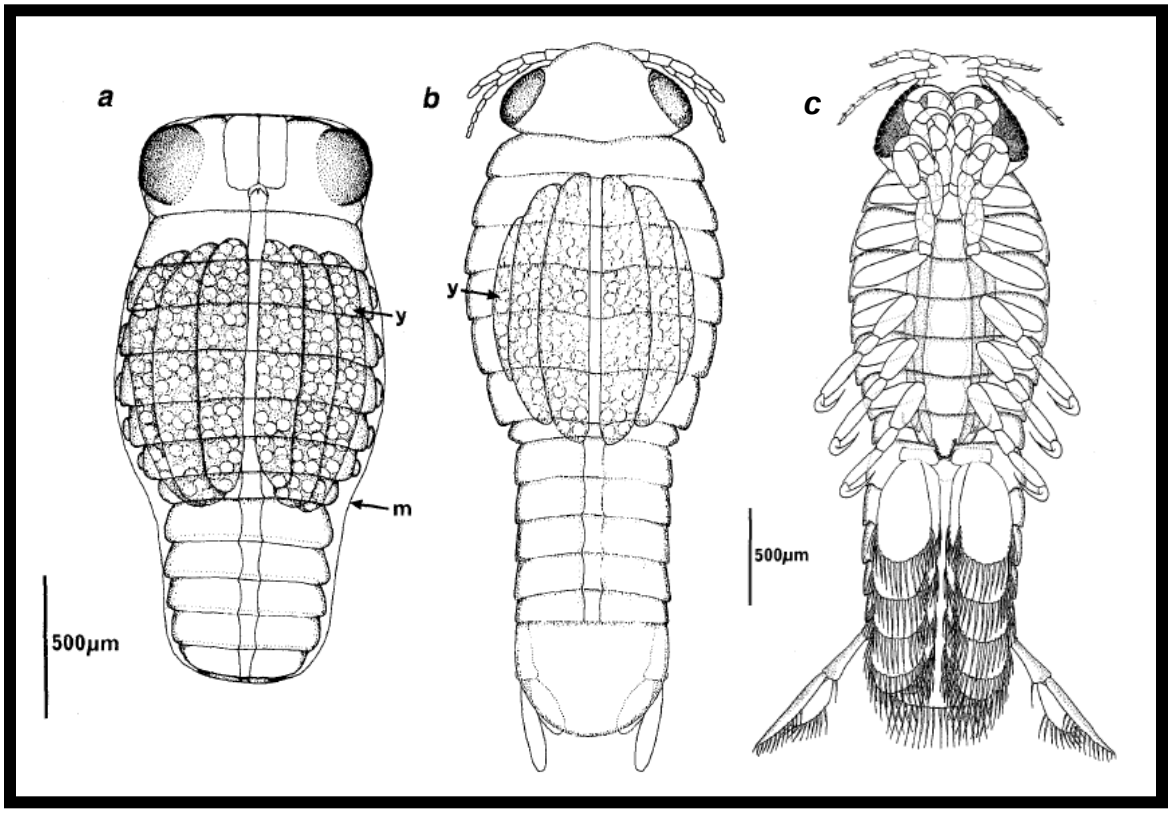

Figure 3. Development stages of *Anilocra pomacentri* within the brood pouch. (a) Prehatch I stage. (b) Prehatch II stage. (c) Manca. m, membrane; y, yolk supply (from Adlard and Lester, 1995)

The mancae is only held in the brood pouch for 2 days (Adlard and Lester, 1995) before being released. Two methods, natural release and burst release, have been observed for the release of mancae from the brood pouch. In the natural release method, the most posterior part of the brood pouch starts to form a gap and the female isopod pushes her body away from the host by straightening the seventh pair of pereopods (Williams and Williams, 1985). By this method, Williams and Williams (1985) observed a single manca being released every time the isopod performed this movement. In the laboratory, Adlard and Lester (1995) observed this same form of release when mancae were released either singly or in small groups over a period of 1-

3 h. An impact-burst release was observed by Williams and Williams (1985) when the host was speared. In this form of release, the female isopod did the same movements as in the natural release method but more violently. Also, they observed the gap in the brood pouch to be much larger, therefore allowing the mancae to burst out in a swarm. Adlard and Lester (1995) also observed a similar form of burst release when pressure was applied to the dorsal surface of the female isopod. In all cases, upon release from the brood pouch, mancae were highly active and able to infect larval or juvenile fishes immediately (Williams, 1984; Adlard and Lester, 1995; Fogelman and Grutter, 2008).

The mancae attach only to larval or juvenile fish hosts, where they start to feed and develop into juveniles (Adlard and Lester, 1995). If the host to which the juveniles are attached is the appropriate one, they will stay attached and continue molting. They eventually develop into a brief male stage and then into a female (i.e. they are protandrous hermaphrodites) which are incapable of leaving their host (Brusca, 1981; Williams, 1984; Adlard and Lester, 1995). These females will alternate between reproductive and feeding/growing stages, since the brood pouch occupies most of the space in the body and the oostegites cover the mouthparts so they cannot feed (see 7d) (Bunkley-Williams and Williams, 1998). Once the reproductive stage is over (i.e. 3 days after the release of mancae from the brood pouch) they molt and start feeding, and 18 days after the release they begin to develop a new brood pouch (Adlard and Lester, 1995). Therefore, the feeding/growing stage lasts only for 15 days, while the reproductive stage is 47 days longs. This cycle alternation allows the female to grow and produce several broods throughout her lifespan.

If the host to which the juvenile attaches is not the appropriate one it will eventually change hosts. Adlard and Lester (1995) suggest that in this case the juvenile becomes a functional male which is able to fertilize permanently attached females, but does not stay attached because a fish carrying an adult female is too large for successful attachment to occur since parasite and host growth are concurrent. This hypothesis that juveniles develop into functional males and are free-swimming was based on observing only one *A. pomacentri* per host. Williams (1984) also described a free-swimming male stage, but the stage was characterized by having 6 pairs of pereopods, fully developed appendix masculina and penis lobes. This free-swimming male stage described by Williams (1984) was called a micromale because the onset of male characters was at a body size and stage of morphological development typical of juveniles. Like in *A. pomacentri,* the micromales of the Caribbean *Anilocra* do not live attached next to the female, but rather seem to attach on “intermediate” hosts. In either scenario, Adlard and Lester’s or Williams’, the functional male will eventually find an appropriate host to which attach permanently and transform into an adult female.

In the Caribbean *Anilocra*, 1-3 females were found attached per host and occasionally small males were found attached to the same host (Williams, 1984). However, in other genera (e.g. *Kuna*, *Renocila*) cymothoids occur on hosts as male-female pairs with the female inhibiting the sexual transformation of their associated males. In these cases, male functionality is thought to be acquired after attachment. Since the attachment is permanent, these males lose their ability to swim and are considered adult males which will remain males until the female dies. Lester (2005)

suggests that the onset of male functionality is related to the isopod's site of attachment (external vs. buccal/gill chamber) and is probably genus specific.

Cymothoid's effects on hosts

Some cymothoids feed on host blood, while others feed on discharges of plasma in wounds. Studies have shown that cymothoid mancae feed voraciously and can damage or kill juvenile fish by the tissue damage they cause (Adlard and Lester, 1994, 1995; Fogelman and Grutter, 2008), while adults can significantly reduce growth, reproduction, and survivorship of the fish they parasitize (Adlard and Lester, 1994, 1995; Fogelman et al. 2009). Cymothoids inhabiting the gill chamber are usually associated with stunted gills caused by pressure atrophy and damage associated with isopod feeding and attachment. In the mouth, they affect the development of the oral structures and may completely replace the tongue (Brusca, 1981; Bunkley-Williams and Williams, 1998; Lester, 2005).

Meadows and Meadows (2003) tested the hypothesis that *A. chaetodontis* altered its host behavior to increase the parasite's fitness. They observed that infected fish had more aggressive interactions with other fish of the same species, had smaller territories, and spent less time feeding and more time in low flow environments; all of which allowed the parasite to infect other fish. Fogelman et al. (2009) noted parasitic castration on the host of *A. apogonae* Bruce 1987. They noted that the castrated hosts had smaller gonads; female hosts had fewer and smaller ova; and male hosts' ability to mouthbrood their young and the number of eggs present in the mouthbrood was greatly reduced.

Host and site specificity in cymothoids

Most cymothoids are host specific varying from a single species (e.g. *A. partiti*, *Kuna insularis* Williams and Williams 1985), to one or two genera in the same family (e.g. *A*. *chaetodontis*, *Nerocila benrosei* Bunkley-Williams and Williams 1999), to various genera throughout several families (e.g. *A. physodes* (Linnaeus 1758), *Ceratothoa steindachneri* Koelbel 1878). In the case of *Anilocra,* in areas of high host species diversity the parasites are found on few host species, while in areas of low host species diversity the isopod parasitizes a higher number of hosts (Bruce, 1987). In other words, the parasites tend to be less host specific when fewer host species are available. Bruce (1987) suggested that this increment in the number of host species was due to reduced competitive pressure. Meanwhile, larval stages seem to be less host specific than the adult stage since they have been reported on hosts other than their “definitive” host (Williams, 1984; Adlard and Lester, 1994; Fogelman and Grutter, 2008).

Cymothoids are also site specific, attaching on the skin, fins, in the gill chamber, mouth or burrowing in the musculature of fishes. Brusca (1981) described three evolutionary lineages (superficial, buccal-gill chamber and burrowing) with respect to their attachment strategies. He depicted the superficial attaching lineage as the most primitive in the family and concluded that the superficial and the buccal-gill chambers lineages each occurred separately as a single event, and that the burrowing lineage may have occurred independently several times.

Bruce (1990) briefly discussed the subfamily classification (Anilocrinae, Ceratothoinae, Cymothoinae, Livonecinae,) in which Schiöedte and Meinert had divided the family. He noted that although position on the host and body shape have influenced

the analysis of relationships of the cymothoids, those lineages proposed by Brusca (1981) were no longer valid since there are cases in which a genus of isopod specific for attaching in one position has species that attach in another position. For example, species of the genus *Mothocya* Costa (in Hope) 1851 attach in the gill chamber but *Mothocya ihi* Bruce 1986 attaches in the buccal cavity. Likewise, species of the genus *Nerocila* are found attached on the skin but *Nerocila lomatia* Bruce 1987 attaches in the gill chamber. Therefore, Bruce (1990) suggests that in classification morphology should take precedence over position on host. Since the morphology for all the subfamily groups proposed by Schiöedte and Meinert have not been reviewed, Bruce (1990) recommends that it is better to avoid their use, except in the case of the subfamilies Anilocrinae and Cymothoinae Schiöedte and Meinert 1881 for which he already established a diagnosis.

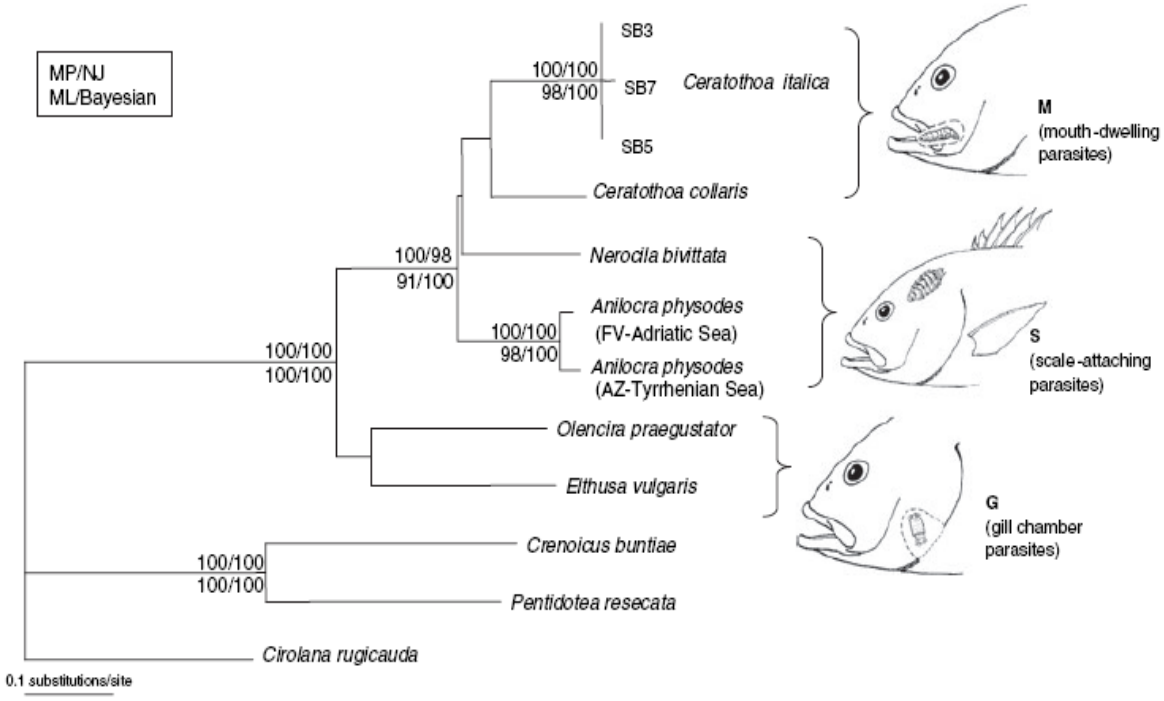

Figure 4. Phylogenetic relationships of three parasitic types based on mtDNA 16s and COI sequences. (from Ketmaier et al., 2007)

Using molecular data, Ketmaier et al. (2007) examined the earlier view that the scale, gill and mouth parasitic types represented three evolutionary lineages (subfamilies Anilocrinae, Livonecinae, and Cymothoinae, respectively) and Brusca's hypothesis that the scale attaching species are the more ancestral lineage. Their result (see Fig. 4) did not support either hypothesis, but indicates that the gill and mouth parasitic types evolved independently. Also, the results of Jones et al. (2008) did not support Brusca's hypothesis but suggested that ancestral cymothoids attached in the buccal or gill cavity and that external attachment is a derived condition that has appeared more than once.

Classificatory overview of the genus *Anilocra* Leach 1818

The genus *Anilocra* differs from the other genera in the family Cymothoidae by the following characteristics:

- cephalon anterior margin usually narrowed and folded ventrally between bases of antennules; posterior margin trilobed; not immersed, or weakly immersed in pereonite 1,
- antennule not broader or longer than antenna,
- posterolateral angle of pereonite 1 and 7 somewhat prominent and produced,
- posterolateral angles of pereonites 2-6 not produced,
- coxal plates short, rarely reaching to posterior margin of their pereonites,
- pereopods increasing in length posteriorly, pereopod 7 abruptly longer than the others,
- pleon not immersed, or slightly immersed in pereonite 7,

- pleopods 3-5 often formed into deep pockets or pleats,
- uropods often extending beyond posterior margin of pleotelson (Richardson, 1905; Brusca, 1981; Bruce, 1987; Kensley and Schotte, 1989).

Bruce (1987) mentions that the genus *Anilocra* is comprised of 37 species, while Schotte et al. (1995 onwards) lists 49 species. A literature revision (see Table 2) revealed that 59 species have been described since the creation of the genus. Of these 59 species, 1 species was declared *species inquirenda* (Bruce and Harrison-Nelson, 1988), 2 species were declared *nomen dubium* (Richardson, 1905; Trilles, 1975a; Brusca, 1981; Williams and Williams, 1981), 8 species have been made synonymies of other species (Richardson, 1905; Trilles, 1975a; Trilles, 1975b; Williams and Williams, 1981; Bruce, 1987; Bruce and Harrison-Nelson, 1988; Espinosa-Pérez and Hendrickx, 2001), 2 species are possibly synonymies of other species (Bruce and Harrison-Nelson, 1988; Williams and Bunkley-Williams, 2003), and for 2 species Bruce (1987) indicates that their status is uncertain due to the unavailability of specimens for his examination. For another 7 species (*A. atlantica*, *A. coxalis*, *A. guinensis*, *A. hedenborgi*, *A. recta*, *A. rissoiana, A. tartoor*), no other mention was found in the literature after the publication of their original description. Therefore, of the 59 species included in the list only 30 should be considered valid. Since Bruce (1987) does not mention which 37 species of *Anilocra* he considered valid, a comparison cannot be made.

The majority of the *Anilocra* species are distributed throughout two areas of high diversity, the Australian-Malaysian region with 18 species and the Caribbean region with 9 species. Previously, the species of the Caribbean were considered a single species, *A. laticauda*, despite the fact that various redescriptions were made (Richardson, 1905;

Table 2. Species of *Anilocra.*

Species	Status	Geographic distribution	Revision reference
abudefdufi Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
acanthuri Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
*acuminat*a Haller 1880	Synonym of *A. capensis*	Indo-Pacific	Bruce (1987), Bruce and Harrison-Nelson (1988)
acuta Richardson 1910	Valid	Gulf of Mexico, US Atlantic Coast	Schultz (1969), Bowman et al. (1977), Brusca (1981), Williams and Williams (1981)
alloceraea Koelbel 1878	Valid	Australia, Indonesia, Singapore	Bruce (1987), Bruce and Harrison-Nelson (1988)
amboinensis Schiöedte and Meinert 1881	Valid	Indonesia, Philippines	Bruce and Harrison-Nelson (1988)
ankistra Bruce 1987	Valid	Australia	Bruce (1987)
apogonae Bruce 1987	Valid	Australia, Papau New Guinea	Bruce (1987), Bruce and Harrison-Nelson (1988)
asilus Walker and Hornell 1896	Synonym of *A. frontalis*		Trilles (1975b)
atlantica Schiöedte and Meinert 1881	*	*	*
australis Schiöedte and Meinert 1881	Status uncertain	Indo-Pacific	Bruce (1987)
capensis Leach 1818	Valid	Southern Europe, Indonesia, West & Southern Africa	Trilles (1975a), Bruce and Harrison-Nelson (1988), Thorsen and Trilles (2002)
carpenteriensis Avdeev 1977	Synonym of *A. dimidiata*	Australia	Bruce (1987)
caudata Bovallius 1887	Valid	Australia, Philippines, Vietnam	Bruce (1987)
cavicauda Richardson 1910	Valid	Australia, Philippines, Vietnam	Bruce (1987), Bruce and Harrison-Nelson (1988)
chaetodontis Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
chromis Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
clupei Williams and Williams 1986	Valid	Japan	Williams and Williams (1986)
coxalis Schiöedte and Meinert 1881	*	*	*
cuvieri Leach 1818	Synonym of *A. physodes*		Trilles (1975b)
dimidiata Bleeker 1857	Valid	Australia, Hong Kong, Indian Ocean, Indo-Malaysia area, Philippines, Vietnam,	Trilles (1975a), Bruce (1987), Bruce and Harrison-Nelson (1988)
edwardii Saint-Loup 1885	Synonym of *A. physodes*		Trilles (1975b)
elviae Winfield, Alvarez and Ortiz 2002	Valid	Gulf of Mexico	Winfield et al. (2002)

Table 2. (continued)

Species	Status	Geographic distribution	Revision reference
frontalis Milne Edwards 1840	Valid	Adriatic Sea, Dutch Coast, North Sea, Mediterranean	Trilles (1975a), Bruce (1987), Ramdane et al. (2007)
gigantea (Herklots 1870)	Valid	Fiji, Hawaii, New Caledonia	Trilles (1975a), Bruce and Harrison-Nelson (1988), Williams and Bunkley-Williams (2003)
guinensis Bovallius 1887	*	*	*
haemuli Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
hedenborgi Bovallius 1887	*	*	*
holacanthi Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
holocentri Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
huacho Rokitsky 1984	Valid	East Pacific	Bruce and Harrison-Nelson (1988)
koolanae Bruce 1987	Valid	Australia, Indonesia	Bruce (1987), Bruce and Harrison-Nelson (1988)
laevis Miers 1877	Valid	Peru	Trilles (1975a), Brusca (1981), Williams and Williams (1981), Espinosa-Pérez and Hendrickx (2001)
laticauda Milne-Edwards 1840	*Nomen dubium*	Caribbean	Richardson (1905), Schultz (1969), Trilles (1975a), Brusca (1981), Williams and Williams (1981) , Espinosa-Pérez and Hendrickx (2001)
leachii Schiöedte 1866	*Nomen dubium*	West Indies	Richardson (1905), Trilles (1975a), Brusca (1981), Williams and Williams (1981)
leptosoma Bleeker 1857	Valid	Australia, Indonesia, Philippines	Trilles (1975a), Bruce (1987)
longicauda Schiöedte and Meinert 1881	Valid	Australia, Philippines, Singapore, Vietnam, Indonesia	Trilles (1975a), Bruce (1987), Bruce and Harrison-Nelson (1988)
marginata (Bleeker 1857)	Possible synonym of *A. amboinensis*	Indonesia	Bruce and Harrison-Nelson (1988)
mediterranea Leach 1818	Synonym of *A. physodes*		Trilles (1975b)
meridionalis Richardson 1914	Tentatively synonymized with *A. gigantea*	Galapagos Island, Hawaii, Tropical Eastern Pacific	Brusca (1981), Williams and Williams (1981), Espinosa-Pérez and Hendrickx (2001), Williams and Bunkley-Williams (2003)
mexicana Saussure 1857	Synonym of *A. laticauda*	Caribbean or Mexico	Richardson (1905), Trilles (1975a), Williams and Williams (1981), Espinosa-Pérez and Hendrickx (2001)

Table 2. (continued)

Species	Status	Geographic distribution	Revision reference
monoma Bowman and Tareen 1983	Valid	Kuwait	Bowman and Tareen (1983), Bruce (1987)
montii Thatcher and Lobos 2001	Valid	Chile	Thatcher and Lobos Blumenfeldt (2001)
morsicata Bruce 1987	Valid	Australia	Bruce (1987), Bruce and Harrison-Nelson (1988)
myripristis Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
nemipteri Bruce 1987	Valid	Australia	Bruce (1987)
occidentalis Richardson 1899	Synonym of *Elthusa vulgaris*	US Pacific Coast	Espinosa-Pérez and Hendrickx (2001)
partiti Williams and Williams 1981	Valid	Caribbean	Williams and Williams (1981)
physodes (Linnaeus 1758)	Valid	Adriatic Sea, Black Sea, Egean Sea, Mediterranean, Tyrrhenian Sea	Trilles (1975a), Bruce (1987), Ketmair et al. (2007), Ramdane et al. (2007)
pilchardi Bariche and Trilles 2006	Valid	Lebanon	Bariche and Trilles (2006)
plebeia Schiöedte and Meinert 1901	Valid	Tropical West Atlantic	Richardson (1905), Schultz (1969), Brusca (1981), Williams and Williams (1981)
pomacentri Bruce 1987	Valid	Australia	Bruce (1987), Bruce and Harrison-Nelson (1988)
prionuri Williams and Williams 1986	Valid	Japan	Williams and Williams (1986)
recta Nierstrasz 1915	*	*	*
rhodotaenia Bleeker 1857	*Species inquirenda*	Indonesia	Bruce and Harrison-Nelson (1988)
rissoiana (Leach 1818)	*	*	*
soelae Bruce 1987	Valid	Australia	Bruce (1987)
tartoor (Pillai 1954)	*	*	*
tropica Avdeev 1977	Status uncertain	Indo-Pacific	Bruce (1987)

*Revision reference not found

Menzies and Glynn, 1968; see Williams, 1984). Williams (1984) noted that these redescriptions were not based on the original material of Milne Edwards, but on multiple morphologically diverse specimens. Williams and Williams (1981) revised the Caribbean *Anilocra,* declared *A. laticauda* a *nomen dubium* and recorded nine new species: *A. abudefdufi, A. acanthuri, A. chaetodontis, A. chromis, A. haemuli, A. holacanthi, A. holocentri, A. myrispristis,* and *A. partiti* (Fig. 5).

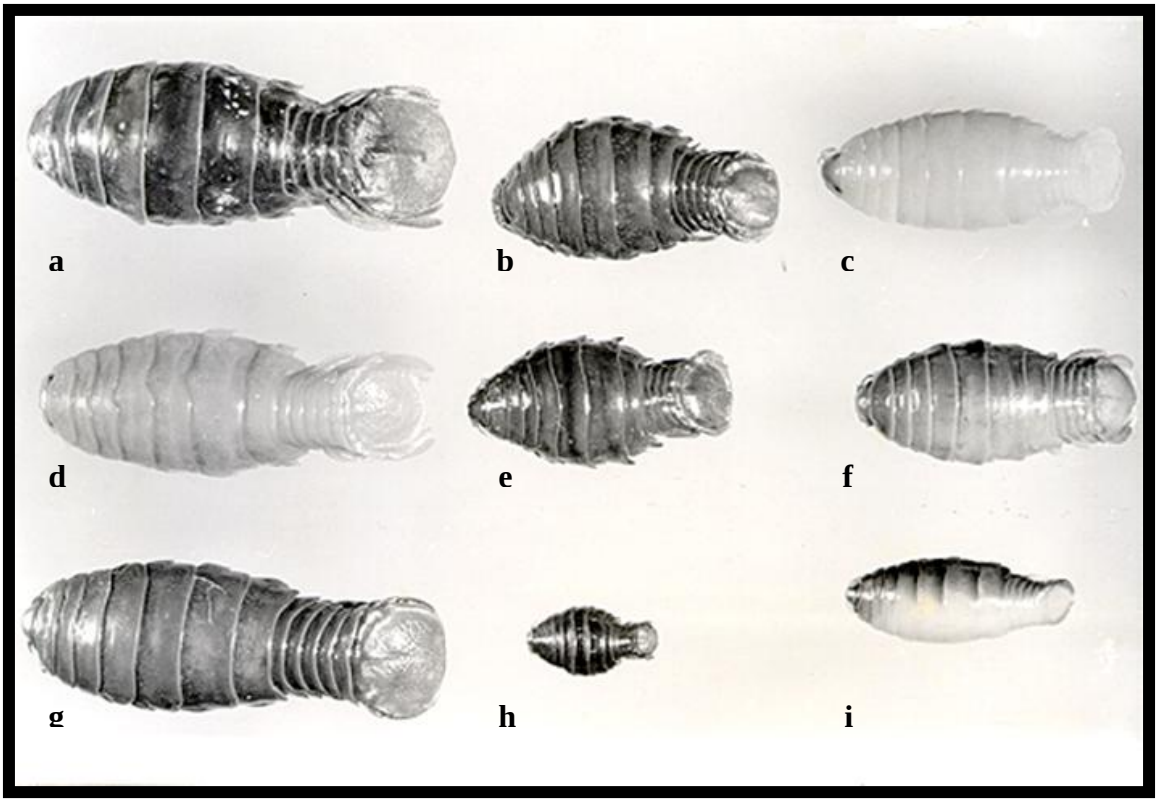

Figure 5. Nine species of *Anilocra* from the Caribbean. (a) *A. holocentri*, (b) *A. holacanthi,* (c) *A. haemuli,* (d) *A. myripristis*, (e) *A. chaetodontis*, (f) *A. abudefdufi*, (g) *A. acanthuri*, (h) *A. partiti,* (i) *A. chromis*. (from Williams and Williams, 1981)

Kensley and Schotte (1989) provided a taxonomic key for the nine species of *Anilocra* in the Caribbean:

1. Pereopods 2-4 with swelling on outer margin of dactylus ……2

   Pereopods 2-4 lacking swelling on outer margin of dactylus ……5

2. Body axis distorted by more than 10˚ ……...*holacanthi*

   Body axis distorted by less than 5˚ ……...3

3. Dactylus of pereopod 7 longer than propodus ……*partiti*

   Dactylus of pereopod 7 shorter than propodus ……..4

4. Posteroventral angle of pereonite 7 overlapping pleonite 1 only ..……..*abudefdufi*

Posteroventral angle of pereonite 7 overlapping pleonites 1 and 2 …*chaetodontis*

5. Posteroventral angle of pereonite 7 produced ……6

   Posteroventral angle of pereonite 7 not produced ……7

6. Uropod reaching posterior margin of pleotelson ……*myripristis*

   Uropod not reaching posterior margin of pleotelson ……..*haemuli*

7. Posteroventral angle of pereonite 7 overlapping pleonite 1 ……..*holocentri*

   Posteroventral angle of pereonite 7 not overlapping pleonite 1 ……...8

8. Uropod reaching posterior margin of pleotelson ……..*acanthuri*

   Uropod not reaching posterior margin of pleotelson ……..*chromis*

In addition to the characters used by Kensley and Schotte (1989) in their taxonomic key, Williams and Williams (1981) also used the shape and size of the telson, length of the endopod of the uropod, number of articles and length of antennae and antennule, shortest and longest pereonite, and quantity of setae on mandibular palp as characters for the diagnosis of each species.

Hosts and geographic distributions for the Caribbean species of *Anilocra*

Anilocra abudefdufi is found attached beneath the eye of one species of the family Pomacentridae, *Abudefdufi saxatilis* (Linneaus 1758) (sergeant major), in the Caribbean coasts of Panamá and Colombia (Williams and Williams, 1981; Williams, 1984).

Anilocra acanthuri parasitizes two species of the family Acanthuridae, *Acanthurus bahianus* Castelnau 1855 (ocean surgeon) and *Acanthurus chirurgus* (Bloch 1787) (doctorfish), under the pectoral fin. The isopod's distribution on each host

is listed in Table 3. Williams (1984) noted that the hosts are parasitized in mutually exclusive geographic ranges, with the exception of one Bahama Island (Long Island) were both hosts had the isopod attached. Since only a single doctorfish was being parasitized, Williams (1984) could not determine if it was a case of redistribution of the isopod or a case of the isopod mistaking its host.

Anilocra chaetodontis attaches beneath the eye on four species of the family Chaetodontidae*: Chaetodon capistratus* Linneaus 1758 (foureye butterflyfish), *Chaetodon ocellatus* Bloch 1787 (spotfin butterflyfish)*, Chaetodon sedentarius* Poey 1860 (reef butterflyfish), *Chaetodon striatus* (banded butterflyfish) Linneaus 1758. This isopod's distribution on each host is listed in Table 3. According to Williams (1984), *A. chaetodontis* appeared to be less host specific and more opportunistic since it tends to parasitize the most abundant butterflyfish in the area, and the parasite-host relationships do not show any geographic variation.

Anilocra chromis occurs beneath the eye of two species of the family Pomacentridae, *Chromis multilineata* (Guichenot 1853) (brown chromis) and *Chromis cyanea* (Poey 1860) (blue chromis). This isopod selectively parasitizes the blue chromis in the western Caribbean and the brown chromis in the eastern Caribbean (Table 3), even though both fish occur sympatrically throughout the Caribbean (Williams and Williams, 1981; Williams et al., 1983; Williams, 1984).

Anilocra haemuli is found attached beneath the eye on three groups of fishes (grunts, groupers, and creole-fish) in two families, Haemulidae and Serranidae. In the family Haemulidae (grunts) only the genera *Haemulon* Cuvier 1829 and *Orthopristis* Girard 1858 are parasitized, while in the family Serranidae it is found on groupers,

genera *Epinephelus* Bloch 1793*, Cephalopholis* Bloch and Schneider 1801*,* and *Mycteroperca* Gill 1862, and on the creole-fish, *Paranthias furcifer* (Valenciennes 1828). Williams and Williams (1981) reported *Cephalopholis cruentata* (Lacepède 1802) (graysby), *E. fulvus* (Linnaeus 1758) (coney), *E. guttatus* (Linnaeus 1758) (red hind), *H. aurolineatum* Cuvier 1830 (tomtate), *H. carbonarium* Poey 1860 (caesar grunt), *H. chrysargyreum* Günther 1859 (smallmouth grunt)*, H. flavolineatum* (Desmarest 1823) (French grunt)*, H. macrostomum* Günther 1859 (Spanish grunt), *H. plumierii* (Lacepède 1801) (white grunt), *H. sciurus* (Shaw 1803) (bluestriped grunt), *O. ruber* (Cuvier 1830) (corocoro grunt), and the creole-fish as hosts for *A. haemuli.* Williams (1984) reported the same hosts as Williams and Williams (1981) and three additional hosts: *E. adscensionis* (Osbeck 1765) (rock hind)*, H. bonariense* Cuvier 1830 (black grunt), *H. steindachneri* (Jordan and Gilbert 1882) (chere-chere grunt). Bunkley-Williams et al. (1998) reported again on the corocoro grunt and Bunkley-Williams et al. (1999) on the creole-fish and on two additional species: *M. rubra* (Bloch 1793) (mottled grouper) and *M. bonaci* (Poey 1860) (black grouper). Ortiz et al. (2003) reported for the first time a host, *E. fulvus*, for *A. haemuli* from Cuba. Bunkley-Williams et al. (2006) reported *A. haemuli* on the black grunt, the chere-chere grunt, the corocoro grunt, the French grunt, and on a new host, *H. boschmae* (Metzelaar 1919) (bronzestripe grunt). Additionally, they reported juveniles *A. haemuli* from *Heteropriacanthus cruentatus* (Lacepède 1801) (glasseye), family Priacanthidae, which they believe was serving as a temporary host for the isopod juveniles (Dr. L.B. Williams, personal communication).

Anilocra haemuli's host and geographic distribution are presented in Table 3. This isopod has not been found to infect grunts and creole-fish at the same locality,

although it parasitizes the grunts and the groupers in the Puerto Rico and the U.S. and British Virgin Islands, and the groupers and the creole-fish at Mona Island, Dominican Republic, Barbados and Colombia. Regarding this geographic distribution, Williams (1984) suggests that the sub-populations may have at one time been isolated from one another and have now redispersed to some overlapping areas.

Anilocra holacanthi parasitizes one species of the family Pomacanthidae, *Holacanthus tricolor* (Bloch 1795) (rock beauty), beneath the eye. This isopod can be found in Mona Island, Puerto Rico, U.S. and British Virgin Islands, Dominican Republic, Bahamas Islands, and Jamaica (Williams and Williams, 1981; Williams et al., 1983; Williams, 1984).

Anilocra holocentri is found on *Holocentrus adscensionis* (Osbeck 1765) (squirrelfish) in the eastern Caribbean, while *Anilocra myripristis* occurs on *Myripristis jacobus* Cuvier 1829 (blackbar soldierfish) in the western Caribbean (see Table 3) (Williams and Williams, 1981; Williams et al., 1983; Williams, 1984; Fernández and Ortiz-Touzet, 2004). These parasites are morphologically similar and both attach in the interorbital region of the head of their respective hosts which belong to the family Holocentridae.

Anilocra partiti occurs only in Jamaica on the subocular region of *Pomacentrus partitus* (Poey 1868) (bicolor damselfish) of the family Pomacentridae (Williams and Williams, 1981; Williams, 1984).

Table 3. Hosts and geographic distributions of the Caribbean species of *Anilocra.*

***Anilocra* species**	**Fish host species**	**Geographic distribution**	**Revision reference**
abudefdufi	*Abudefdufi saxatilis*	Colombia, Panamá	Williams and Williams (1981); Williams (1984)
acanthuri	*Acanthurus bahianus*	Bahamas Island, Dominican Republic, Mona Island	Williams and Williams (1981); Williams (1984)
	Acanthurus chirurgus	Anegada, Bahamas Islands, Culebra Island, Florida, Puerto Rico, St. John, St. Thomas	Williams and Williams (1981); Williams (1984)
chaetodontis	*Chaetodon capistratus*	Bahamas Islands	Williams and Williams (1981)
		Culebra Island, Mona Island, Mosquito Island, Puerto Rico, St. Croix, St. John, Virgin Gorda,	Williams and Williams (1981); Williams (1984)
		Dominican Republic	Williams (1984)
	Chaetodon ocellatus	Bahamas Islands, Puerto Rico, St.Thomas	Williams and Williams (1981); Williams (1984)
		St. Croix	Williams (1984)
	Chaetodon sedentarius	Puerto Rico	Williams and Williams (1981); Williams (1984)
		Cuba	Fernández and Ortiz-Touzet (2004)
	Chaetodon striatus	Anegada, Bahamas Islands, Mona Island, Puerto Rico	Williams and Williams (1981); Williams (1984)
		Dominican Republic	Williams (1984)
		St. Croix, St. John	Williams (1984)
chromis	*Chromis cyanea*	Bahamas Islands, Dominican Republic	Williams and Williams (1981); Williams (1984)
		Florida, Haiti	Williams (1984)
	Chromis multilineata	Anegada, Culebra Island,	Williams and Williams
		Mona Island, Mosquito Island, Puerto Rico, St. Croix, St. John, Vieques Island, Virgin Gorda,	(1981); Williams (1984)
		Montserrat	Williams (1984)
haemuli	*Cephalopholis cruentata*	Bahamas Islands, Dominican Republic, St. John	Williams and Williams (1981); Williams (1984)
		Barbados	Williams (1984)
	Epinephelus adscensionis	Barbados, St. Croix,	Williams (1984)
		Cuba	Ortiz et al. (2003)
	Epinephelus guttatus	Anegada, Puerto Rico, St. John, St. Thomas	Williams and Williams (1981); Williams (1984)
		Dominican Republic, St. Croix, Vieques Island	Williams (1984)
	Epinephelus fulvus	Bahamas Islands, Dominican Republic, Guadeloupe, Mona Island, Puerto Rico, St. John, St. Thomas, St. Croix	Williams and Williams (1981); Williams (1984)
		Barbados	Williams (1984)
	Haemulon aurolineatum	Jamaica, Puerto Rico	Williams and Williams (1981); Williams (1984)
		St. Thomas, Trinidad	Williams (1984)

Table 3. (continued)

Anilocra species	*Fish host species*	Geographic distribution	Revision reference
	Haemulon bonariense	Puerto Rico, Venezuela	Williams (1984); Bunkley-Williams et al. (2006)
	Haemulon boschmae	Venezuela	Bunkley-Williams et al. (2006)
	Haemulon carbonarium	Puerto Rico, St. John	Williams and Williams (1981); Williams (1984)
		Tobago	Williams (1984)
	Haemulon chrysargyreum	Culebra Island, PR	Williams (1984)
		Puerto Rico	Williams and Williams (1981)
		St. John	Williams and Williams (1981); Williams (1984)
	Haemulon flavolineatum	Culebra Island, Puerto Rico, St. John, St. Thomas, Virgin Gorda, Mosquito Island, Florida	Williams and Williams (1981); Williams (1984)
		Venezuela	Bunkley-Williams et al. (2006)
	Haemulon macrostomum	Puerto Rico	Williams and Williams (1981); Williams (1984)
		St. John, St. Thomas	Williams (1984)
	Haemulon plumierii	Mexico, Florida	Williams and Williams (1981); Williams (1984)
		Trinidad	Williams (1984)
	Haemulon sciurus	Florida	Williams and Williams (1981); Williams (1984)
		Puerto Rico	Williams (1984)
	Haemulon steindachneri	Trinidad	Williams (1984)
		Venezuela	Bunkley-Williams et al. (2006)
	Heteropriacanthus cruentatus	Venezuela	Bunkley-Williams et al. (2006)
	Mycteroperca rubra	Colombia	Bunkley-Williams et al. (1999)
	Mycteroperca bonaci	Colombia	Bunkley-Williams et al. (1999)
	Orthopristis ruber	Venezuela	Williams and Williams (1981); Williams (1984); Bunkley-Williams et al. (1998); Bunkley-Williams et al. (2006)
	Paranthias furcifer	Barbados, Bonaire, Curacao	Williams (1984)
		Mona Island, Dominican Republic	Williams and Williams (1981)
		Colombia	Williams and Williams (1981); Williams (1984); Bunkley-Williams et al. (1999)
		Dominican Republic, Desecheo Island, Mona Island	Williams and Williams (1981); Williams (1984)

Table 3. (continued)

***Anilocra* species**	***Fish host species***	**Geographic distribution**	**Revision reference**
holacanthi	*Holacanthus tricolor*	Bahamas Island, Dominican Republic, Jamaica, Mona Island, Puerto Rico, St. John, Virgin Gorda	Williams and Williams (1981); Williams (1984)
		Culebra Island, PR	Williams (1984)
holocentri	*Holocentrus adscencionis*	Puerto Rico, St. John	Williams and Williams (1981); Williams (1984)
		St. Croix	Williams (1984)
		St. Thomas	Williams and Williams (1981); Williams (1984)
myrispristis	*Myrispristis jacobus*	Bahamas Islands, Mona Island	Williams and Williams (1981); Williams (1984)
		Cuba	Fernández and Ortiz-Touzet (2004)
		Dominican Republic	Williams and Williams (1981); Williams et al. (1983); Williams (1984)
partiti	*Pomacentrus partitus*	Jamaica	Williams and Williams (1981); Williams (1984)

MATERIALS AND METHODS

Specimen collection

Seventy-five specimens representing five species of *Anilocra* were collected by Dr. Ernest H. Williams, Jr. and Dr. Paul Sikkal. The isopods were manually removed from the fish host, which were trapped in a net, and then released. The isopod species sampled were*: A. acanthuri, A. chaetodontis, A. chromis, A. haemuli, A. holocentri* from the Virgin Islands, and *A. chaetodontis, A. chromis, A. haemuli* from Puerto Rico. Collection details are listed in Table 4 and collection areas shown in Fig. 6. Isopods were placed in a cooler upon collection and transported to the laboratory at the University of Puerto Rico-Mayagüez, where they were preserved in CTAB buffer (2 % cetyltrimethylammonium-bromide, 100 mM Tris-Cl, 20 mM EDTA, and 1.4 M NaCl) and stored at -20 ˚C until further processing.

Figure 6. Map of the localities of sample collection in this study.

Table 4. Collection data and sequence identification of specimens used in this study.

Isopod species	Specimen Code	Locality	Collection date	Host	COI sequence identification
A. acanthuri	DF30	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI1
A. acanthuri	DF31	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI2
A. acanthuri	DF32	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI3
A. acanthuri	DF33	St. Thomas, USVI	07/2008	*A. chirurgus*	-
A. acanthuri	DF34	St. Thomas, USVI	07/2008	*A. chirurgus*	-
A. acanthuri	DF35	St. Thomas, USVI	07/2008	*A. chirurgus*	-
A. acanthuri	DF36	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI4
A. acanthuri	DF37	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI5
A. acanthuri	DF38	St. Thomas, USVI	07/2008	*A. chirurgus*	*A. acanthuri* USVI6
A. chaetodontis	BFF1	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR1
A. chaetodontis	BFF2	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR2
A. chaetodontis	BFF3	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR3
A. chaetodontis	BFF4	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR4
A. chaetodontis	BFF5	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR5
A. chaetodontis	BFF6	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR6
A. chaetodontis	BFF7	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR7
A. chaetodontis	BFF8	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR8
A. chaetodontis	BFF9	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR9
A. chaetodontis	BFF10	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR10
A. chaetodontis	BFF11	La Parguera, PR	01/2007	*C. capistratus*	*A. chaetodontis* PR11
A. chaetodontis	BFF20	St. John, USVI	07/2007	*C. capistratus*	*A. chaetodontis* USVI1
A. chaetodontis	BFF21	St. John, USVI	07/2007	*C. capistratus*	*A. chaetodontis* USVI2
A. chaetodontis	BFF30	Tortola, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI1
A. chaetodontis	BFF31	Tortola, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI2
A. chaetodontis	BFF32	Tortola, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI3
A. chaetodontis	BFF33	Tortola, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI4
A. chaetodontis	BFF34	Tortola, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI5
A. chaetodontis	BFF40	Guana Island, BrVI	07/2008	*C. capistratus*	*A. chaetodontis* BrVI6
A. chromis	BC1	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR1
A. chromis	BC2	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR2
A. chromis	BC3	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR3
A. chromis	BC4	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR4
A. chromis	BC5	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR5
A. chromis	BC6	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR6
A. chromis	BC7	La Parguera, PR	03/2007	*Ch. multilineata*	*A. chromis* PR7
A. chromis	BC20	USVI	07/2007	*Ch. multilineata*	*A. chromis* USVI1
A. chromis	BC21	USVI	07/2007	*Ch. multilineata*	*A. chromis* USVI2
A. chromis	BC22	USVI	07/2007	*Ch. multilineata*	*A. chromis* USVI3
A. chromis	BC23	USVI	07/2007	*Ch. multilineata*	*A. chromis* USVI4
A. chromis	BC50	Culebra Island, PR	01/2008	*Ch. multilineata*	*A. chromis* PR8
A. haemuli	FG1	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR1
A. haemuli	FG2	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR2
A. haemuli	FG3	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR3
A. haemuli	FG4	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR4
A. haemuli	FG5	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR5
A. haemuli	FG6	La Parguera, PR	01/2007	*H. flavolineatum*	*A. haemuli* PR6
A. haemuli	FG20	USVI	07/2007	*H. flavolineatum*	*A. haemuli* USVI1
A. haemuli	FG21	USVI	07/2007	*H. flavolineatum*	*A. haemuli* USVI2
A. haemuli	FG22	USVI	07/2007	*H. flavolineatum*	*A. haemuli* USVI3
A. haemuli	FG30	Tortola, BrVI	07/2008	*H. flavolineatum*	*A. haemuli* BrVI1
A. haemuli	FG31	Tortola, BrVI	07/2008	*H. flavolineatum*	*A. haemuli* BrVI2
A. haemuli	FG32	Tortola, BrVI	07/2008	*H. flavolineatum*	*A. haemuli* BrVI3
A. haemuli	FG33	Tortola, BrVI	07/2008	*H. flavolineatum*	*A. haemuli* BrVI4
A. haemuli	FG34	BrVI	07/2008	*H. flavolineatum*	*A. haemuli BrVI5*
A. haemuli	FG35	BrVI	07/2008	*H. flavolineatum*	*A. haemuli BrVI6*

Table 4. (continued)

Isopod species	Specimen Code	Locality	Collection date	Host	COI sequence identification
A. haemuli	FG40	Guana Island, BrVI	07/2008	*H. flavolineatum*	-
A. haemuli	FG50	Culebra Island, PR	01/2008	*H. flavolineatum*	*A. haemuli* PR7
A. haemuli	FG51	Culebra Island, PR	01/2008	*H. flavolineatum*	*A. haemuli* PR8
A. haemuli	RH20	St. John, USVI	07/2007	*E. guttatus*	*A. haemuli* USVI4
A. haemuli	RH21	St. John, USVI	07/2007	*E. guttatus*	*A. haemuli* USVI5
A. haemuli	RH22	St. John, USVI	07/2007	*E. guttatus*	*A. haemuli* USVI6
A. haemuli	RH23	St. John, USVI	07/2007	*E. guttatus*	*A. haemuli* USVI7
A. haemuli	RH24	St. John, USVI	07/2007	*E. guttatus*	*A. haemuli* USVI8
A. haemuli	RH30	St. John, USVI	07/2008	*E. guttatus*	*A. haemuli* USVI9
A. haemuli	RH40	Guana Island, BrVI	07/2008	*E. guttatus*	*A. haemuli* BrVI7
A. haemuli	RH41	Guana Island, BrVI	07/2008	*E. guttatus*	*A. haemuli* BrVI8
A. haemuli	RH42	Guana Island, BrVI	07/2008	*E. guttatus*	*A. haemuli* BrVI9
A. haemuli	RH43	Guana Island, BrVI	07/2008	*E. guttatus*	*A. haemuli* BrVI10
A. haemuli	RH44	Guana Island, BrVI	07/2008	*E. guttatus*	-
A. holocentri	SF30	St. John, USVI	07/2008	*Ho. adscencionis*	*A. holocentri* USVI1
A. holocentri	SF31	St. John, USVI	07/2008	*Ho. adscencionis*	*A. holocentri* USVI2
A. holocentri	SF32	St. John, USVI	07/2008	*Ho. adscencionis*	*A. holocentri* USVI3
A. holocentri	SF33	Tortola, BrVI	07/2008	*Ho. adscencionis*	*A. holocentri* BrVI1
A. holocentri	SF40	Guana Island, BrVI	07/2008	*Ho. adscencionis*	-
A. holocentri	SF41	Guana Island, BrVI	07/2008	*Ho. adscencionis*	*A. holocentri* BrVI2

BC = brown chromis; BFF = foureye butterflyfish; BrVI = British Virgin Islands; DF = doctorfish; FG = French grunt; PR = Puerto Rico; RH = red hind; SF = squirrelfish; USVI = U.S. Virgin Islands

Figure 7. Specimens of *Anilocra acanthuri* and *Anilocra holocentri* collected in this study. *A. acanthuri* (a) dorsal side, (b) ventral side showing oostegites. *A. holocentri* (c) dorsal side, (d) ventral side showing brood pouch covering the mouthparts.

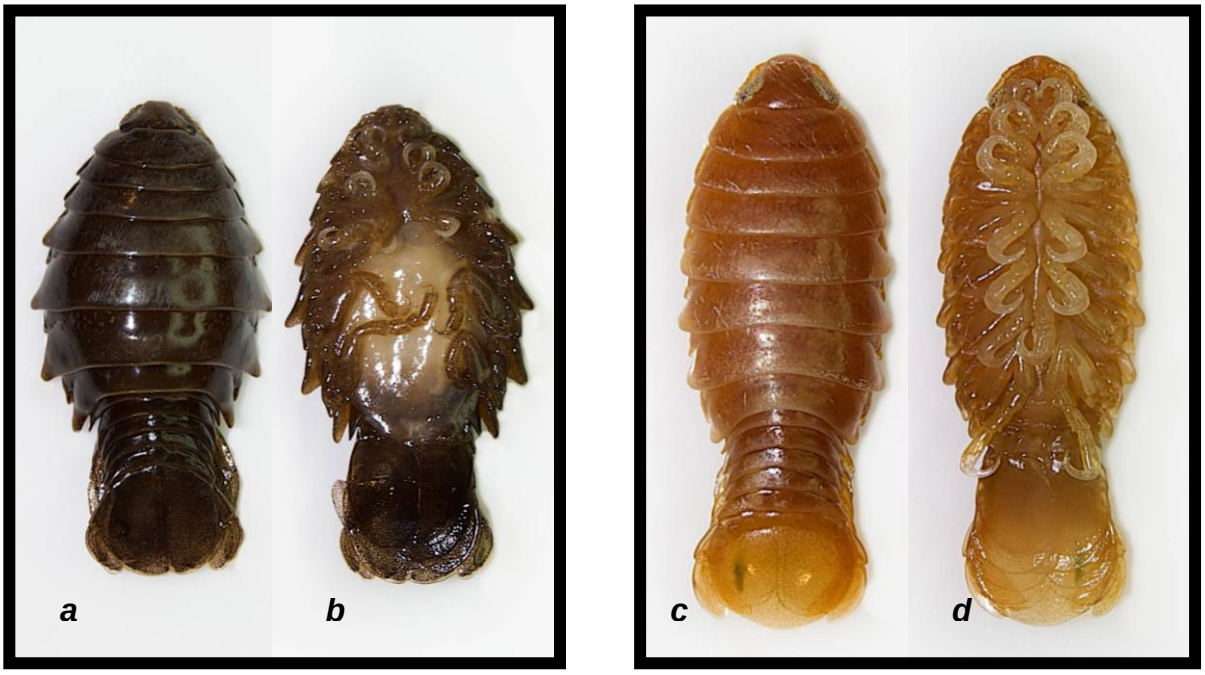

Figure 8. Specimens of *Anilocra chaetodontis* and *Anilocra haemuli* collected in this study. *A. chaetodontis* (a) dorsal side, (b) ventral side showing developing brood pouch. *A. haemuli* (c) dorsal side, (d) ventral side without brood pouch.

DNA extraction, amplification, and sequencing

Originally, DNA was to be extracted using a CTAB extraction protocol in which the CTAB preserved samples are subjected to several cycles of heating and quick freezing to break cells prior to phenol-chloroform extraction. Later, the extraction protocol was changed to the DNAzol® Genomic DNA Isolation Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) which has proven more effective in obtaining DNA from fresh or preserved specimens than phenol-chloroform extraction (Junqueira et al., 2002). To remove the CTAB in which they were preserved, specimens were washed with sterile deionized water. The specimen was then grounded in 500 µl of DNAzol® with a sterile pestle in a sterile 1.5 ml microcentrifuge tube. Another 500 µl of DNAzol® was added to the tube and the contents mixed twice by inversion. The mix was centrifuged at 16,000 *g* for 10 min and the supernatant transferred to a new sterile

microcentrifuge tube. To precipitate the DNA, 500 µl of ice-cold absolute ethanol was added to the supernatant, the contents mixed 5 times by inversion, incubated for 5 min at room temperature, and centrifuged at 10,000 *g* for 5 min. The supernatant was discarded and the pellet washed twice with 800 µl of ethanol (95 % and 75 %, respectively) and centrifuged at 1,000 *g* for 1.5 min. The DNA was then dried for 10 min at room temperature in a Vacufuge™ Concentrator5301 (Eppendorf AG, Hamburg, Germany) and afterwards resuspended in 50 µl of sterile deionized water. This DNA was cleaned using the Elu-Quik® DNA Purification Kit (Schleicher & Schuell BioScience, Inc., Keene, NH, USA) following the manufacturer's protocol before being subjected to amplification.

For this study, the mitochondrial cytochrome c oxidase subunit 1 (COI) gene was chosen for this study for its usefulness in determining relationships among closely related species (Hwang and Kim, 1999) and its successful use in isopod phylogenetic studies (e.g. Wetzer, 2001, 2002; McGaughran et al. 2006; Teske et al., 2006; Ketmaier et al., 2007).

Table 5. Primers used for DNA amplification. (from Teske et al., 2006)

Gene	Direction	Primer	Sequence
COI	forward	CrustCOIF	5'- TCA ACA AAT CAY AAA GAY ATT GG-3'
	reverse	PeraCOIR	5'- TAT WCC TAC WGT RAA TAT ATG ATG-3'

The polymerase chain reation (PCR) cocktail consisted of 5 µl of 5X GoTaq Flexi Buffer, 3.5 mM $MgCl_2$, 0.4 mM dNTP mix, 0.4 µM of each primer (Table 5), 0.1 µl of *Taq* DNA polymerase (5 U μl^{-1}) (Promega Corporation, Madison, WI, USA), 10 µl template DNA (1:50 or 1:100 dilution), and deionized water to yield a final volume of 25 µl.

PCR reactions were run on a Mastercycler (Eppendorf AG, Hamburg, Germany). The PCR profile of Teske et al. (2006) was followed: initial denaturation at 94 ˚C for 3 min, followed by 35 cycles for 30 sec at 94 ˚C, annealing for 45 sec at 45 ˚C, elongation for 90 sec at 72 ˚C, and a final elongation at 72 ˚C for 10 min. Three microliters of each amplified product were evaluated by electrophoresis on a 1 % agarose gel stained with ethidium bromide (10 mg ml^{-1}) in a 1X TAE buffer (40 mM Tris, 20 mM Acetic Acid, 1 mM EDTA). PCR products were separated by electrophoresis in a low melting agarose (Molecular Sigma Biology, St. Louis, MO, USA) gel and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, USA) following the manufacturer's protocol. Some difficult and resilient samples were cloned using the pGEM-T Easy Vector System II cloning kit (Promega Corporation, Madison, WI, U.S.A.) and the plasmids extracted with the Wizard® *Plus* SV Minipreps DNA Purification System (Promega Corporation, Madison, WI, USA) following the manufacturer's protocol.

Cleaned PCR products and plasmids were sent to the Nevada Genomic Center (University of Reno, Nevada, USA) to be sequenced. The samples were sequenced in both, forward and reverse, directions on an ABI Prism 3730 DNA Analyzer using the ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1. The DNA from the cleaned COI PCR products were sequenced with the same primers used for amplification (see Table 5), while the plasmids were sequenced with primers T7 and SP6 provided by the Nevada Genomic Center.

Sequence assembly and analyses

Sequence reads were verified as being derived from isopod DNA using the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) webpage (http://www.ncbi.nlm.nih.gov/). Contigs were assembled, edited, and aligned using Sequencher 4.2 (Gene Codes Corporation©, Ann Harbor, MI, USA). Outgroup sequences (see Table 6) were retrieved from GenBank from the NCBI webpage. They included available COI gene sequences of species from the family Cymothoidae and its sister group, family Bopyridae (Dreyer and Wägele, 2001). These sequences, and those generated in this study, were aligned using ClustalW (Thompson et al., 1994) as implemented in MEGA (Molecular Evolutionary Genetics Analysis) version 4.0.2 (Tamura et al., 2007) under the default parameters. The resulting alignments were manually improved and verified that the translated protein sequence alignments did not include termination codons.

Table 6. Outgroups used in this study.

Species	Family	GenBank accession number	Number of base pairs (bp)
Bopyroides hippolytes (Krøyer 1838)	Bopyridae	DQ889082	632
Anilocra physodes (Linnaeus 1758)	Cymothoidae	EF455817	446
Ceratothoa collaris Schiöedte and Meinert 1883	Cymothoidae	EF455816	445
Ceratothoa italica Schiöedte and Meinert 1883	Cymothoidae	EF455813	443
Ceratothoa oestroides (Risso 1826)	Cymothoidae	GQ240280	440
Elthusa vulgaris (Stimpson 1857)	Cymothoidae	AF255790	583
Nerocila bivittata (Risso 1816)	Cymothoidae	EF455819	446
Olencira praegustator (Latrobe 1802)	Cymothoidae	AF260844	583

The data was analyzed under parsimony, maximum likelihood (ML), and Bayesian inference (BI) in order to estimate a plausible phylogeny describing the evolutionary

relationships between species (Holder and Lewis, 2003). The parsimony analysis was performed using PAUP* (Phylogenetic Analysis Using Parsimony) version 4.0 (Swofford, 2002). The starting tree for the heuristic search was obtained via simple stepwise addition and 10 replicates of the heuristic search were performed. Nodal support was calculated by bootstrap (BP) analysis with a heuristic search of 1000 pseudoreplicates via random stepwise addition. The resulting trees were summarized in a 50% majority rule tree.

Under maximum likelihood (ML) analyses the data was analysed by codon partitions and without partitions. The unpartitioned data was analysed in PAUP* with the best fit model for nucleotide substitution being estimated under the Akaike Information Criterion (AIC) (Posada and Buckley, 2004) in Modeltest version 3.7 (Posada and Crandall, 1998). The starting tree for the heuristic search was obtained via random stepwise addition and 10 replicates of the search were performed. Nodal support was calculated by BP analysis with a faststep-heuristic search of 1000 pseudoreplicates via random stepwise addition. On account that PAUP* does not provided for analysis by data partitions, raxmlGUI (Silvestro and Michalak, 2011), a user friendly graphical front-end of Stamatakis' (2006) RAxML program, was used to analyze the data by codon position under the best fit model estimated by the same program. The starting tree for each analysis was obtained via random stepwise addition under maximum parsimony and 1000 replicates of ML searches performed with nodal support calculated by rapid bootstrap algorithm (Stamatakis et al., 2008). The resulting trees for the partitioned analyses are summarized in a 50% majority rule tree, whereas for the unpartitioned analysis the most resolved of the 2 resulting trees is presented.

Finally, Bayesian inference (BI) analyses were performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) which allows for the analyses by codon position under different evolutionary models. The best fit models for each of the three partitions and for the unpartitioned data were estimated under the Bayesian Information Criterion (BIC) (Posada and Buckley, 2004) in Modeltest version 3.7. Both, the partitioned and unpartitioned analyses, were run for 2 million generations with a sample frequency of trees every 100^{th} generation for each of the 4 Markov chain Monte Carlo (MCMC) chains. The likelihood values of the MCMC chains were visualized with the trace function of the program Tracer version 1.5 (Rambaut and Drummond, 2009) and the number of trees that should be removed as burn-in determined. The first 2,000 and 2,500 trees were removed as burn-in for the partitioned and unpartitioned analyses, respectively. Posterior probabilities (PP), the frequency of recovering a particular tree or clade during the course of the chain (Huelsenbeck and Ronquist, 2001), were generated using MCMC. The resulting trees were summarized in a 50% majority rule tree for both analyses.

RESULTS

Sampling

All isopod specimens were collected off hosts from which they had already been reported in the literature. This study expanded the locality records of *A. chaetodontis, A. haemuli,* and *A. holocentri* to include Tortola and Guana Island in the British Virgin Islands. In both islands, *A. chaetodontis* was collected from *Chaetodon capistratus*, *A. haemuli* from *Haemulon flavolineatum*, and *A. holocentri* from *Holocentrus adscencionis*. Meanwhile, *A. haemuli* from *Epinephelus guttatus* was only collected from Guana Island.

Data Properties

The products amplified for the COI gene were approximately 900 bp, but all sequences were trimmed to 760 bp to match the shortest generated sequence. Only partial sequences were obtained for samples DF33, DF34, DF35, SF40, due to unsuccessful sequencing of the reverse complement strand and were not included in the analyses. Also excluded were sequences obtained from samples FG40 and RH44 due to uncertainty in handling and identification of specimens.

The data matrix used for the phylogenetic analyses consisted of 77 isopod sequences, 69 ingroup and 8 outgroup. In the parsimony analysis, 404 characters were constant, 104 variable characters were parsimony uninformative, and 252 variable characters were parsimony informative. This analysis yielded 360 parsimonious trees that were 886 steps in length with a consistency index of 0.6298, and a retention index

Table 7. Model parameters used in Maximum Likelihood analyses.

	Partition by codon position			No partition
	1st	2nd	3rd	
Model	GTR	GTR	GTR	TVM+G
Base frequencies				
A	0.293566	0.163710	0.403597	0.2939
C	0.167373	0.244745	0.068366	0.1862
G	0.282330	0.158959	0.064588	0.1482
T	0.256731	0.432586	0.463450	0.3718
Substitution rates				
A-C	1.009930	0.386873	5.355377	1.0174
A-G	4.030463	3.336067	119.404500	11.7477
A-T	2.024592	0.054082	5.523781	1.3573
C-G	0.707292	2.564654	92.246028	3.1672
C-T	13.328450	1.464460	90.302499	11.7477
G-T	1.000000	1.000000	1.000000	1.000
ti/tv ratio	-	--	-	-
Gamma	0.359098	0.214829	2.137888	0.2677

of 0.8483. The model parameters for each codon position and unpartitioned matrix estimated under AIC for ML and under BIC for BI are given in Table 7 and Table 8, respectively (see Huelsenbeck and Crandall (1997) for model descriptions). For the ML and the BI, the shape of the gamma distribution for the 1st and 2nd codon positions show a strong among-site variation, while the high value of the gamma distribution for

Table 8. Model parameters used in Bayesian Inference.

	Partition by codon position			No partition
	1st	2nd	3rd	
Model	TrN+G	HKY+G	TVM+G	HKY+G
Base frequencies				
A	0.3191	0.1423	0.3593	0.2899
C	0.1741	0.2602	0.1542	0.1898
G	0.2617	0.1984	0.0942	0.1517
T	0.2451	0.3991	0.3923	0.3686
Substitution rates				
A-C	1.0000	-	0.4716	-
A-G	3.3870	-	13.8281	-
A-T	1.0000	-	0.3518	-
C-G	1.0000	-	4.5502	-
C-T	11.6191	-	13.8281	-
G-T	1.0000	-	1.0000	-
ti/tv ratio	-	1.2445	-	3.7162
Gamma	0.3309	0.2829	2.5329	0.2770

the 3rd position indicates a lower heterogeneity among sites (Huelsenbeck and Crandall, 1997). Also, for both sets of unpartitioned data, the gamma distribution indicates high rates of among-site variation.

Phylogenetic analyses

Phylogenetic relationships among clades were consistent within the topologies obtained with parsimony (Fig. 9), ML (Figs. 10-11), and BI (Figs. 12-13) analyses. The phylogenetic relationships between the ingroup and outgroup clades show that *A. physodes* is not the sister species to the Caribbean *Anilocra* species, but instead is nested within the outgroup clade. All topologies show *N. bivittata* as the closest relative to the Caribbean *Anilocra* species with variable support in each analysis (BP 64%, BP 76%, BP 52%, PP 0.99, PP 1.00). Nonetheless, all three inference methods consistently recovered the Caribbean *Anilocra* species as a well supported monophyletic group (BP 100%, BP 99%, BP 79%, PP 1.00, PP 1.00). Within the Caribbean *Anilocra* group four well supported groups in three monophyletic clades (A, B, C) were recovered (Figs. 9, 12-13). Parsimony and BI analyses present monophyletic clade A (*A. chromis*), clade B (*A. holocentri*) and clade C (*A. acanthuri*, *A. haemuli, A. chaetodontis*), while ML analyses only recovered clades A and C.

Anilocra chromis is strongly supported as a monophyletic group in all analyses (Figs. 9-13). In the parsimony (BP 100%) (Fig. 9) and the partitioned BI (PP 1.00) analyses (Fig. 12), it appears as the basal species of the Caribbean *Anilocra*. Meanwhile, in the unpartioned BI analysis (Fig. 13) its relationship is unresolved compared to clade C, however, it presents the best support within the Caribbean

Anilocra group. No population structure based on geographic origin of the samples was recovered for *A. chromis* from Puerto Rico and the US Virgin Islands in these analyses.

Anilocra holocentri is also recovered as a well supported monophyletic species with identical internal resolutions in both, parsimony (BP 99%) (Fig. 9) and BI (PP 0.98 and 0.71) analyses (Figs. 12-13). In the partitioned BI analysis (Fig. 12), the internal resolution has the strongest support (PP 0.99 and 0.86) and it appears as sister taxon to clade C. Only in both ML analyses *A. holocentri* fails to form a monophyletic group, with sample USVI1 grouping with the clades A and C (Figs. 10-11). In these analyses, *A. holocentri* samples are unresolved at the base of the Caribbean *Anilocra* clade.

Clade C, composed of *A. acanthuri, A. chaetodontis*, and *A. haemuli*, is consistently recovered in all analyses (Figs. 9-13). This clade has a monophyletic *A. acanthuri* and another group containing the other two species. *Anilocra acanthuri* is depicted as a well supported monophyletic species (BP 100%, BP 100%, BP 93%, PP 1.00, PP 1.00) and as the sister taxon to the *A. haemuli-A. chaetodontis* clade within clade C in all topologies. Although in all topologies the clade is internally resolved, the partitioned BI analysis (Fig.12) shows the best nodal supports. Since all samples were from the same host and geographic location, no population structure can be inferred.

The other two species in clade C, *A. haemuli* and *A. chaetodontis,* are always recovered as the most internal species of the Caribbean group (Figs. 9-13). Although the relationships of some samples are not clearly resolved, it still depicts three well defined and significant clades: *A. chaetodontis* from Puerto Rico, *A. chaetodontis* from the Virgin Islands, and *A. haemuli* from the red hind. The *A. haemuli* from the red hind group is well supported only in ML (BP 98%, BP 60%) and BI (PP 1.00, PP 1.00)

analyses. Meanwhile, the *A. haemuli* from the French grunt group, although lacking internal resolution, is well supported as sister taxon (BP 74%, BP 53%, PP 0.65, PP 0.96) to the *A. haemuli* from the red hind group, except in the unpartitioned ML analysis (Fig. 11). All analyses show a close relationship between the *A. haemuli* from the French grunt group and the *A. chaetodontis* samples. The unpartitioned ML (Fig. 11) and the partitioned BI analyses (Fig. 12) depict a weakly supported polytomy (BP <50%, PP 0.59) that includes the *A. haemuli* samples BRVI1 and USVI1, the *A. chaetodontis* from Puerto Rico clade, and the *A. chaetodontis* from the Virgin Islands clade, while the other analyses depict the *A. chaetodontis* from the Virgin Islands clade as having diverged prior to the *A. haemuli* samples BRVI1 and USVI1 (BPs <50%, PP 0.79). Both *A. chaetodontis* clades are well defined although the samples from Puerto Rico are only well supported in the BI topologies (PP 0.71, PP 0.91) (Figs. 12-13), while the *A. chaetodontis* from the Virgin Islands clade is well supported in all analyses with the strongest supported seen in the unpartitioned BI analysis (PP 0.99) (Fig. 13). *Anilocra chaetodontis* shows a clear population structure based on geographic origin of the samples in all analyses. Meanwhile, *A. haemuli* lacks a population structure based on geographic origin of samples, but clearly recovers a population structure based on host species in all analyses.

Overall, the clades were consistently recovered with all the inference methods used, except for the *A. holocentri* clade in the ML analyses. Partitioned and unpartitioned analyses did not show significant variation in clades and nodal support. The BI analyses produced the most resolved topologies and the ML topologies had the weakest nodal support.

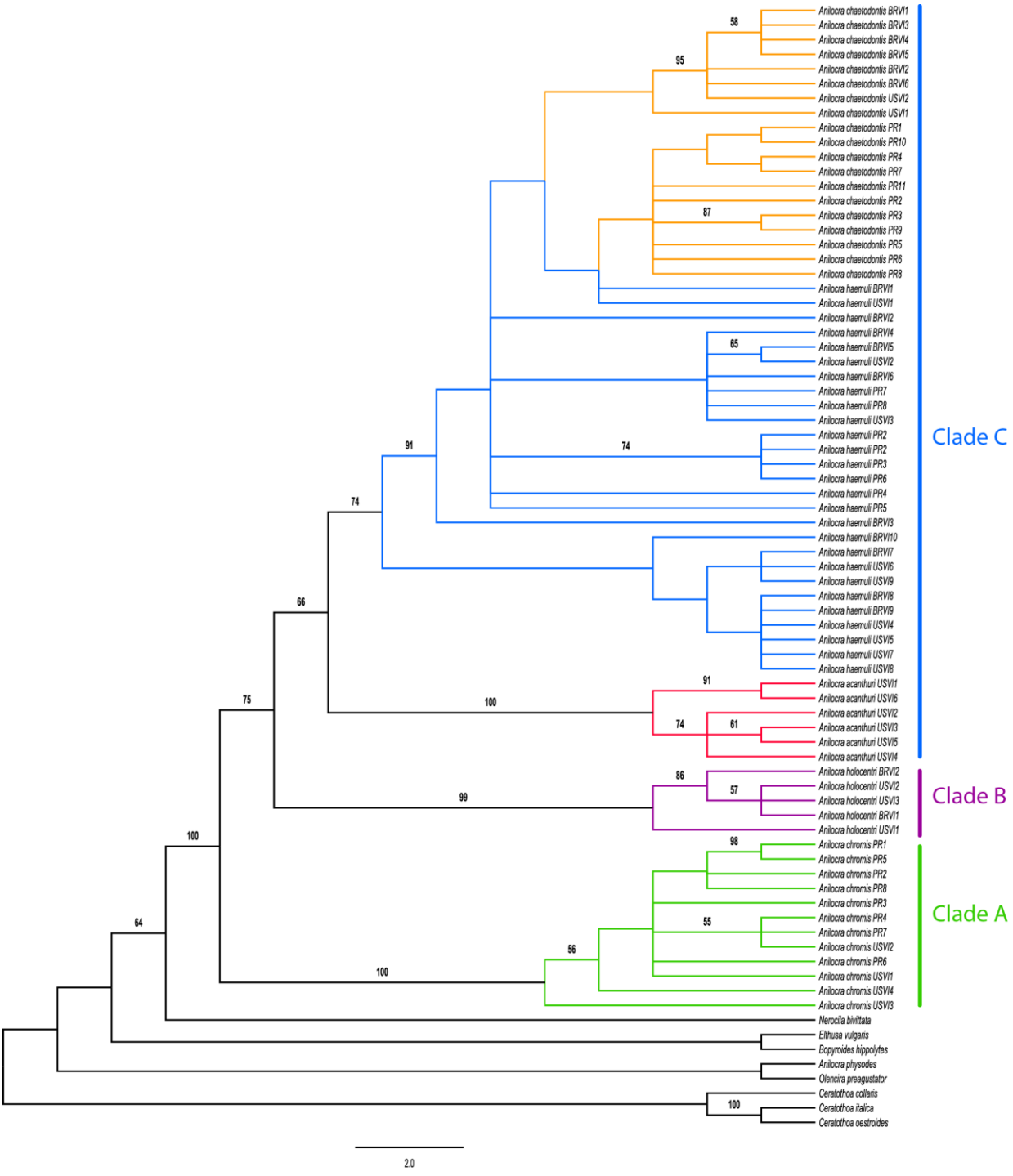

Figure 9. Majority rule tree of the most parsimonious trees resulting from the parsimony analysis. Bootstrap values of ≥ 50 % are indicated at the nodes.

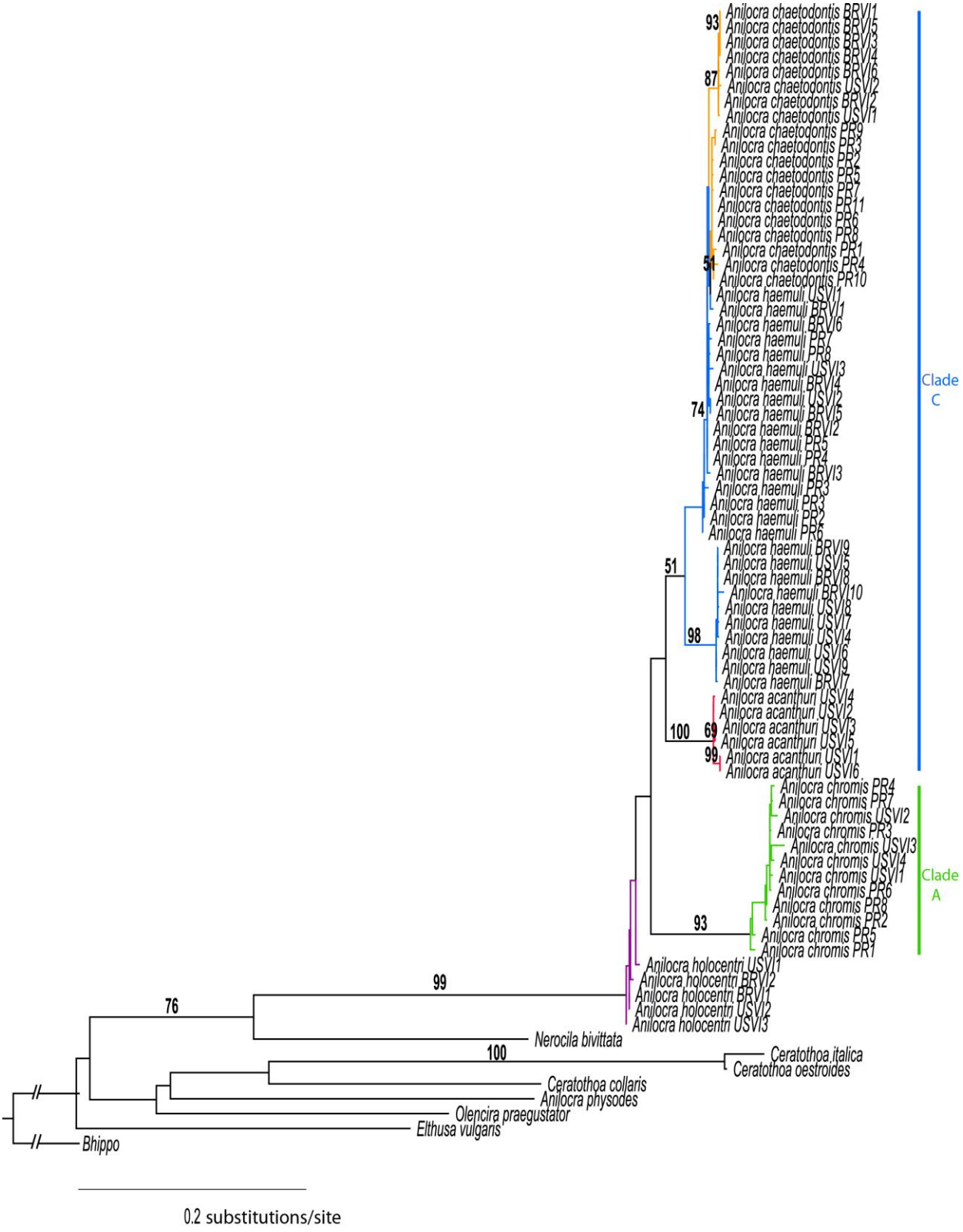

Figure 10. Majority rule tree resulting from the partitioned analysis under maximum likelihood. Bootstrap values of ≥ 50 % are indicated at the nodes.

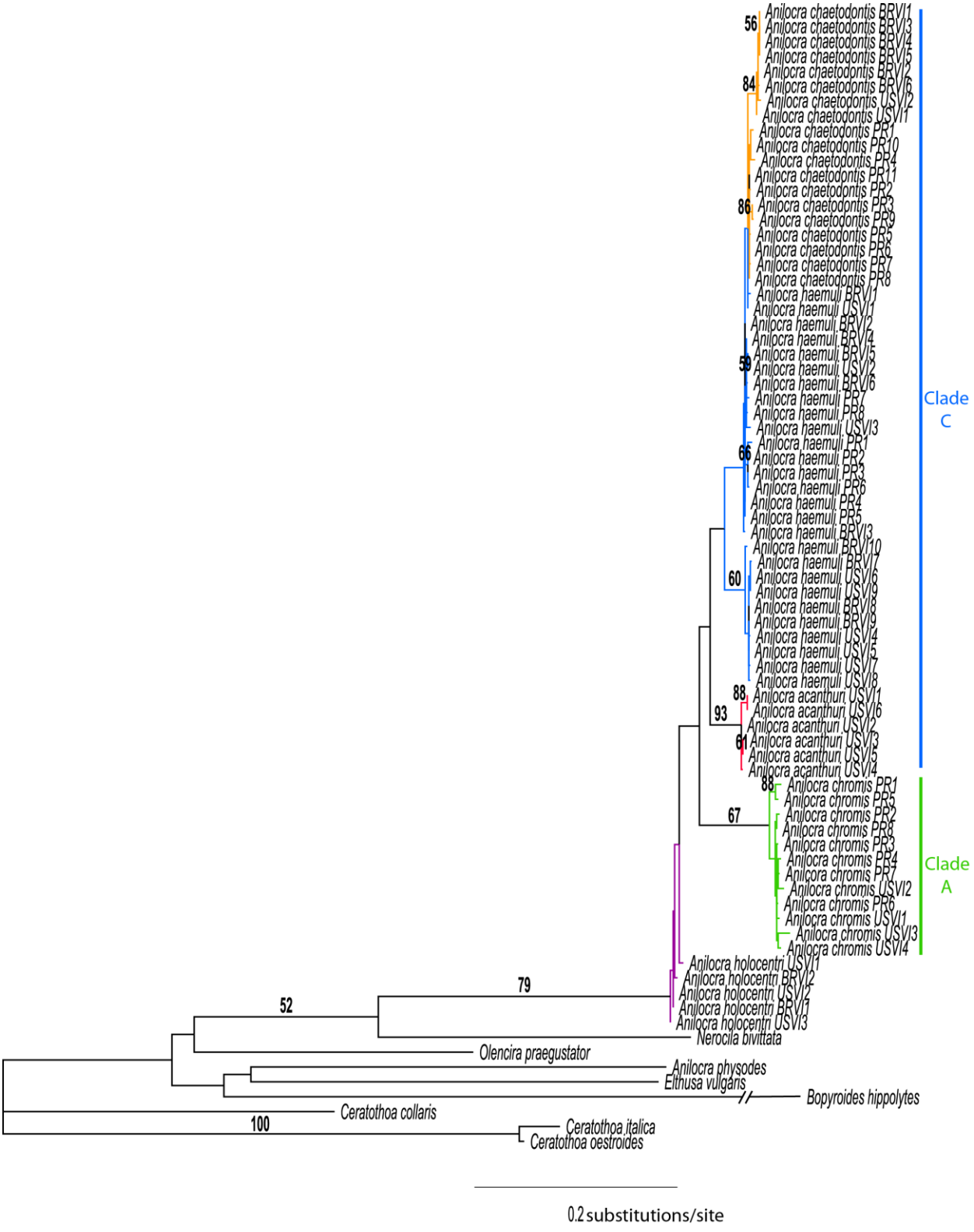

Figure 11. Phylogenetic tree resulting from the unpartitioned analysis under maximum likelihood. Bootstrap values of ≥ 50 % are indicated at the nodes.

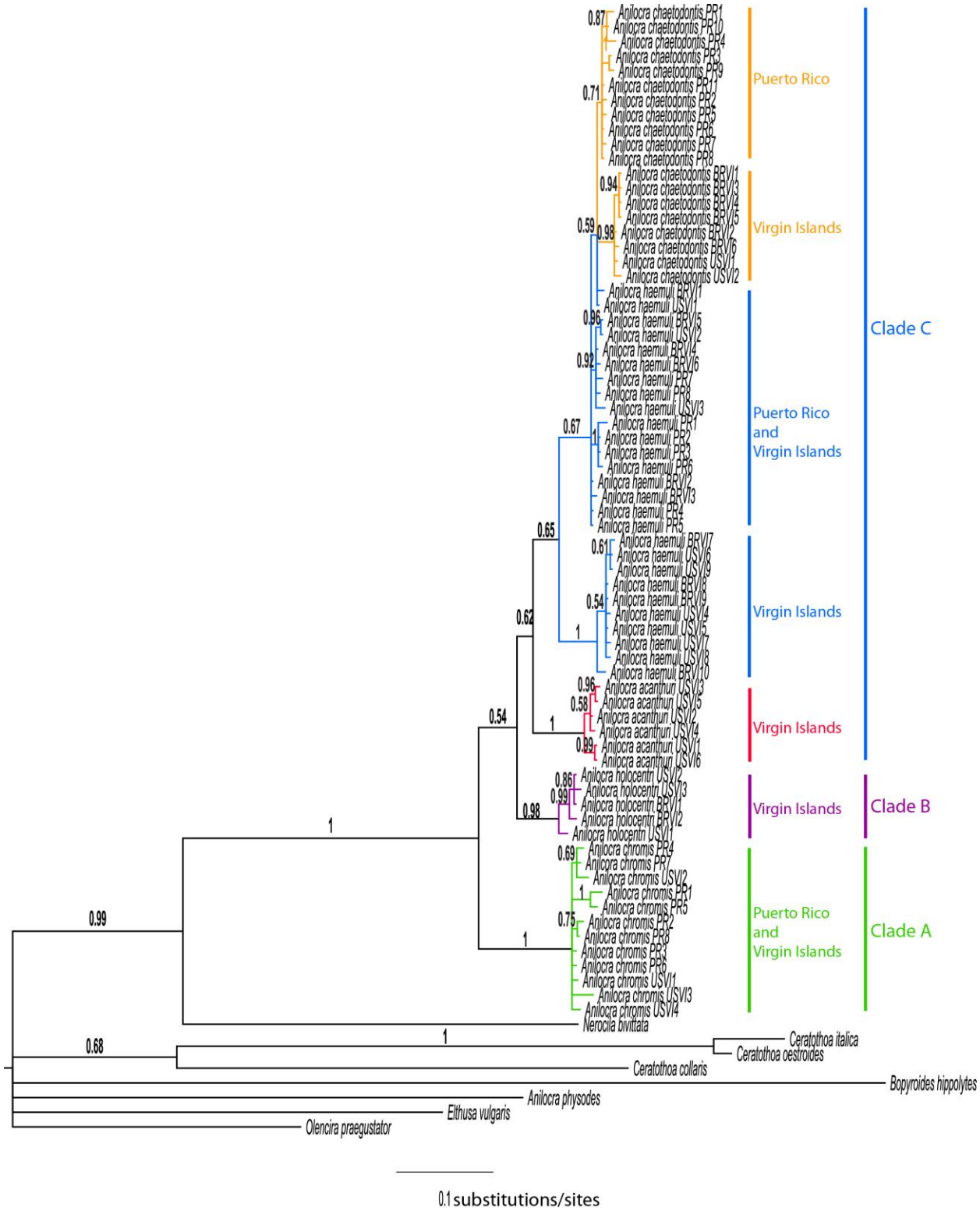

Figure 12. Majority rule tree resulting from the partitioned analysis under Bayesian inference. Posterior probabilities of ≥ 50 % are indicated at the nodes. Collection locality is indicated next to each species group.

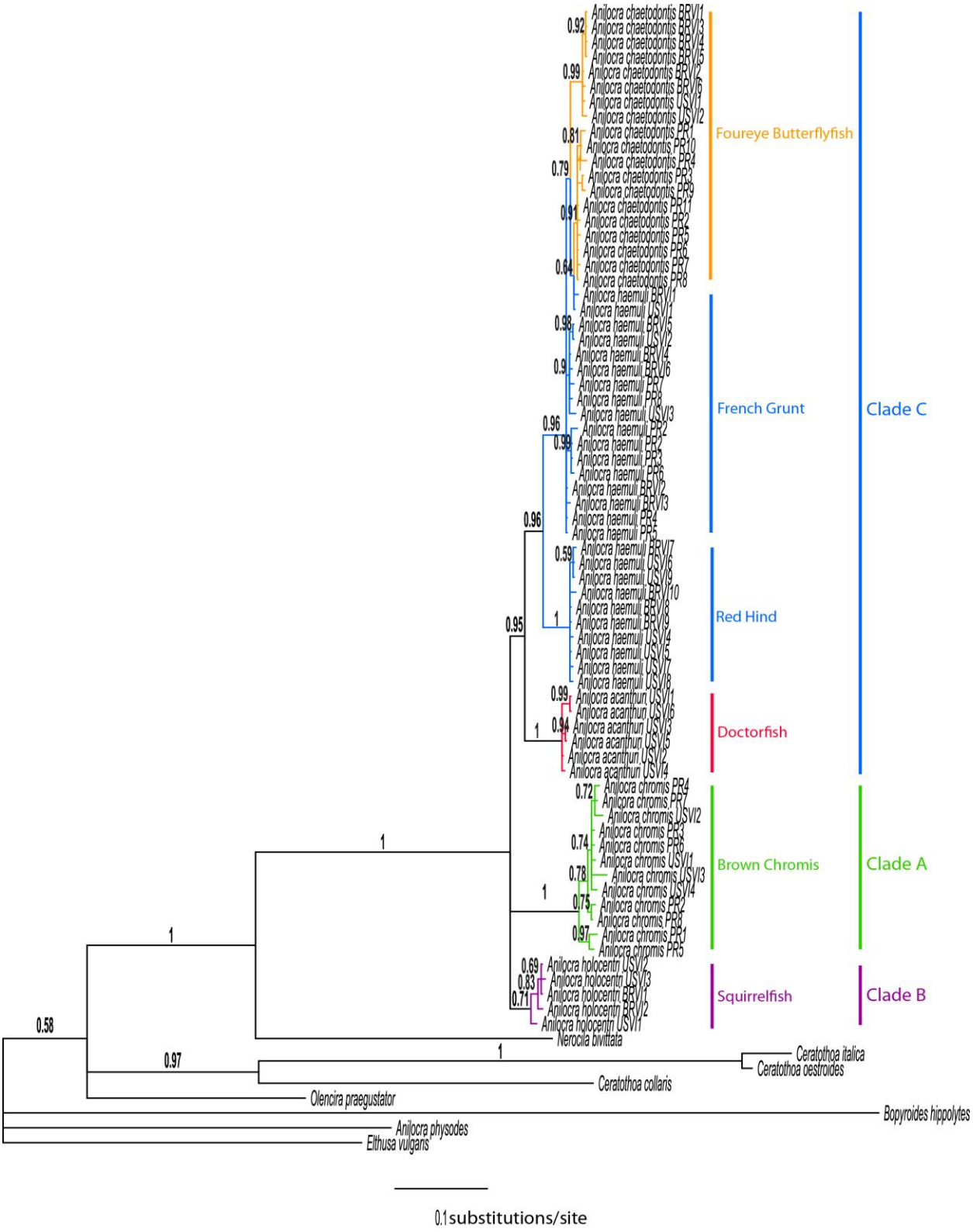

Figure 13. Majority rule tree resulting from the unpartitioned analysis under Bayesian inference. Posterior probabilities of ≥ 50 % are indicated at the nodes. Host species' common name is indicated next to each species group.

DISCUSSION

Phylogenetic reconstructions of the five species of *Anilocra* from the Caribbean Sea examined in this study show that they form a monophyletic group. Although four additional described species of *Anilocra* exist in the Caribbean they were not included because not all species occur in the localities sampled in this study (see Table 3 for geographic distributions of each species). The analyses consistently recovered three monophyletic clades: clade A comprised only of *A. chromis*, clade B comprised only of *A. holocentri*, and clade C comprised of *A. acanthuri, A. chaetodontis*, and *A. haemuli.* All samples for these species were collected from hosts and localities previously reported by Williams and Williams (1981) and Williams (1984), with the addition of Tortola and Guana Island in the British Virgin Islands, which expand the geographic distribution of *A. chaetodontis, A. haemuli,* and *A. holocentri*.

Relationship with *Anilocra physodes*

The COI sequence analyses suggest that the Caribbean *Anilocra* is a recently evolved group compared to *A. physodes* and the other outgroup species. *Anilocra physodes* was included in the analyses since it was the only available COI sequence of the genus *Anilocra* in GenBank and is the type species for the genus. Since it belongs to the same genus as the ingroup, it was expected to be positioned as sister taxon to the Caribbean *Anilocra* in the phylogenetic reconstructions even though it is found in the Mediterranean Sea. However, the phylogenetic reconstructions clearly show the lack of a close relationship between *A. physodes* and the Caribbean *Anilocra,* and shows

Nerocila bivittata as the sister species of the Caribbean *Anilocra* even though it is also found in the Mediterranean and its morphology is notably different from *Anilocra.*

Based on these phylogenies and the life history of the Caribbean *Anilocra* compared to that of *A. physodes,* we might speculate that they may belong to two different genera. Evidence for this includes a significantly different life history pattern seen in the males of the Caribbean species versus *A. physodes*. The Caribbean *Anilocra* are characterized by a free-swimming functional male stage with six pairs of pereopods, fully developed appendix masculina and penis lobes at a size and development relative to mancae of other species (Williams, 1984). In *A. physodes,* males are larger than Caribbean males and live attached next to the female (Trilles, 1975b). In species with female-male pairs, male functionality is thought to be acquired after attachment. Since the attachment is permanent, the natatory setae are lost and therefore their ability to swim is also lost (Brusca, 1981; Lester, 2005). Also, in other non-Caribbean *Anilocra* species, males with a fully developed appendix masculina and penis lobes have seven pairs of pereopods (Brusca, 1981; Adlard and Lester, 1994), thus making the Caribbean *Anilocra* males different from the other males in the genus. Further studies are needed on the development of individuals from more species in the genus in order to assess whether the Caribbean *Anilocra* indeed represents a different genus based on male morphology and development.

Morphological considerations

The basal position of *A. chromis* suggests that of the five species sampled from the Caribbean, it was the first to have evolved. Unfortunately, due to lack of a

phylogenetic analysis based on morphological characters we cannot say what the ancestral characters are for the Caribbean species. In consulting the original descriptions of the Caribbean *Anilocra* species, it seems that *A. chromis* does not present some of the distinguishing characters among the species (see Table 1 in Williams and Williams, 1981). Some of the characters included in that table are: (A) uropod reaching posterior margin of telson, (B) endopod of uropod extending beyond posterior end of exopod, (C) posterior ventral angles slightly produced in pereonites, (D) posterior ventral angles produced in pereonites, (E) posterior ventral angle of pereonite 7 overlapping pleonites, (F) pereopods 2-4 with swelling on outer margin of dactyl. *Anilocra chromis* only shares characters with three of the other Caribbean species. It shares character A only with *A. myripristi* (not sampled in this study), character B only *A. partiti* (not sampled in this study), and like *A. acanthuri* (sampled in this study) it lacks the expression of characters C and E.

In Williams and Williams' (1981) Table 1, we observed that *A. chaetodontis* seems to have more unique characters than the other eight described Caribbean species. In this species the posterior ventral angles start to become produced from pereonite 4 (character C), are completely produced in pereonites 5-7 (character D), and the posterior ventral angle of pereonite 7 overlaps as far down as pleonite 2 (character E). Also, *A. chaetodontis* is the only species sampled that presents character F, which it shares with three other species, *A. myripristi*, *A. abudefdufi* and *A. partiti,* not sampled in this study. If we assume that *A. chaetodontis* is the most derived species, we can speculate that having characters C, D, and E, and lacking character A is the derived

condition in the Caribbean group. Future analyses should combine morphological and molecular data to further determine the primitive and derived characters of the group.

Clade A: *Anilocra chromis*

Anilocra chromis uses two host species, however, samples were only collected from *Chromis multilineata* (brown chromis) since it does not parasitize *Chromis cyanea* (blue chromis) in the same geographic areas, even though both host species occur sympatrically (Williams, 1984). The brown chromis and blue chromis are not sister species, instead *Chromis atrilobata* Gill 1862 (scissortail damselfish) from the eastern Pacific is the sister species of the brown chromis (Quenouille et al., 2004). However, the scissortail damselfish is not parasitized by *A. chromis*, suggesting that the isopod colonization occurred after the divergence of these host species. It can also be speculated that a host switch from the blue chromis to the brown chromis occurred afterwards, but it cannot be determined if it was an ancient or recent host switch due to lack of data of *A. chromis* from the blue chromis. For the geographic area sampled no differences were expected between the Puerto Rican and Virgin Islands specimens since they are on a continuous insular shelf that lacks physical barriers and fish populations are expected to interact. Due to the lack of samples from the other host, no conclusions on speciation can be drawn that support the presence of cryptic species in *A. chromis.*

Clade B: *Anilocra holocentri*

Anilocra holocentri is found on a single host species, *Holocentrus adscensionis,* from Puerto Rico to the British Virgin Islands (see Table 3). The sampled area for this study only included the US and British Virgin Islands and no differences among those populations were observed in the phylogenetic reconstructions. Only parsimony and Bayesian inference topologies recovered the *A. holocentri* clade, however no conclusion can be inferred due to the very limited number of samples obtained in this study. A wider geographic area should be sampled to determine if this species has population structure based on geographic area and confirmed that samples from this species form a clade.

Clade C: *Anilocra acanthuri, Anilocra chaetodontis, Anilocra haemuli*

Anilocra acanthuri was only collected from *Acanthurus chirurgus* in St. Thomas, since it does not parasitize its other host, *Acanthurus bahianus*, in this area although both host species occur sympatrically throughout the Caribbean (Williams, 1984). DNA analyses of *A. acanthuri* from *Acanthurus bahianus* are needed to determine if this parasite is a cryptic species which has morphological stasis, i.e. lack of change in characteristics of gross external anatomy (Bickford et al., 2007), or is a true multi-host parasite which has failed to speciate (Banks and Paterson, 2005).

Within clade C, *Anilocra chaetodontis* and *A. haemuli* form a paraphyletic group. Phylogenetic reconstructions suggest that *A. chaetodontis* diverge from *A. haemuli*. However, the placement of *A. haemuli* samples BRVI1 and USVI1 after the divergence of *A. chaetodontis* Virgin Islands clade in some topologies (Figures 9, 10, and 13)

makes the prior assumption inconclusive. The phylogenetic analyses indicate that they are closely related species, whereas their morphology suggests they are very different, not closely related species (Williams and Williams, 1981), with *A. chaetodontis* appearing to be morphologically more related to *A. myrispristis* and *A. haemuli* to *A. chromis*.

Anilocra chaetodontis forms two well defined clades based on the two geographic areas sampled, which suggest a population structure based on geographic origin since all samples were collected from the same host species, *Chaetodon capistratus*. The geographic areas sampled are part of a continuous insular shelf that lacks the physical barriers that would normally limit the interactions between host populations and reduce the gene flow of their isopod parasite. Still, gene flow between isopods in these regions would be expected to continue since it also uses *Chaetodon ocellatus* and *Chaetodon striatus* as hosts in these areas. These three host species are sister species (Fessler and Westneat, 2007) that occur sympatrically and the isopod does not present specificity towards any of these hosts for a particular geographic area (Williams, 1984). The fourth host species, *Chaetodon sedentarius*, used by *A. chaetodontis* is genetically distant from the other three host species (Fessler and Westneat, 2007) and has only been reported as host in Puerto Rico (Williams and Williams, 1981) and Cuba (Fernández and Ortiz-Touzet, 2004). In summary, if samples from one host recovered two distinct clades of *A. chaetodontis*, it is possible that future analyses on samples from the other hosts might reveal even more clades, which can clarify if parallel speciation or host switching has occurred. The present data only suggest that *A. chaetodontis* might be a cryptic species with morphological stasis.

Anilocra haemuli samples formed two groups, a well defined group of the samples collected from the red hind and a polytomy (Figures 9-13) with the samples collected from the French grunt, which suggests population structure based on host. The samples of *A. haemuli* from the red hind group were collected only in the Virgin Islands and the samples of *A. haemuli* from the French grunt were collected from Puerto Rico and the Virgin Islands but do not present a disjunct distribution in the collection localities. The geographic distribution of *A. haemuli* on the grunts, Family Haemulidae, and the groupers, Family Serranidae, differs greatly. The groupers are being parasitized in more widespread regions of the Caribbean while parasitized grunts are being found in several localized pockets of populations. It is worth noting that this sampling includes the only geographic area where the distribution of *A. haemuli* on the grunts and on the groupers overlaps. The phylogenetic reconstructions depict the samples taken from the French grunt as having diverged more recently, which could suggest a host switch event from the red hind to the French grunt. The two distinct clades of *A. haemuli* based on host recovered in the analyses could also suggest the presence of two species, where each species parasitizes one host family. Even if they are considered two species, it would be possible that a more extensive sampling will recover clades that might suggest additional cryptic speciation within each of them. This could be plausible due to high number of hosts that *A. haemuli* parasitizes and its restrictive distribution on some hosts (see Table 3). Furthermore, in the family Serranidae, *A. haemuli* parasitizes *Paranthias furcifer* (creole-fish) which differs greatly from the other groupers in its ecological habits. The disjunct distribution the parasite presents on the creole-fish might also suggest the presence of another cryptic species. In summary, a more inclusive

sampling is needed to determine if host switch events have occurred and which was the ancestral host. Also, parallel host-parasite phylogeny of all host species with their respective isopod and measures of genetic diversity within populations are needed to determine if *A. haemuli* is a cryptic species or a true multi-host parasite, i.e. there is gene flow between parasite populations on divergent hosts due to failure to speciate or incomplete host switching (Banks and Paterson, 2005). The present data only suggest that *A. haemuli* might be a cryptic species with morphological stasis.

Isopod Speciation

An example of another species with a high number of hosts over a wide range distribution is *A. physodes*, which is found parasitizing 25 genera in 13 families distributed throughout the northeastern Atlantic and the Mediterranean (Bruce, 1987). Only Ketmaier et al. (2007) have provided evidence to suggest that *A. physodes* is composed of cryptic species. With DNA data they showed the presence of two distinct haplotypes of *A. physodes* collected from the same host species in two different geographically disjunct populations, Adriatic Sea and Tyrrhenian Sea. Free-living isopod species with varying ecological traits, e.g. *Acanthaspidia* (Raupach and Wägele, 2006), *Austridotea* (McGaughran et al., 2006), *Ceratoserolis* (Held, 2003), *Exosphaeroma* (Tesk et al., 2006), *Glyptonotus* (Held and Wägele, 2005), have also been found to present significant genetic differences that recognize them as cryptic species.

Although molecular data seem to be providing hints at ongoing speciation without morphological changes, the morphological characters used to identify species in the genus *Anilocra* should be revised. The lack of a definitive and unifying set of characters

to identify species has provided grounds for a high amount of synonyms and invalid species. Table 2 provides some examples which have been clarified, while others like *A. elviae* (Winfield et al., 2002) and *A. montii* (Thatcher and Lobos-Blumenfeldt, 2001), which should be classified in the genera *Pleopodias* and *Cymothoa*, respectively (Dr. L.B. Williams, personal communication), have not been properly reviewed and reclassified. Also, overlooking morphological characters now considered of taxonomic importance and using highly variable characters to determine species (Brusca, 1981) results in very distinct specimens being placed under the same species name (e.g. *Anilocra* sp. in the Caribbean Sea prior to Williams and Williams (1981) descriptions). The Caribbean is a high diversity area for *Anilocra*, with nine formally described (Williams and Williams, 1981) and one undescribed species (Dr. L.B. Williams, personal communication). Another area of high diversity is the Australian-Malaysian region with 18 species currently recognized (Bruce, 1987). Several of these have been previously placed as synonyms of other *Anilocra* species in the same region, which underestimated the parasitic isopod diversity. An inclusive taxon sampling of the genus, particularly from these two areas of reported high diversity, for molecular and morphological analyses should be performed to assess diversity and determine the type of speciation process *Anilocra* species are undergoing.

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