Digenean metacercariae of fishes from the lagoon flats of Palmyra Atoll, Eastern Indo-Pacific

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Abstract

Although many studies on the taxonomy of digenean trematodes of marine fishes have been completed in the Eastern Indo-Pacific (EIP) marine ecoregion, only a few have considered metacercarial stages. Here, the results are presented of a taxonomic survey of the digenean metacercariae of fishes from Palmyra Atoll, a remote and relatively pristine US National Wildlife Refuge located 1680 km SSW of Hawaii. Up to 425 individual fish were collected, comprising 42 fish species, from the sand flats bordering the lagoon of the atoll. Quantitative parasitological examinations of each fish were performed. Morphological descriptions of the encountered digenean metacercariae are provided, together with their prevalence, mean intensities, host and tissue-use. Up to 33,964 individuals were recovered representing 19 digenean metacercaria species from eight families. The species composition of digeneans in lagoon fishes at Palmyra Atoll is a subset of what has previously been reported for the EIP. Further, the large diversity and abundance of metacercariae reported in this study highlight the utility of including this group in future ecological research in the EIP marine ecoregion.

Introduction

Digenean trematode species richness appears to be particularly high in the Eastern Indo-Pacific (EIP) and Central Indo-Pacific (CIP) marine ecoregions (Yamaguti, 1970; Cribb et al., 1994). Several studies have examined adult digenean trematodes of marine fishes in these ecoregions, including Australia, the South Pacific regions of French Polynesia, New Caledonia and Hawaii (see, for example, Cribb et al., 1994, 2002; Nolan & Cribb, 2005). However, only a few such studies have considered digenean metacercariae (e.g. Yamaguti, 1970; Køie & Lester, 1985, 1985; Cribb, 1998 and references therein), perhaps partly because metacercariae are more difficult to locate, recognize and identify than adult worms. However, knowledge of metacercariae is obviously necessary for understanding the population dynamics of both digeneans and hosts, the ecological impacts on hosts and the broader role of digeneans in ecosystems. For example,
metacercariae, being trophically transmitted, require their host to be eaten by a final host and they therefore have an inherently different relationship with their hosts than do adult trematodes (which die if their host is eaten). Metacercariae can dominate the parasite community of fishes in both numbers of individuals and species (Salgado-Maldonado & Kennedy, 1997; Rodriguez-González & Vidal-Martínez, 2008). More generally, trophically transmitted parasites, including abundant metacercariae, had substantially greater biomass than did adult parasites in a study in three estuarine ecosystems (Kuris et al., 2008).

Palmyra Atoll is one of the northern Line Islands, located in the East Indo-Pacific marine ecoregion (sensus Spalding et al., 2007), 1680 km SSW of Hawaii (5°52′55.54″N; 162°04′59.05″W). During the Second World War, Palmyra supported a few thousand military personnel. The atoll has otherwise never had a permanent human population. All fishing has been prohibited at Palmyra since it became a US National Wildlife Refuge in 2000 (before that, its remoteness kept fishing pressure low).

As a part of a larger research project on the role of parasites in EIP atoll food webs (Lafferty et al., 2008), a survey of the parasites of the fishes from the lagoon flats of Palmyra Atoll was conducted. A substantial diversity and abundance of digenean metacerciae infecting fish as second intermediate hosts was found. The lack of comprehensive taxonomic studies of digenean metacerciae in the EIP prompted us to provide morphological descriptions of these parasites, and to summarize their prevalence, mean intensity and host and tissue use. The primary aim of this paper is to provide a document that facilitates ecological, taxonomic and other biological research involving these trematodes at Palmyra Atoll, and perhaps more broadly throughout the EIP.

Materials and methods

Between 13 October – 10 November 2009 and 22 June – 28 July 2010, fish were collected by seine, spear, and hook and line from the intertidal sand flats bordering the Palmyra Atoll lagoon (see Fig. 1 in Papastamatiou et al., 2009). Fish were individually bagged and examined fresh within 24 h (except for four bonefish, which were frozen for 4 months at −60°C before examination).

Parasitological examinations were conducted using stereomicroscopes, using 0.7% saline solution as necessary. Metacercaria abundance was quantified by squashing tissues, organs and body parts between two glass plates. For most body parts with clear bilateral symmetry (i.e. body surface, muscles, pectoral and pelvic fins, eyes and gills), metacercariae were enumerated in the left half and multiplied by two to estimate the expected intensity for both sides. Brain, liver, gall bladder, spleen, kidney, intestine, stomach, heart and swim bladder were squashed in their entirety. For body parts > 25 g (primarily large jack muscle fillets and shark livers), tissue interspersed throughout the body part area was excised to squash a maximum of 25 g. The observed intensities were multiplied by the appropriate weight proportion to estimate intensity in the whole body part. Both prevalence and mean intensity values were calculated using ‘expected’ data, while reported total number of individuals recovered reflect only directly ‘observed’ metacercariae.

Metacercariae generally lack diagnostic adult structures that characterize most species descriptions. Thus, it is generally not possible to identify metacercariae to the species level without previous life cycle or molecular work linking them to adults. However, metacercariae do have a suite of morphological characters. This makes it possible frequently to place them into the appropriate family and genus, and to designate them as different morphospecies. Even if morphospecies designations are somewhat provisional, they provide a clear, working hypothesis of specific identity that can immediately facilitate research. To identify metacercariae, up to 10–20 cysts of a putative species were removed from host tissues and individually isolated on a slide with a drop of saline solution. The site of infection, shape, movements, colour and number of metacercarial cysts were recorded. The metacercarial cyst dimensions were measured for those that withstood coverslip pressure. Surgical needles were used to free metacercariae from cysts that did not excyst with coverslip pressure. A coverslip was added to flatten the worm, and the corners of the coverslip were fixed to the slide with Du-Noyer sealant (1 part dehydrated lanolin and 4 parts melted resin) that was melted to the slide using an alcohol lamp. Then, a small amount of ammonium picrate fixative (1:1 saturated ammonium picrate and glycerin) was added to one side of, and drawn under, the coverslip using a Kimwipe® on the opposite side. Although metacercariae suffer some deformation by flattening, fixation with ammonium picrate works well for fieldwork because of its simplicity, rapid staining and its ability to clarify internal morphology and external sclerotized structures. This technique produces semi-permanent slides which can be preserved for 1–2 years by adding small amounts of fixative after monthly inspection (for further details see Scholz & Aguirre-Macedo, 2000). Sketches or high-quality digital photographs were used to document morphological details. Measurements of metacercariae were based on flattened specimens fixed with ammonium picrate, unless otherwise indicated. In some cases, which are indicated, we acquired morphometrics from excysted specimens that were killed in hot water, fixed in 4% formalin, stained with carmine and mounted in Canada balsam. Images of the metacercariae were captured using a digital camera and drawn on a digitizing tablet using Adobe Illustrator software. All other helminthological methods followed were those recommended by Vidal-Martínez et al. (2001). Synonyms for each host species were obtained from FishBase (http://www.fishbase.com) and helminth records for each of the possible host synonyms were researched using Web of Science (ISI Web of Knowledge®; http://wok.mimas.co.uk), Google Scholar (http://scholar.google.com), and the Host–Parasite Database of the Natural History Museum, London (Gibson et al., 2005). In the tables, fish species are arranged by family, in taxonomic order following Randall et al. (1997). Voucher specimens of the metacercariae have been deposited in the United States Parasitological collection (USNPC), Beltsville, Maryland. All measurements below are in micrometres
The species with the highest mean intensity in our study, and others with low host sample size, there
were calculated following Bush et al. (1997). Observations of other parasite taxa and stages will be reported
elsewhere.

**Results**

Table 1 presents the species and numbers of host fishes examined, and the prevalence and mean intensity
(number of fish examined = 4), Albula glossodonta (Forsskål, 1775) (12), Gymnothorax pictus (Ahl, 1789) (2),
G. rupepelliae (McClelland, 1844) (1), Chanos chanos (Forsskål, 1775) (3), Epinephelus merra Bloch, 1793 (1),
Acanthocotyla myriaphis Cuvier, 1833 (2), Parasperis sp. (16), Oplomus eplomus (Valenciennes, 1837) (19), istigobius
rigillus (Herre, 1953) (1), Ptolobogus prolatus Watson & Lachner, 1985 (4) and Astoreopterix semipunctata Ruppell,
1830 (5).

Of the 33,964 metacercariae counted, 19 digenean species were recognized comprising 8 families. The
digenean families recovered, in decreasing order of number of species and individuals, were Bucephalidae
Poche 1907 (8 species with 29,480 individuals), Cyatho-
cotylidae Mühling 1898 (2 and 2209), Cryptogonimidae
Asterropterix semipunctata Lachner, 1985 (4) and
Rhipidocotyla sp. (fins); Tetraodontidae: Acanthuridae: Acanthurus triostegus (Linnaeus, 1758) (1),
Istigobius rigillus (Herre, 1953) (1), Ptolobogus prolatus Watson & Lachner, 1985 (4) and Astoreopterix semipunctata Ruppell,
1830 (5).

In general, the most prevalent taxon was Bucephalus
sp. 2 with prevalence values between 15% in Valenci-
nea sexguttata (Valenciennes, 1837) and 91% in Arothron
hispidus (Linnaeus, 1758), followed by other bucephala-
such as Rhipidocotyla sp. 1–3 (table 1). This range does not
include the 100% prevalence of Bucephalus sp. 2
observed in three Stegastes nigrans (Lacepède, 1802).
In this case, and others with low host sample size, there is
substantial error around prevalence estimates. The species with the highest mean intensity in our samples
was also Bucephalus sp. 2 with 1053 ± 3655 individuals in A. hispidus, followed by Cyathocotylidae with
714 ± 372 individuals in Hyperphampus affinis (Günther, 1866) and Rhipidocotyle sp. 1 with 147 ± 187 in A. hispidus.

Table 1 presents 167 host records for the 19 digenean
species, none of which have been recorded from Palmyra
Atoll. The following section presents morphological
descriptions for each of the recovered species, including
host information and site(s) of infection.

**Family Acanthocolpidae**

**Stephanostomum sp. (fig. 1a)**

**Description.** Based on three flattened individuals, one from Arothron hispidus (USNPC No. 104843) and two from
Acanthorus triostegus. Body length 810–2050 (1230 ± 710),
maximum width 220–350 (264 ± 74) towards posterior
end of the body, tegument densely covered with spines
at anterior half. Eye-spot pigment present scattered in
anterior part of the body. Oral sucker 92–210 (132 ± 68)
long and 117–320 (187 ± 115) wide, with double row of 30
spines and without ventral hiatus. Acetabulum equatorial,
90–200 (134 ± 58) long and 85–190 (121 ± 60) wide.
Prepharynx 132–250 (207 ± 64) long. Oesophagus short,
20–62 (44 ± 22) long. Caeæa bifurcating immediately
preacetabularly, extending to anterior excretory vesicle.
Developing testes median, tandem. Anterior (right) testis
55–150 (102 ± 67) long, 50–125 (87 ± 53) wide. Posterior
(left) testis 70 (n = 1) long, 55 (n = 1) wide. Ovary not
distinguishable. Excretory vesicle V-shape, excretory pore
terminal.

**Hosts.** Carangidae: Caranx pappensis Alleyne &
MacLeay, 1877 (encysted in intestine wall); Pomacentri-
dae: Abufeduf sp. septemfasciatus (Cuvier, 1830) (muscle);
Mugilidae: Valamugil engeli (Bleekeer, 1858–59) (fins);
Acanthoridae: Acanthurus triostegus (Linnaeus, 1758) (fins);
Tetraodontidae: A. hispidus (fins).

**Comments.** Stephanostomum Looss, 1899 is a large genus
However, due to the number of spines around the oral
sucker (30) and the lack of a ventral hiatus, only two
species previously described in the Indo-Pacific region
could be the potential adult stages: S. madhaviae Bray et
Cribb, 2003 (30–34 spines) from Caranx ignobilis
(Forsskål, 1775) from Queensland and S. rachycentronis
Shen, 1990 (30–34 spines) from Rachycentron canadum
(Linnaeus, 1766) from Hainan Island, China (Bray &
Cribb, 2003).

**Acanthocolpidae gen. sp. (fig. 1b)**

**Description.** Based on two flattened individuals; one from Mullolidichthys vanicolensis (Valenciennes, 1831)
(USNPC No. 104844) and one from Valamugil engeli.
Body length 510–715, maximum width 75–82 at posterior
part of body, tegument densely covered with fine spines,
especially at anterior half. Eye-spot pigment present at prepharynx level. Oral sucker subterminal
75–82 long and 80–87 width. Acetabulum postequatorial,
67 long and 72 width. Prepharynx 267 long, pharynx
34 long and 33 wide, nearly equatorial. Oesophagus and
caeæa not distinguishable. Genitalia non-developed.
Excretory vesicle clavate, occupying last quarter of
body.

**Hosts.** Mullilidae: Mullolidichthys vanicolensis (fins); Mugi-
lidae: Valamugil engeli (brain, heart, muscle).

**Comments.** This metacercaria is likely an acanthocolpoid,
based on the tegument covered with fine spines, lack of
circumoral spines, neck region enlarged, pharynx present
and small acetabulum close to midbody. This species may be
one of the several acanthocolpoid genera that lack oral
spines, e.g. Acanthocaltus Luhe, 1906; Lepidauchen Nicoll,
1913; Neophysis Stafford, 1904; Spinolagoidorus Skribin et
Koval, 1958; and Ningaloolia Bray et Cribb, 2007 (Bray &
Cribb, 2007).
Table 1. Prevalence (above, in percentage) and mean intensity (below ± SD) of digenean metacercariae of marine fishes from Palmyra Atoll. All fish species are in phylogenetic order following Randall et al. (1997), and digeneans following Olson et al. (2003). Acronyms are as follows: n, number of fish sampled; St, Stephanostomum sp.; Ac, Acanthocolpidae gen. sp.; C1–C2, Cryptogonimidae gen. spp. 1–2; Sl, Stellantchasmus sp.; Zo, Zoogonidae gen. sp.; B1–B3, Bucephalus spp. 1–3; Do, Dollfustrema sp.; R1–R3, Rhipidocotyle spp. 1–3; Pr, Prosorhynchus sp.; Me, Mesostephanus sp.; Cy, Cyathocotylidae gen. sp.; Ca, Cardiocephaloides sp.; Mo, Monolicaecum-type; To, Torticaecum-type.

| Family    | n  | St | Ac | C1 | C2 | Sl | Zo | B1 | B2 | B3 | Do | R1 | R2 | R3 | Pr | Me | Cy | Ca | Mo | To |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Hemiramphidae |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Hemiramphus lutkei | 5  | 1  | 4  | 1  |  2 |  4 |  8 |  6 |  9 |  2 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Hyporhamphus affinis | 5  | 1  | 4  | 80 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |  8 |
| Belonidae | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Platyrhineus argalus | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Zebroids | 5  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Carangidae | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Caranx ignobilis | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Caranx papuensis | 5  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Lutjanidae | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Lutjanus fulvus | 19 | 19 | 19 | 19 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Lutjanus monostigma | 5  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Mullidae | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Mullidae | 28 | 28 | 28 | 28 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Mulluscula viridescens | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Upeneus arge | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Kyphosidae | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Kryptos | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Chaetodontidae | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Chaetodon auriga | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Pomacentridae | 11 | 11 | 11 | 11 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |
| Pomacentrus adelus | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Stegastes nigricans | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Pomacentrus adelus | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Stegastes nigricans | 3  | 3  | 3  | 3  |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |  3 |
| Mugilidae | 1  | 1  | 1  | 1  |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |  1 |

V.M. Vidal-Martínez et al.
Table 1 – continued

| n  | St | Ac | C1 | C2 | Sl | Zo | B1 | B2 | B3 | Do | R1 | R2 | R3 | Pr | Me | Cy | Ca | Mo | To |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Creminugil crenilabris | 40 | – | – | 5 | – | – | 65 | 50 | 7 | 2 | 57 | 50 | 2 | – | 12 | 7 | 47 | – | – |
| Liza triserialis | 44 | – | – | 1 ± 1 | – | – | 38 ± 75 | 31 ± 57 | 1 ± 3 | 1 | 12 ± 27 | 6 ± 62 | 1 | – | 1 ± 9 | 1 ± 7 | 13 ± 29 | – | – |
| Valamugil engeli | 50 | 2 | 6 | 1 ± 3 | 1 | 7 ± 38 | 6 ± 21 | – | – | 1 ± 3 | 6 ± 8 | – | – | – | 1 | 1 | – | – | – |
| Sphyraenidae | | | | | | | | | | | | | | | | | | | | |
| Sphyraena barracuda | 2 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 50 | – | – | – |
| Gobiidae | | | | | | | | | | | | | | | | | | | | |
| Amblygobius phalaena | 12 | – | – | – | – | 8 | 33 | 17 | 8 | – | 8 | – | – | – | – | – | – | 8 | – |
| Istigobius ornatus | 9 | – | – | – | – | 1 | 2 ± 5 | 3 ± 21 | 1 | – | 1 | – | – | – | – | – | – | 1 | – |
| Valenciennea sexguttata | 13 | – | – | – | – | 15 | 8 | 8 | – | – | 8 | – | – | – | – | – | – | – | – |
| Acanthuridae | | | | | | | | | | | | | | | | | | | | |
| Acanthurus triostegus | 23 | 5 | – | 5 | 41 | – | – | 61 | 85 | 15 | 61 | – | 39 | 24 | 12 | 2 | 24 | 2 | – | – |
| Acanthurus xanthopterus | 10 | – | 2 | 102 ± 85 | – | – | 65 ± 169 | 76 ± 68 | 11 ± 8 | 52 ± 69 | – | 6 ± 8 | 17 ± 11 | 22 ± 4 | 1 | 2 | 26 | – | – |
| Balistidae | | | | | | | | | | | | | | | | | | | | |
| Rhinecanthus aculeatus | 7 | – | – | – | – | 30 ± 53 | 206 ± 163 | – | – | 14 | 29 | 14 | – | – | – | 14 | – | – | – |
| Tetraodontidae | | | | | | | | | | | | | | | | | | | | |
| Arothron hispidus | 11 | 9 | – | 18 | 9 | – | – | 91 | 54 | 64 | 9 | 45 | 54 | – | – | – | – | – | – | – |
| Arothron hystrix | 1 | – | 1 ± 2 | 1 | – | – | 1053 ± 1365 | 10 ± 12 | 147 ± 187 | 3 | 21 ± 36 | 12 ± 33 | – | – | – | – | – | – | – |
Family Cryptogonimidae

Cryptogonimidae gen. sp. 1 (fig. 1c)

Description. Based on two excysted specimens; one flattened (USNPC No. 104845) from Arothron hispidus and one unflattened from Valamugil engeli. Body oval, 260–350 long, 150–200 wide, tegument covered with spines. Eye spots scattered in anterior third. Oral sucker 20–82 by 35–77; acetabulum postequatorial, 25–35 in diameter. Sucker ratio 1:0.45–0.71. Prepharynx 37–45 long, pharynx 30–42 by 42; oesophagus short, 5 long; caeca bifurcation at equatorial level, caeca extending near to posterior end; excretory vesicle V-shaped, observed in fresh material but not apparent in fixed individuals.

Hosts. Mullidae: Mulloidichthys vanicolensis (eye, intestine, kidney); Chaetodontidae: Chaetodon lunula (Lacépède, 1802) (fins), Abudefduf sordidus (Forsskål, 1775) (fins); Mugilidae: Crenimugil crenilabris (Forsskål, 1775) (kidney, spleen), Valamugil engeli (intestine); Acanthuridae: Acanthurus triostegus (fins), Acanthurus xanthurus Valenciennes, 1835 (fins); Tetraodontidae: Arothron hispidus (fins).

Comments. This metacercaria has key characteristics of the family Cryptogonimidae, such as a spiny body with eye spots, a well-developed oral sucker and pharynx and an acetabulum smaller than oral sucker (Miller & Cribb, 2008). Unfortunately, a V-shaped excretory vesicle was observed only in fresh material but was not apparent in fixed individuals.

Cryptogonimidae gen. sp. 2 (fig. 1d)

Description. Based on two flattened specimens; one (USNPC Nos 104846 and 104847) from Hemiramphus lutkei Valenciennes, 1847, and one from Hyporhamphus affinis. Body elongated, slightly clavate without spined tegument, maximum length 490–820, maximum width 147–155 at equatorial level. Eye-spot pigment scattered in the anterior third of the body. Oral sucker subterminal,
70–97 long and 80–82 wide. Acetabulum postequatorial, 70–80 in diameter. Sucker ratio 1:0.87–0.97. Prepharynx absent or very short, pharynx 32–35 by 22–25; oesophagus short or absent; caeca long, reaching posterior end of body; excretory vesicle Y-shaped and excretory pore subterminal.

Hosts. Hemiramphidae: Hemiramphus lutei (liver); Mullidae: Mulloidichthys vanicolensis (intestine, stomach); Pomacentridae: Abudelfaf septemfasciatus (liver), Abudelfaf sordidus (liver); Mugilidae: Liza vaigiensis (Quoy & Gaimard, 1825) (intestine), Valamugil engeli (liver); Acanthuridae: Acanthusus triostegus (fins, muscle); Tetraodontidae: Arothron hispidus (fins).

Comments. This metacercaria has key characteristics of the family Cryptogonimidae, such as a spiny body with eye spots, a well-developed oral sucker and pharynx, acetabulum smaller than oral sucker, and excretory vesicle Y-shaped (Miller & Cribb, 2008).

Family Heterophyidae

Stellantchasmus sp. (fig. 1e)


Comments. This metacercaria was identified based on the presence of nearly symmetrical testes, the seminal vesicle modified as a muscular expulsor and the caeca reaching the testis anlagen. The lowest values of the range for each one of the measurements obtained are well in the range of those reported by Yamaguti (1970) and Scholz et al. (1991). In contrast, the largest values for each measurement are, in general, out of ranges reported by these authors, which is possibly explained by our specimens being slightly flattened. Yamaguti (1970) reported the metacercariae of Stellantchasmus falcatus Onji et Nishio, 1915 from Mugil cephalus Linnaeus, 1758 from Hawaii. There are several reports of Stellantchasmus for the CIP region because it is zoonotic (Rekharani & Madhavi, 1985; Hong, 2000).

Family Zoogonidae (Odhner, 1902)

Zoogonidae gen. sp. (fig. 1f)

Description. Based on one specimen from water in the holding bag of Liza vaigiensis. Cyst oval, 328 long, 304 wide. Body pear-shaped, 550 long, 209 wide; tegument with spines extending to up to ventral sucker, and sparse posteriorly. Eye-spot pigment absent. Oral sucker small, 71 long, 70 wide; ventral sucker very large, 117 long, 111 wide. Sucker ratio 1:1.58. Prepharynx 56 long; pharynx 54 long, 37 wide; oesophagus short, 51 long; caeca short, extending to about midlevel of ventral sucker. Excretory vesicle saccular. Excretory pore terminal.

Hosts. Mugilidae: Liza vaigiensis (water in the holding bag).

Comments. The morphological characteristics of this metacercaria, such as the absence of a prepharynx, the small size of the pharynx, the short caeca and sucker ratio (1:1.58), are similar to those of the members of Zoogonidae. This is a cosmopolitan family, also widely distributed in the Indo-Pacific region (see Bray, 1987). The metacercariae of a member of this family have been recorded encysted on plants (Bray, 1987), which could have been eaten by the mullet and regurgitated after capture. Unfortunately, the specimen was lost during processing.

Bucephalidae

Bucephalus sp. 1 (fig. 1g)


Hosts. Apogonidae: Cheilodipterus quinquelineatus (heart); Carangidae: Caranx ignobilis (intestine); Caranx papuensis (heart, intestine); Lutjanidae: Lutjanus fulvus (Forster, 1801) (heart); Mugilidae: Mulloidichthys vanicolensis (heart, eye); Chaetodontidae: Chaetodon auriga Forsskål, 1775 (heart), Chaetodon lunula (heart); Pomacentridae: Abudelfaf septemfasciatus (gills, heart, kidney), Abudelfaf sordidus (heart, kidney, tissue surrounding eye), Pomacentrus adelus Allen, 1991 (fins), Stegastes nigricans
(heart); Mugilidae: *Crenimugil crenilabris* (fins, heart, kidney, spleen), *Liza vaigiensis* (heart, kidney), *Valamugil engeli* (heart, kidney); Gobiidae: *Amblygobius phalaena* (Valenciennes, 1837) (eye); Acanthuridae: *Acanthurus triostegus* (fins, muscle), *Acanthurus xanthopterus* (fins, heart); Balistidae: *Rhinecanthus aculeatus* (Linneaus, 1758) (kidney, tissue surrounding eye).

**Comments.** These metacercariae were identified as belonging to the genus *Bucephalus* Baer, 1827 because the rhabdus was a simple sucker with tentacles around the anterodorsal rim, the mouth was between middle- and hind-body, the caecum was sac-like and variedly directed from pharynx, the pars prostatica was straight (not figured), and the excretory vesicle was variable in length. This metacercaria mainly infected host hearts. It is 0.58 times smaller in length than *Bucephalus* sp. 2 (Student’s *t* 0.05,21 test: 8.34, *P* < 0.05), which also infected the heart. The only way to distinguish these species without excystation is by cyst length, *Bucephalus* sp. 1 being 0.55 smaller than that of *Bucephalus* sp. 2 (Student’s *t* 0.05,18 test: 6.22, *P* < 0.01 for length; *t* 0.05,18 test: 3.87, *P* = 0.001 for width). *Bucephalus* sp. 1 was not found as an adult in the predatory fishes examined at Palmyra (see table 1).

Although it was possible to distinguish *Bucephalus* spp. 1–3 as separate species, it was not possible to assign specific species names without adults. However, these metacercariae could belong to one of the species already described in the Central Indo-Pacific (CIP) or East Indo-Pacific (EIP) marine ecoregions. *Bucephalus* species recorded for these marine ecoregions are: *B. fragilis* Velasquez, 1959 for *Megalaspis cordyla* (Linneaus, 1758); *B. leognathi* Velasquez, 1959 for *Leognathus sp.*; *B. pseudovarius* Velasquez, 1959 for *Caranx sp.* and *B. paraheterotentacularis* Velasquez, 1959 for *Serialina nigrofasciata* (Rüppell, 1829); all of them from the Philippines (Velasquez, 1959). Other species described in these ecoregions are: *B. varicus* Manter, 1940 from *Caranx sp.* from Fiji Islands (Manter, 1963); *B. sphyraeae* Yamaguti, 1952 for *Sphyraea sp.*, and *B. retractilis* Yamaguti, 1952 for *Caranx sp.* from Celebes Islands (Yamaguti, 1952); *B. carangis* Yamaguti, 1970 for *Caranx lugubris* Poey, 1860, *B. carangoides* Yamaguti, 1970 for *Caranxoides sp.*, *B. kaku* Yamaguti, 1970 for *Sphyraena barracuda* (Edwards, 1771), *B. sextentaculatus* Yamaguti, 1970 for *Caranx sexfasciatus* Quoy & Gaimard, 1825, *B. ulua* Yamaguti, 1970 for *Caranxoides ferdau* (Forsskål, 1775), all from Hawaii (Yamaguti, 1970); and *B. gorgon* (Linton, 1905) Eckmann, 1932 for *Seriola lalandii* Valenciennes, 1833 from New South Wales, Australia (Hutson et al., 2007). It is also possible that not enough predatory fish were examined to find adult bucephalids. Adult bucephalids have been recorded previously from jacks (*C. sexfasciatus* and *C. ignobilis*) in Japan (Sakaguchi, 1966).

*Bucephalus* sp. 2 (fig. 2a)

**Description.** Based on 13 individuals: five from *Abudefuluf septemfasciatus*, five from *Abudefuluf sordidus* (one sent to USNPC, Nos. 104850, 104851 and 104852) and three from *Acanthurus triostegus*. Cyst oval, 190–290 (215 ± 29) long, 120–200 (146 ± 25) wide. Body shape ellipsoid, total length 300–445 (385 ± 47), maximum width 90–152 (118 ± 23). Width as % of body length: 24–39% (31 ± 5%). Segument with fine spines. Pharynx sucker-like, 55–100 long, 47–80 width (69 ± 11 long, 68 ± 9 width) with 7–10 tentacular appendages. Pharynx length as % of body length: 14–23 (18 ± 2%). Moutn 155–345 (271 ± 54) from anterior end. Pre-mouth distance as % of body length: 40–81% (71 ± 12%). Pharynx 20–37 (28 ± 5) in diameter. Intestine saccular, 25–208 long (98 ± 61 long), oriented anteriorly. Testis primordia smooth. Post-testicular distance 0–34 (11 ± 30). Post-testicular distance as % of body length: 0–28% (2 ± 8%). Cirrus sac 55–107 (74 ± 15) long, 10–25 (16 ± 5) width. Anterior-most reach of cirrus sac as % of body length: 15–26% (18 ± 7%). Excretory vesicle elongate, saccular, with undulating margins, 195–342 (267 ± 64 long).

**Hosts.** Hemiramphidae: *Hyporhamphus affinis* (muscle); Apogonidae: *Cheiloidipterus quinquelineatus* (heart, muscle); Carangidae: *Carangoides ferdau* (Forsskål, 1775) (fins), *Caranx ignobilis* (intestine), *Caranx papuensis* (heart, intestine); Lutjanidae: *Lutjanus monostigma* (Cuvier, 1828) (fins, liver); Mullidae: *Mullolichthys vunicolensis* (heart, eye); Kyphosidae: *Kyphosus cinerascens* (Forsskål, 1775) (fins, liver); Chaetodontidae: *Chatoedon auriga* (heart), *Chaetodon lunula* (heart); Pomacentridae: *Abudefuluf septemfasciatus* (heart), *Abudefuluf sordidus* (heart, gills), *Pomacentrus adelus* (heart, kidney), *Stegastes nigricans* (eye, fins, gills, heart, kidney); Mugilidae: *Crenimugil crenilabris* (heart, *Liza vaigiensis* (eye, fins, gills, heart, intestine, muscle, stomach), *Valamugil engeli* (body cavity, brain, heart, muscle, kidney); Gobiidae: *Amblygobius phalaena* (tissue surrounding eye), *Istigobius ornatus* (Rüppell, 1830) (eye, gills, muscle), *Valamugil nigricans* (gills, muscle); Acanthuridae: *Acanthurus triostegus* (fins, gills, heart, kidney), *Acanthurus xanthopterus* (fins); Balistidae: *Rhinecanthus aculeatus* (fins, tissue surrounding eye); Tetraodontidae: *Arctagon hispidus* (fins, heart).

**Comments.** See comments for *Bucephalus* sp. 1.

*Bucephalus* sp. 3 (fig. 2b)


**Hosts.** Carangidae: *Caranx papuensis* (stomach); Kyphosidae: *Kyphosus cinerascens* (heart); Pomacentridae: *Abudefuluf sordidus* (heart, kidney), *Pomacentrus adelus* (eye, muscle); *Stegastes nigricans* (eye, kidney, liver, muscle); Mugilidae: *Crenimugil crenilabris* (fins, heart, kidney, muscle), *Valamugil engeli* (muscle); Gobiidae: *Amblygobius...
phalaena (eye, muscle), Valenciennea sexguttata (muscle); Acanthuridae: Acanthurus triostegus (gills), Acanthurus xanhopterus (fins); Tetraodontidae: Arothron hispidus (fins).

Comments. This is the largest metacercaria of the three members of Bucephalus in the present study. However, only a few specimens were recovered. Additional comments as for Bucephalus sp. 1.

Rhipidocotyle sp. 1 (fig. 2c)

Description. Based on ten individuals; three from Hyporhamphus affinis, one from Crenimugil crenilabris (USNPC, No. 104854) and six from Liza vaigiensis. Cyst elliptical, 400–625 long, 175–273 wide. Body shape cylindrical, total length 2380–3630 (2843 ± 387), maximum width 210–510 (320 ± 83). Width as % of body length: 6–18% (11 ± 3%). Tegument with fine spines. Rhynchus funnel shaped, 90–160 long (133 ± 24), 100–170 width (130 ± 26) with a crown of five lobes. Rhynchus length as % of body length: 4–6% (5 ± 1%). Mouth opening 890–1890 (1275 ± 291) from anterior end. Pre-mouth distance as % of body length: 33–52% (45 ± 7%). Pharynx 40–80 (57 ± 12) in diameter. Intestine tubular 240–810 (571 ± 176), oriented posteriorly. Pars prostatica slightly bent. Post-testicular distance 270–530 (412 ± 85). Post-testicular distance as % of body length: 10–19% (15 ± 3%). Cirrus sac 190–370 long (283 ± 53), 40–80 width (55 ± 13). Anterior-most reach of cirrus sac as % of body length: 7–13% (10 ± 2%). Ovary pre-testicular (not measured). Vitelline glands two bands along the body sides anterior to ovary.

Hosts. Hemiramphidae: Hemiramphus lutkei (body cavity, brain, kidney, muscle, tissue surrounding brain), Hyporhamphus affinis (fins, spleen, tissue surrounding eye and intestine); Belonidae: Platyclenea argalus (Lesueur, 1821) (kidney); Carangidae: Caranx ignobilis (intestine), Caranx papuensis (fins, intestine); Lutjanidae: Lutjanus
Rhipidocotyle sp. 2 (fig. 2d)

Description. Based on seven individuals; five from Hemiramphus lutkei, one from Crenimugil crenilabris and one from Liza vaigiensis (USNPC No. 104855). Cyst oval, 520–680 length (617 ± 85), 150–420 width (313 ± 144). Body shape cylindrical, total length 890–1860 (1410 ± 305), maximum width 170–490 (281 ± 107). Width as % of body length: 10–30% (20 ± 7%). Segmentation with fine spines. Rhynchus sucker-like, 60–200 long (116 ± 51 long), 70–240 wide (124 ± 59) with 3–4 rounded lobes on the anterior end. Rhynchus length as % of body length: 5–12% (8 ± 3%). Mouth opening 410–1370 (737 ± 247) from anterior end. Pre-mouth distance as % of body length: 43–87% (57 ± 17%). Pharynx 40–120 (59 ± 28) in diameter. Intestine tubular, 150–880 long (313 ± 263), oriented posteriorly. Vitelline glands two bands along body sides anterior to ovary. Post-testicular distance 40–370 (196 ± 113). Post-testicular distance as % of body length: 2–24% (14 ± 7%). Cirrus sac 60–190 long (139 ± 45), 30–90 width (47 ± 21). Anterior-most reach of cirrus sac as % of body length: 5–14% (10 ± 4%). Excretory vesicle 1170–1350 (n = 2) length.

Hosts. Hemiramphidae: Hemiramphus lutkei (fins); Hyporhamphus affinis (gills, heart, liver); Belonidae: Platybelone argalus (kidney, water); Apogonidae: Cheilodipterus quinquelineatus (eye); Carangidae: Caranx ignobilis (intestine), Caranx pampus (intestine, stomach); Lutjanidae: Lutjanus monostigma (fins); Mullidae: Mulliodichthys vanicolensis (heart, eye); Pomacentridae: Abudelfuf septemfasciatus (gills), Abudelfuf sordidus (fins, kidney, muscle); Mugilidae: Crenimugil crenilabris (fins, heart, kidney, muscle); Liza vaigiensis (muscle, heart, kidney, tissue surrounding brain, ovary), Valamugil engeli (tissue surrounding brain, intestine), Valamugil ascens (fins, heart, kidney, liver), Mugil cephalus, Mugil cephalus deliciosus (fins, heart, liver); Acanthuridae: Acanthurus triostegus (fins, gills), Acanthurus xanthopterus (fins); Balistidae: Rhinecanthus aculeatus (heart, muscle); Tetraodontidae: Arothron hispidus (fins).

Comments. This species is smaller than Rhipidocotyle sp. 1.

Rhipidocotyle sp. 3 (fig. 2e)


Hosts. Hemiramphidae: Hyporhamphus affinis (gills); Carangidae: Caranx pampus (fins); Mullidae: Mulliodichthys vanicolensis (fins, heart, kidney, muscle); Mugilidae: Crenimugil crenilabris (kidney), Valamugil engeli (heart, kidney); Gobiidae: Valencienni sexguttata (muscle); Acanthuridae: Acanthurus triostegus (body cavity, fins, heart, kidney, muscle), Acanthurus xanthopterus (fins); Balistidae: Rhinecanthus aculeatus (muscle); Tetraodontidae: Arothron hispidus (fins, heart).

Comments. See comments for Rhipidocotyle sp. 1.

Prosorhynchus sp. (fig. 2f)

Description. Based on four individuals from Acanthurus xanthopterus (one sent to USNPC, No. 104857). Cyst oval to elliptical, 270–500 (378 ± 105; n = 5) long, 190–200 (197 ± 6; n = 3) wide. Total body length 800–1475 (1184 ± 317), maximum width 210–300 (271 ± 42). Width as % of body length: 20–29% (24 ± 4). Segmentation with fine spines. Rhynchus funnel-shaped, 100–180 (144 ± 33) long, 150–185 (174 ± 16) wide. Rhynchus length as % of body length: 7–19% (13 ± 5%). Mouth 620–1063 (883 ± 217) from anterior end. Pre-mouth distance as % of body length:
Metacercariae from Palmyra Atoll

Dollfustrema sp. (fig. 3a)

Description. Based on seven individuals; three from Mullloidichthys vanicolensis, and four (one sent to USNPC, No. 104858) Acanthurus xanthopterus. Body ellipsoidal, 188–450 (288 ± 96) long, 63–130 (106 ± 30) wide. Width as % of body length: 29–45% (38 ± 6%). Tail with fine spines. Pharynx a shallow funnel topped with two cirri, 30–57 (48 ± 10) long, 40–95 (70 ± 19) wide. Rhynchus length as % of body length: 12–24 (17 ± 4). Mouth 138–310 (218 ± 67) from anterior end. Pre-mouth distance as % of body length: 67–75% (72–77% (75 ± 2%). Pharynx 68–103 (80 ± 16) in diameter. Intestine tubular, 270–490 (380 ± 156) long, oriented posteriorly. Testes smooth, 40–82 (61 ± 30) long, 30–52 (41 ± 16) wide. Post-testicular distance 50–112 (n = 2). Post-testicular distance as % of body length: 3–8% (n = 2). Cirrus sac 110–122 (n = 2) long, 20–40 (n = 2) wide. Seminal duct coiled. Pars prostatica straight. Anterior-most reach of cirrus sac as % of body length: 8% (n = 2). Excretory vesicle 1287–1340 long.

Host. Mullidae: Mullloidichthys vanicolensis (muscle); Chaetodontidae: Chaetodon lunula (heart); Pomacanthidae: Abudedefduf septemfasciatus (eye, fins); Abudedefduf sordidus (fins); Pomacentrus adels (fins); Mugilidae: Crenimugil crenilabris (kidney); Gobiidae: Amblygobius phalaena (liver), Valenciennea sexguttata (muscle); Acanthuridae: Acanthurus triostegus (fins, gills, tissue surrounding eye), Acanthurus xanthopterus (fins); Tetraodontidae: Arothron hispidus (fins).

Comments. The recovered material of Dollfustrema had two cirrpts of spines around the pharynx. This characteristic and those of the terminal genitalia correspond well with the generic description provided by Overstreet & Curran (2002). Two species of Dollfustrema Eckmann, 1934 (Dollfustrema bipapillosum Manter et Pritchard, 1961 of Gymnothorax petelli (Bleeker, 1856) and D.strombyrunichum Manter et Pritchard, 1961 of G. petelli) have been described from Hawaii by Manter & Pritchard (1961). Recently, Nolan & Cribb (2010) described Dollfustrema gibsoni from Gymnothorax woodwardi McCulloch, 1912 from southwest Western Australia. Due to the geographical proximity, any of the species mentioned above could be the adult of the Dollfustrema sp.

Family Cynothocyrtidae

Mesostephanus sp. (fig. 3b)


Hosts. Belonidae: Platybelone argalus (muscle); Mugilidae: Crenimugil crenilabris (brain, heart, muscle), Valamugil engeli, (muscle); Sphyraenidae: Sphyraena barracuda (Edwards, 1771) (gills); Acanthuridae: Acanthurus triostegus (fins).

Comments. These metacercariae were identified as Mesostephanus Lutz, 1935 due to the presence of a spherical oral sucker, a ventral sucker slightly pre-equatorial and smaller than the oral sucker, the voluminous tribocytic organ and the genital primordial excretory vesicle l-shaped. Four species of Mesostephanus have been described for the region: M. halasturis...
Tubangui et Masilungan, 1941 for *Haliastur indus intermedius* Blyth, 1865 from the Philippines (Tubangui & Masilungan, 1941); *M. neophocae* Dubois et Angel, 1976 for *Neophoca cinerea* (Péron, 1816) and *Mirounga leonina* (Linnaeus, 1758) from Australia (Dubois & Angel, 1976); *M. haliasturis* and *M. scottae* Cribb, Barker et Beuret, 1995 for *Phalacrocorax sulcirostris* (Brandt, 1837) and *P. varius* (Gmelin, 1789), and *M. pelecami* Cribb, Barker et Beuret, 1995 for *Pelecanus conspicillatus* Temminck, 1824 from Queensland, Australia (Cribb et al., 1995). The metacercariae recovered from *C. crenilabris* could belong to any of these species. Lutz (1935) reported the presence of *Mesostephanus* from the booby *Sula brasiliensis* Spix, 1825 from Brazil. Several species of boobies, such as the brown boobies (*Sula leucogaster* Boddaert, 1783), nest at Palmyra Atoll and could serve as potential definitive hosts.

**Cyathocotylidae gen. sp.** (fig. 3c)

*Description.* Based on seven slightly flattened individuals; one (USNPC No. 104860) from *Hemiramphus lutkei* and six from *Hyperhamphus affinis*. Body foliaceous to elliptical, slightly folded dorsally at its anterior end; length 680–1080 (877 ± 147), maximum width 320–525 (425 ± 71) at posterior quarter of body. Tegument smooth with granular pigments on anterior part of body, pre-acetubulary. Oral sucker subterminal, long 49–72 (65 ± 8), width 52–72 (62 ± 8). Acetabulum postequatorial, 46–65 long (58 ± 6) and 42–74 width (59 ± 10). Prepharynx absent; pharynx small, elliptical 35–47 long (41 ± 5) and 25–35 (30 ± 4) wide. Oesophagus 50–122 long (76 ± 30). Caeca narrow, close to middle ventral line of body, extending to posterior end. Tribocytic organ fully developed. Developing testes median, in tandem at

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Fig. 3. Excysted metacercariae recovered from lagoon-flat fishes of Palmyra Atoll (all worms in ventral view). (a) *Dollfustrema* sp.; (b), *Mesostephanus* sp.; (c) *Cyathocotylidae gen. sp.*; (d) *Cardiocephaloides* sp.; (e) *Monolicaecum*-type; (f) *Torticaecum*-type. All scale bars are in micrometres.
posterior end, overlapping one to each other, globular. Anterior testis 67–125 long (92 ± 24) and 110–185 (149 ± 28). Posterior testis 52–110 long (84 ± 24) and 112–180 (143 ± 34) wide. Excretory pore subterminal.

Hosts. Hemiramphidae: Hemiramphus lutkei (muscle), Hyporhamphus affinis (eye, fins, muscle); Belonidae: Platybelone argolus (eye, muscle); Mugilidae: Crenimugil crenilabris (muscle), Valamugil engeli (body cavity, heart, kidney, intestine, muscle); Acanthuridae: Acanthurus triostegus (eye, fins, muscle).

Comments. These metacercariae were morphologically different from Mesostephanus due to the foliaceous body shape, granular pigment on anterior part of body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general morphology, it might be a cyathocotylid such as Crenimugil. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ. Based on its general body, very narrow caeca, and primordia of testes in tandem posterior to tribocytic organ.

Host. Hemiramphidae: Hemiramphus lutkei (fins); Carangidae: Carangoides orthogrammus (Jordan & Gilbert, 1882) (muscle, water in holding bag); Lutjanidae: Lutjanus monostigma (fins); Mullidae: Upeneus arge Cuvier, 1829 (water in holding bag, scales).

Comments. This ‘metacercaria’ was tentatively recognized as belonging to the ‘Torticaecum-type’ given the absence of stomach, absence of pharynx and lack of gland-cells around oesophagus. See additional comments for ‘Monolicaecum-type’ sp.

Discussion

Up to 425 individual host fish were surveyed belonging to 42 species and 33,964 metacercariae of 19 digenean species of 8 families were recovered. Three digenean families were particularly common in the metacercaria assemblage: Bucephalidae, Cryptogonimidae and Cyathocotylidae (table 1). Bucephalid species appear to have the broadest specificity, infecting up to 13 host families, 26 host species, and 14 sites within hosts (tables 1 and 2). Four other digenean families appeared less frequently (Acanthocolpidae, Heterophyidae, Strigeidae and Didymozoidae). All these families are a subset of those previously detected as adults or metacercariae throughout the CIP or EIP marine ecoregions (e.g. Yamaguti, 1970; Cribb et al., 1994). Metacercariae in the abundant and less-abundant families originate from first intermediate host infections in bivalves or snails (Yamaguti, 1975), both of which are common on Palmyra’s lagoon flats (unpublished observations). Trematode species in the common and rarer families use fishes and birds as final hosts (Yamaguti, 1975), indicating the role of both fishes and birds as predators on fish at Palmyra.

A few surveys have examined metacercariae infecting marine fishes in the Central and East Indo-Pacific ecoregions (Yamaguti, 1970; Køie & Lester, 1985; Cribb, 1998 and references therein). Our study differed from these previous surveys in several respects, and this perspective frames the rest of our discussion. First, the fish assemblage was broadly surveyed, aiming to examine all the potential host species of the lagoon flats. Most other studies have focused on particular host taxa, or have obtained hosts haphazardly (e.g. via sporadic sampling at fish markets). We have thus far sampled 42 of 47 recorded fish species (unpublished data) from Palmyra’s lagoon flats. Sampling a major portion of the local fish diversity helps to establish an accurate baseline expectation for the diversity and composition of the parasite community occurring in the entire ecosystem. In fact, a randomized species accumulation curve suggests that we have encountered most of the metacercaria species of the Palmyra Lagoon flats (fig. 4).

Second, each individual fish was thoroughly and systematically processed, squashing different tissues to count and identify individual metacercariae. This processing facilitated the detection of rare trematode

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<th>Number of fish species</th>
<th>Number of sites within hosts</th>
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<tr>
<td>Didymozoidae</td>
<td>Monolicaecum-type</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Torticaecum-type</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

*‘Sites within hosts’ provides a crude indication of variability in tissue/organ site use, which may vary within or among host species. See metacercaria species descriptions for detailed information.
species within individual hosts. This intensive, within-host effort combined with our broad sampling of the fish community to further help to capture adequately the metacercaria diversity of the lagoon flats.

Third, the parasitological survey was conducted on fishes from a largely unexplored habitat: the lagoon flats. Previous efforts in the CIP and EIP marine ecoregions have been restricted to fore-reef habitats. To our knowledge only one of these fore-reef surveys (Lafferty et al., 2008) processed individual fish in a manner similar to our own. Lafferty et al. (2008) sampled five fish species from the fore-reef at Palmyra Atoll. From the 110 individuals they processed, they recovered only a single morphospecies of metacercaria. If this represents fore-reef metacercaria diversity, then it is low compared to the 19 species that we documented on the sand flats. Although Lafferty et al. did not broadly sample the fore-reef fish assemblage, data from the present study were re-sampled to make sampling efforts more comparable. Ten thousand random draws (without replacement) of 110 individual fish (matching the number of individuals from Lafferty et al.) from our lagoon-flat data set recovered a minimum of 13 metacercaria species, with a mean ± SD of 16.0 ± 0.9. Additionally, controlling for the number of species examined by randomly sampling 11 individuals (Lafferty et al.’s minimum per species sample size) from 5 of the 14 fish species in our data set with adequate sample sizes, we recovered a minimum of four metacercaria species, with a mean ± SD of 13.0 ± 1.7. Further supporting the hypothesis that fore-reef fish may harbour lower metacercaria diversity, Lafferty et al. (2008) also only encountered a single metacercaria species during their sampling of the fore reef of a nearby Atoll (Christmas Island).

Why might lagoon flats have relatively high diversity and abundance of metacercariae? Compared to the fore reef, the lagoon flats are characterized by shallow, relatively still waters and high densities of probable first intermediate host bivalves and snails (unpublished data). These factors may contribute to greater infectious cercaria densities and higher transmission rates to second intermediate host fishes on the lagoon flats compared to the fore reef.

Another unique attribute of the present study is that it took place in a remote, small, relatively undisturbed atoll. As a trophically intact system, Palmyra Atoll may help establish baseline expectations for undisturbed community structure of both hosts and parasites. This may be particularly useful for efforts to develop parasites as ecological indicators of free-living diversity. For instance, trematode parasites in easily sampled first intermediate hosts can indicate more difficult to sample free-living invertebrates, fishes, birds and mammals ( Huspeni et al., 2005; Lafferty & Dunham, 2005; Hechinger et al., 2007). The host specificity documented herein can inform future efforts to use trematode assemblages in first intermediate hosts to indicate surrounding free-living diversity in the CIP and EIP marine ecoregions.

The low variability of the morphological measures used to differentiate metacercariae suggests that each taxon identified is a discrete species. However, cryptic species may, of course, occur within any of our delineated species. This may be most likely for those metacercariae with few developed morphological characters (e.g. the two didymozoids). Further cryptic diversity may be associated with those species characterized by very discrete and disjunct tissue or host use. The descriptions of morphology, and tissue and host use for these metacercariae should help guide future taxonomic research on these trematodes.

In conclusion, metacercariae appear to be abundant and diverse in Palmyra lagoon-flat fishes. The descriptions provided here will facilitate future ecological work by providing names and descriptions. The descriptions and information on host use may also help guide future life cycle and taxonomic research focused on EIP trematodes. Additionally, we hope that the diversity and abundance of metacercariae documented here highlight their importance and the need to include them in future studies of fish–helminth interactions or of the role of parasites in ecosystems.

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