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# Prevalence of Zoonotic Metacercariae in Two Species of Grouper, *Epinephelus coioides* and *Epinephelus bleekeri*, and Flathead Mullet, *Mugil cephalus,* in Vietnam

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**Abstract:** Fishborne zoonotic metacercariae have not been reported from brackish water and marine fish from Vietnam waters although these parasites are common in the country's freshwater fish. Both wild-caught and cultured grouper (*Epinephelus coioides* and *Epinephelus bleekeri*), and mullet (*Mugil cephalus*) from brackish and marine waters located in Khanh Hoa province in central coastal Vietnam were examined, and found positive for zoonotic trematode metacercariae. From grouper, *Heterophyopsis continua* and *Procerovum varium* were recovered. The prevalence of *H. continua* ranged from 2.0 to 6.0% and that for *P. varium* ranged from 11.6 to 15.8%. Mullet were infected with *Pygidiopsis summa* and *H. continua*; both of these species are new records for Vietnam. The prevalence of *P. summa* in mullet was generally high, ranging from 17.6 to 75.5%, and was significantly higher than the prevalence of *H. continua* (2.5 to 32.4%). There were no significant differences in the prevalence of metacercariae between grouper from natural or cultured habitats, indicating that the highest risk of infection occurs in the wild-caught state prior to their placement in culture. Further, there was no difference in metacercarial prevalence between the 2 species of grouper. Infected wild-caught seed were only observed from January to October. Monthly variation in prevalence suggests seasonal variation in mullet infections occurs in this region with the highest transmission taking place from October to December. Basic investigations on the ecology and epidemiology of these intestinal flukes need to be carried out to determine their significance as a public health problem and the aspects of their biology that may be vulnerable to control interventions.

**Key words:** *Heterophyopsis continua*, *Procerovum varium*, *Pygidiopsis summa*, fishborne metacercariae, zoonoses, grouper, mullet, Vietnam

## INTRODUCTION

Parasites are a ubiquitous phenomenon in the marine environment, and it is probable that all marine fish are infected with parasites [1]. Among these, fishborne zoonotic parasites, especially the trematodes (flukes) are a public health and food safety risk throughout the world [2]. While zoonotic parasites transmitted by freshwater fish are well-documented and have received considerable attention, especially the liver flukes, zoonotic intestinal flukes from marine and brackish water are far less characterized, although in many regions they are well-documented as major public health problems [3]. The zoonotic flukes identified in countries, such as Korea, Japan, China, and Thailand, indicate that digeneans belonging to the Heterophyidae and Echinostomatidae are the most common intestinal flukes seen in humans [2]. A recent review demonstrates that the number of intestinal flukes transmitted to humans specifically from marine and brackish water fish is substantial [4].

Recently zoonotic species of fishborne intestinal flukes were reported for the first time in both humans and freshwater fish in Vietnam [5-9]. However, no reports on these parasites in marine or brackish water fish in Vietnam have appeared. Because of the growing economic importance of marine and brackish water aquaculture in Vietnam, it was of interest to investigate the trematode food safety risk associated with grouper (*Epinephelus coioides* and *Epinephelus bleekeri*), a very high value cultured fish. For comparison, wild-caught grouper seed which are captured for grow-out in culture, and wild-caught mullet were also investigated.

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## MATERIALS AND METHODS

### Fish collection sites

Wild-caught and cultured grouper and mullet were collected at various times from 3 coastal districts in the Khanh Hoa area in central coastal Vietnam from October 2005 to December 2006 (Fig. 1). The grouper belonged to 2 species, *E. coioides* and *E. bleekeri*. The wild-caught grouper, termed wild-caught seed, are commonly collected from the brackish water Cua Be estuary in Nha Trang city, Khanh Hoa province, at a small size around 2.6-300 g (Table 1) and sold to fish farmers to grow-out to commercial size in either brackish water earthen ponds or in cages anchored in the Nha Trang bay (marine water) (Fig. 1). Wild-caught seed grouper examined for zoonotic metacercariae were purchased from seed dealers; grouper at various stages of grow-out (sizes) were purchased from pond fish farmers (brackish water) and from farmers who grow groupers in cages placed in marine water. The ponds are located within the tidal zones, and are fed with brackish tidal water from the Cua Be estuary. Ponds normally have an area of 1,500-3,000 $m^2$, and are 1.0-1.5 m in depth. The cages used for rearing fish are floating net cages usually far offshore. Each cage is approximately 9 $m^2$ with a depth of 3-4 m. Cages are often linked together in groups.

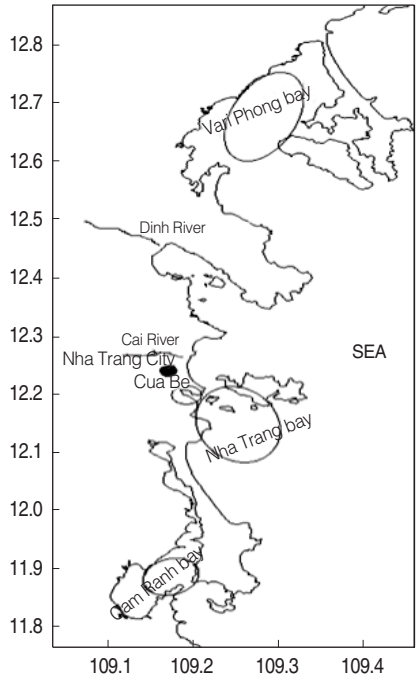


Fig. 1. Coastal area of southcentral coastal Vietnam showing fish collection sites. The latitudes are indicated on the map's vertical border.

Wild-caught mullet (*Mugil cephalus*) were collected between November 2005 to December 2006 from fish markets where the sellers provided information on their origin which included the marine habitats of Van Phong and Cam Ranh bays, and from the marine (Nha Trang bay) and brackish water (Cua Be estuary) sites in Nha Trang city (Fig. 1). The sizes and weights of the mullet examined are given in Table 2.

### Fish examination for metacercariae

Fish were collected and transported live and kept in aerated tanks before examination; fish were normally examined within 48 hr

Table 2. Number and size of mullet (*Mugil cephalus*) examined for zoonotic metacercariae

| District of origin | Fish collected at markets | | | |
|---|---|---|---|---|
| | Nha Trang | | Cam Ranh | Van Ninh |
| Habitat | Brackish water | Marine water | Marine water | Brackish water |
| No. fish | 80 | 139 | 28 | 34 |
| Length (mm) | 184 ± 78[a] (105 – 380)[b] | 251 ± 84[a] (120 – 570)[b] | 200 ± 13[a] (180 – 240)[b] | 263 ± 29[a] (200 – 325)[b] |
| Weight (g) | 112 ± 157[a] (13 – 610)[b] | 216 ± 347[a] (40 – 1,850)[b] | 90 ± 16[a] (60 – 130)[b] | 182 ± 79[a] (95 – 400)[b] |

[a]Means and standard deviations, [b]Ranges.

Table 1. Number and sizes of groupers examined for zoonotic metacercariae

| Origin | Wild-caught seed | | Cultured fish (Grow-out) | | | |
|---|---|---|---|---|---|---|
| | Cua Be estuary | | Brackish/pond (Cua Be estuary) | | Marine/cage (Nha Trang bay) | |
| Fish species | *E. coioides* | *E. bleekeri* | *E. coioides* | *E. bleekeri* | *E. coioides* | *E. bleekeri* |
| No. | 183 | 153 | 47 | 54 | 34 | 50 |
| Length (mm) | 138 ± 44[a] (60 – 290)[b] | 160 ± 58[a] (60 – 280)[b] | 342 ± 57[a] (190 – 420)[b] | 311 ± 75[a] (127 – 435)[b] | 341 ± 40[a] (215 – 420)[b] | 304 ± 59[a] (173 – 431)[b] |
| Weight (g) | 43 ± 49[a] (2.6 – 300)[b] | 69 ± 66[a] (2.7 – 310)[b] | 662 ± 292[a] (85 – 1,210)[b] | 560 ± 355[a] (27 – 1,370)[b] | 579 ± 226[a] (165 – 1,280)[b] | 442 ± 233[a] (65 – 1,220)[b] |

[a]Mean and standard deviations, [b]Range.

of arrival. A total of 608 grouper and 283 mullet were examined. The fish were measured (length) and weighed individually (data shown in Tables 1, 2). Flesh and fins were removed from individual fish and subjected to pepsin digestion as described previously [2, Annex 6]. For large fish (> 300 g), the muscle was cut into small pieces before grinding with a mortar and pestle, or homogenized in a blender. The resultant ground fish tissue was then digested in pepsin for 1-2 hr at 37℃. After recovery, the metacercariae were separated from the digest by several washing and sedimentation cycles, counted, drawn, photographed, and fixed as reference material. The metacercariae were identified using the keys and descriptions published in Velasquez [10,11].

### Data analysis

Differences in prevalence of zoonotic metacercariae between fish species, parasite species, and infections rates between the different locations and production systems were determined using a multiple chi-square test with Yates' correction. A significance level of $P = 0.05$ was used.

## RESULTS

### Grouper

The examination of grouper from the Nha Trang area revealed the presence of metacercariae of 2 potentially zoonotic trematodes, *Heterophyopsis continua* and *Procerovum varium* (Table 3). The primary metacercarial diagnostic characters were; *H. continua*, cyst round, 0.38 mm in diameter, ventral sucker larger than oral sucker, genital sucker dextro-posterior to ventral sucker, and excretory bladder somewhat Y-shaped; *P. varium*, cyst elliptical, 0.14-0.22 × 0.11-0.19 mm, a pair of eyespots lateral to pharynx, oral sucker larger than ventral sucker, ventrogenitial sac median, expulsor 0.50 mm in length, a single testis anlage, and excretory bladder with an inverted triangle shape, with excretory granules. The prevalence of these 2 flukes did not differ between the collection sites or age groups, indicating most infections were acquired by the smaller seed fish in the wild before they were caught and introduced into cage or pond culture. As seen in Fig. 2, infected wild grouper were only observed from January to October. The number of wild-caught grouper collected each month was; November 05 (n = 3), December (18), January 06 (23), February (22), March (19), April (11), May (15), June (31), July (39), August (30), September (58), October (52), November (15), and December (15).

Table 3. Prevalence (%) of zoonotic metacercariae in grouper collected from natural and aquaculture habitats in the Nha Trang district

| Parasite species | Wild-caught seed | Pond-reared fish | Cage-reared fish | *P*-value |
|---|---|---|---|---|
| *Heterophyopsis continua* | 5.1 (17/336)[a] | 2.0 (2/101) | 6.0 (5/84) | 0.35 |
| *Procerovum varium* | 11.6 (39/336) | 15.8 (16/101) | 11.9 (10/84) | 0.52 |

[a]Number infected / number examined.

Table 4. Prevalence of *Heterophyopsis continua* and *Procerovum varium* in different grouper species wild-caught and from farm ponds in Nha Trang district

| Habitat | Grouper species | Prevalence (%) | | *P*-value |
|---|---|---|---|---|
| | | *H. continua* | *P. varium* | |
| Wild-caught | *E. bleekeri* | 4.6 (7/153)[a] | 4.6 (7/153) | 0.78 |
| | *E. coioides* | 5.5 (10/183) | 17.5 (32/183) | 0.002 |
| Pond | *E. bleekeri* | 0.0 (0/54) | 20.4 (11/54) | 0.0005 |
| | *E. coioides* | 4.3 (2/47) | 10.6 (5/47) | 0.43 |

[a]Number infected/number examined.

Although the prevalence of *H. continua* in the 2 species of wild-caught seed grouper, *E. bleekeri* and *E. coioides*, did not differ, the prevalence of *P. varium* was significantly higher in *E. coioides* (Table 4). In contrast, the prevalence of *P. varium* was significantly higher in pond-reared grouper of the *E. bleekeri* species than in *E. coioides*. There were no differences in the prevalence of the 2 parasites in cage-reared grouper (data not shown).

### Mullet

Two zoonotic species of metacercariae were recovered from mullets, *H. continua* and *Pygidiopsis summa*. The identification

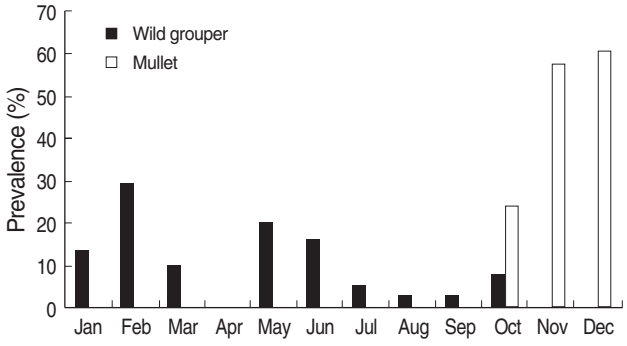


Fig. 2. Monthly prevalence of zoonotic metacercariae in grouper and mullet collected from November 05 to December 06. The grouper data reflects total metacercariae for *E. bleekeri* and *E. coioides*. Data for all species of trematode metacercariae are combined.

Table 5. Prevalence of metacercariae in wild-caught mullet (*Mugil cephalus*)

| Collection sites | Prevalence (%) | | *P*-value[a] |
|---|---|---|---|
| | *H. continua* | *P. varium* | |
| Nha Trang brackish water | 2.5 (2/80)[b] | 55.0 (44/80) | < 0.0001 |
| Nha Trang marine water | 10.8 (15/139) | 75.5 (105/139) | < 0.0001 |
| Cam Ranh marine water | 25.0 (7/28) | 67.8 (19/28) | 0.0013 |
| Van Ninh marine water | 32.4 (11/34) | 17.6 (6/34) | 0.26 |

[a]Comparisons are between parasite species at each collection site, [b]Number infected/number examined.

of *P. summa* was based on; elliptical cyst, 0.25-0.29 × 0.23-0.27 mm, eyespots present, ventrogenital sac elliptical or crescent-shaped, lying dextro-anterior at the margin of the ventral sucker, excretory bladder X-shaped. The prevalence of *H. continua* varied from 2.5% in Nha Trang brackish water locations, to 32.4% in mullet collected from the marine environment of Van Ninh. *P. summa* was less frequent in mullet from Van Ninh (17.6%) than in those from Nha Trang marine water (75.5%) (Table 5). The prevalence of *P. summa* was greater than that of *H. continua* at all locations except Van Ninh, were the difference not significant. Mullet infected with one or both species of metacercariae were only encountered in late 2006 (Table 5; Fig. 2). The number of mullet collected by month was; November 05 (n = 9); December (3); August 06 (20); September (36); October (38); November (111); and December (64).

## DISCUSSION

The results of the present survey for zoonotic metacercariae in grouper and mullet demonstrate that in the Nha Trang marine and brackish waters 3 potentially zoonotic trematodes, *H. continua*, *P. summa*, and *P. varium* are present. The first 2 species are new metacercariae records for Vietnam. Adult *H. continua* have been reported previously from a sea gull (*Larus genei*) in Vietnam [12]. *Procerovum varium* was only recently reported from freshwater fish in Vietnam [9]. Although *P. varium* is reported as a human parasite in Japan [2], Chai [4] reports that it has only been demonstrated to be infective for humans experimentally. No previous reports of *P. summa* have appeared in Vietnam.

The presence of these metacercariae in grouper represents a significant food safety concern, since raw grouper is a popular fish dish in Vietnam and elsewhere. Our data on prevalence rates in grouper raised in aquaculture indicate that options for prevention of infection of grouper may be limited. The prevalence of *H. continua* and *P. varium* did not increase significantly overall from the levels seen in the wild-caught seed during the grow-out period in either ponds or cages, indicating that the major risk to infection occurs when the smaller fish live in the brackish water environment. Control efforts in these circumstances are severely handicapped by lack of basic information on the natural life cycles of the parasites. Information on the snail vectors (taxonomy, ecology, and host-parasite dynamics) for these fluke species is nearly completely lacking. The generally broad host ranges for heterophyid trematodes make interventions to prevent egg contamination of the aquatic ecosystem problematic. For example, gulls, ducks, cats, dogs, and humans are listed as hosts for *P. summa*, the reservoir host ranges for *H. continua* and *P. varium*, however, are poorly characterized [4]. One example of the potential benefit of research on the ecology of these parasites could be in changes in selection of sources for wild-caught seed. Currently, brackish water wild-caught seed is the main source of grouper for grow-out in the Nha Trang area. Sourcing of wild-caught seed from marine water or their production by culture (nursery) could offer viable prevention options as the snail vectors may be absent in the saline marine waters. Without more research effort on these aspects of these zoonotic flukes, prevention of human infection is limited to targeting risk factors such as attempting changes in cultural eating behavior, and/or the development of post-harvest fish inspection and parasite decontamination.

Overall, there were no differences between the 2 grouper species in metacercarial prevalence, irrespective of size. However, in wild-caught seed grouper, the prevalence of *P. varium* was significantly greater than *H. continua* in *E. coioides*, but not in *E. bleekeri*. Although there have not been any spawning habitat studies reported on these 2 grouper species in the Nha Trang aquatic ecosystem, based on their biological characters there is no indication of differences in spawning and subsequent development habitats; for example, the juvenile/fingerlings are caught from the same aquatic locations. But because of the difference we observed in *P. varium* infection rates, ecological studies are needed, particularly on snail vectors occupying the same habitat as that of the grouper, and exploring a possible microhabitat preference by different grouper species that causes differences in exposure to *P. varium* cercarial infection. The explanation for the significant increase in *P. varium* only in *E. bleekeri* in the pond

grow-out stage is also not obvious, but as with the wild-caught seed, ecological investigations on snail vectors and grouper behavior in grow-out pond habitats are needed.

In the Republic of Korea, the intestinal fluke *Pygidiopsis summa* is found in mullets (*Mugil cephalus*) and gobies (*Acanthogobius flavimanus*) along the coastal areas [13] and *H. continua* is recorded from perch, goby, and shad; these fluke species have all been documented to cause human infections [14,15]. Chai [15] provides estimates that there are 10,000 cases of *H. continua* and 50,000 cases of *P. summa* in Korea. The variation in *P. summa* prevalence between geographical locations seen in this study is not unusual, and was also reported by Guk et al. [13]. The prevalence rates in the Vietnamese mullets are very high, compared to those reported in Korea [13,16]. The important definitive and intermediate hosts for these intestinal flukes in Vietnam need to be identified to better understand the factors responsible for the infection rates found, and the strong seasonal tendency for transmission. As cited above, sea gulls and cats are definitive hosts for *H. continua*, and they are also known reservoir hosts for *P. summa* [17].

Unpublished observations from Korea suggest that brackish water snails belonging to the genera *Cerithidea* and *Tympanotonus* are vectors for *P. summa* in Korea [15]. This information could be helpful in guiding investigations on the hosts for the 3 intestinal flukes identified in grouper and mullet in Vietnam. Without a reliable understanding of the life cycles and host ranges, the development of effective approaches to prevention of fishborne fluke transmission to fish and humans will be severely handicapped. The actual risk of infection to humans from consuming these fish species also needs to be determined; the recent publication of Dung et al. [6] on recovery of intestinal flukes from humans infected from eating freshwater fish in Vietnam can serve a guide to designing such studies.

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