ベトナム・ニャチャン湾の卵・仔魚に内部寄生する渦鞭毛虫Ichthyodinium sp.感染の長期変動

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Long-term Dynamics of Infection of Fish Eggs and Larvae with the Endoparasite *Ichthyodinium* sp. (Dinoflagellata) in Nha Trang Bay, Vietnam

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ABSTRACT—Results of long-term investigation of infection prevalence of fish eggs and larvae with the endoparasite *Ichthyodinium* sp. (Dinoflagellata) in Nha Trang Bay (South China Sea, Vietnam) are presented. The parasite was identified on the base of morphological characteristics and phylogenetic analysis with 18S rRNA sequences. After the first record in 1993 the infection rate steadily increased till 2004, remainded high in 2004–2007, decreased in 2008–2009 and again boosted in 2010. Seasonal and annual dynamics of the infection rate was described. The parasite showed different degrees of prevalence in different taxonomic groups of fishes. The infection rates were different among closely related species. This is the first report on long-term dynamics of *Ichthyodinium* infection in Southeast Asia.

Key words: *Ichthyodinium*, Dinoflagellate, endoparasite, phylogenetic analysis, prevalence, Vietnam

Ichthyodinium is an endoparasitic protist infecting fish eggs and resulting in 100% mortality. Infection by *Ichthyodinium chabelardi* was first reported for two fish species – *Sardina pilchardus* and *Maurolicus pennanti* in the Mediterranean Sea near the Algerian coast (Hollande and Cachon, 1952, 1953). Later, this infection has been recorded for a number of fishes from the Atlantic Ocean, among which there were commercially important species, such as mackerel *Scomber scombrus* and gilthead seabream *Sparus aurata* (Marinaro, 1971; Meneses *et al.*, 2003). At present fish infection by *I. chabelardi* is considered to be a reason for the decrease of the sardine abundance and the high mortality of eggs and larvae of mackerel in the Northwest Atlantic (Stratoudakis *et al.*, 2000; Meneses *et al.*, 2003; Gestal *et al.*, 2006). Recently *Ichthyodinium* has been discovered in the Pacific Ocean infecting different fish species in the South China Sea (Shadrin *et al.*, 2001, 2002), leopard coral grouper *Plectopomus leopardus* from Japan (Mori *et al.*, 2007) and yellow fin tuna *Thunnus albacares* from waters of Indonesia (Yuasa *et al.*, 2007). Until now it is not yet clear whether Atlantic and Pacific populations of *Ichthyodinium* are conspecific. Recently conducted molecular study revealed that the small-subunit ribosomal RNA gene sequences of *I. chabelardi* from the Atlantic Ocean were 97% similar to those of *Ichthyodinium* sp. from the Pacific (Skovgaard *et al.*, 2009).

In Nha Trang Bay (South China Sea) fish embryos and larvae infected by an endoparasites species were first recorded in 1993 (Shadrin *et al.*, 2001). Further investigations showed that this parasite morphologically was closely related to *I. chabelardi* (Shadrin, 2006). In 1993 the infection prevalence did not exceed 1%, nevertheless from year to year the infection rate has been increasing and in the second half of the 1990s most of the samples demonstrated the high prevalence of the infection. Since this infection is lethal for fish larvae, it can be one of the important factors determining the population dynamics of fishes from the coastal waters of Vietnam.

Since most previous studies of *Ichthyodinium* were based on the one host-fish species only, this paper is the first attempt to show the distribution of the infection in the diverse fish community. The aim of the paper is also to assess taxonomic selectivity of *Ichthyodinium* sp. and to discover the main tendency in long-term dynamics in the infection rate. Identification of the parasite was conducted on the basis of morphological characteristics and molecular analysis of 18S rRNA sequences.

Materials and Methods

The work was performed at the station of the Joint

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Vietnamese-Russian Tropical Research and Technological Center in Nha Trang located at Nha Trang Bay, the South China Sea. Ichthyoplankton samples were collected in the surface layer (0–5 m) in the vicinity of Nha Trang Bay by Juday net with the opening diameter of 39 cm. These samples were immediately put in the thermostatic 10 L containers and transported into the laboratory, where living fish eggs (embryos) and yolk sac larvae were sorted out. Eggs and larvae were incubated at a temperature of 25°C. Primordial schizonts of *Ichthyodinium* sp. located in the yolk sacs of embryos and larvae had a size of about 10 μm and were hardly distinguishable. After 15 h of incubation growing parasites turned into schizonts of 40–130 μm in size, which could be easily identified by light microscopy. At this stage, all the infected embryos and larvae could be easily distinguished from uninfected ones.

Material used in the present study was collected from 2001 to 2010 during three seasons: winter-spring (the first half of dry season: February–April), summer (middle of dry season: June–July) and autumn (rainy season: October–December). In some years samples were obtained during two or one seasons only (Table 1). In total, 404 samples comprising 131,421 eggs and newly hatched larvae were analyzed. The prevalence of infection was determined as the ratio of the number of infected eggs and larvae to the total number of eggs and larvae of all species presented in the sample. For two species of the genus *Encrasicholina* (Engraulidae), *E. punctifer* and *E. heteroloba,* the prevalence was determined separately. The prevalence for every season was determined as the ratio of the number of infected eggs and larvae to the total number of eggs and larvae of all species which had been collected during the season.

For taxonomic identification of eggs and early larvae, the atlas of eggs and fish larvae of coastal waters of the southern Vietnam (Shadrin *et al.*, 2003) was used.

For the molecular identification of parasite, sequences of two fragments of the 18S rRNA gene were used. Genomic DNAs of the parasite were prepared from three samples of infected yolk–sack larvae of unidentified fish from the ichthyoplankton samples, collected in Nha Trang Bay in 2007 (specimen #Din3) and 2008 (specimens #Din4 and #Din5) and fixed in 96% ethanol.

One sample with non-infected yolk sac larvae (#Din2) was used as a control. Total DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN) according to the manufacture's "Purification of total DNA from Animal Tissues Spin-Column" protocol. The templates were amplified with Smart *Taq* DNA polymerase (Dialat, Russia) and primers, designed for *Ichthyodinium* (Mori *et al.*, 2007): 96f (5′–ACT TGG CGG TTA TTC TCTAC–3′) as forward and 1679r (5′– TCG GTT CAG ACT GAA CCA AG –3′) as reverse one. PCR amplification was performed in a thermal cycler "TETRAD-2" (Biorad) using the following program: initial denaturation for 3 min at 94°C, followed by 35 cycles of 94°C for 10 s, 58°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 10 min. Sequencing was carried out on an ABI Prism 3130 sequencer (Applied Biosystems) with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) with the same primers as in PCR-amplification. Sequences were aligned by eye with the help of BioEdit Editor program (Hall, 1999). For sequence comparison BLAST program from NCBI (www.ncbi.nlm.nih.gov) was used. For data processing and trees reconstruction MEGA 4 (Tamura *et al.*, 2007) was used.

Table 1. Details of sampling for eggs and larvae of fish at Nha Trang Bay in this study

Years	Seasons	Periods	Days	Times	Numbers of eggs/larvae sampled	Mean numbers of eggs/larvae per a sampling
2001	Winter – Spring	22 March – 28 March	2	6	498	83
2002	Winter – Spring	18 March – 15 April	7	7	4,536	648
2003	Winter – Spring	1 April – 5 May	5	5	3,965	793
2004	Winter – Spring	22 Febrary – 12 April	10	12	10,837	903
2004	Summer	17 June – 23 July	5	15	2,492	166
2004	Autumn	1 November – 22 November	7	14	1,823	130
2005	Winter – Spring	2 March – 14 April	15	30	31,504	1,050
2005	Summer	18 June – 4 July	18	36	16,333	454
2006	Winter – Spring	18 Febrary – 3 April	15	45	12,285	273
2006	Autumn	31 October – 15 November	8	24	3,548	148
2007	Winter – Spring	20 Febrary – 2 April	12	36	17,625	490
2007	Autumn	28 October – 20 November	13	39	573	15
2008	Winter – Spring	13 Febrary – 12 April	20	60	6,228	104
2008	Autumn	4 November – 27 November	13	39	620	16
2009	Autumn	12 October – 8 November	12	36	4,356	121
2010	Spring	27 March – 28 April	12	36	14,198	394

Results

Earliest stages of infection that could be detectable under light microscopy were recognizable as usually single spherical structures of 10–15 μm in size (Fig. 1A). Their quantity could be 2–4 or slightly more, and more than 20 primordial schizonts were rarely observed (Fig. 1B). The parasite at its early stages of development could be detected in the middle of the periderm epiboly, as well as in yolk sac larvae soon after hatching. Each parasite grew up and began to divide as it reached 60–120 μm in size or more (Fig. 1C & F). After 10–15 h, dividing parasite cells filled three-quarter of yolk sac volume and the oil droplet started to disintegrate (Fig. 1G). In 25–34 h after the detection of the early stages of the infection, the yolk sac was filled with cells mass entirely (Fig. 1H), and all larvae or embryos died due to a burst of theirs walls. The time of complete filling of the yolk sacs with parasites in embryos with different yolk sac volumes differed unremarkably. The spherical cells with a diameter of about 20 μm released into the sea water from larvae (Fig. 1I) and began to move after 15–20 min. If the infected embryo had not been hatched out, the zoospores died inside the egg envelope.

PCR results with DNA extracted from non-infected yolk sac larvae were negative, whereas they were positive from all the infected samples. Total length of amplified fragments of gene for 18S rRNA was 1,537 bp.

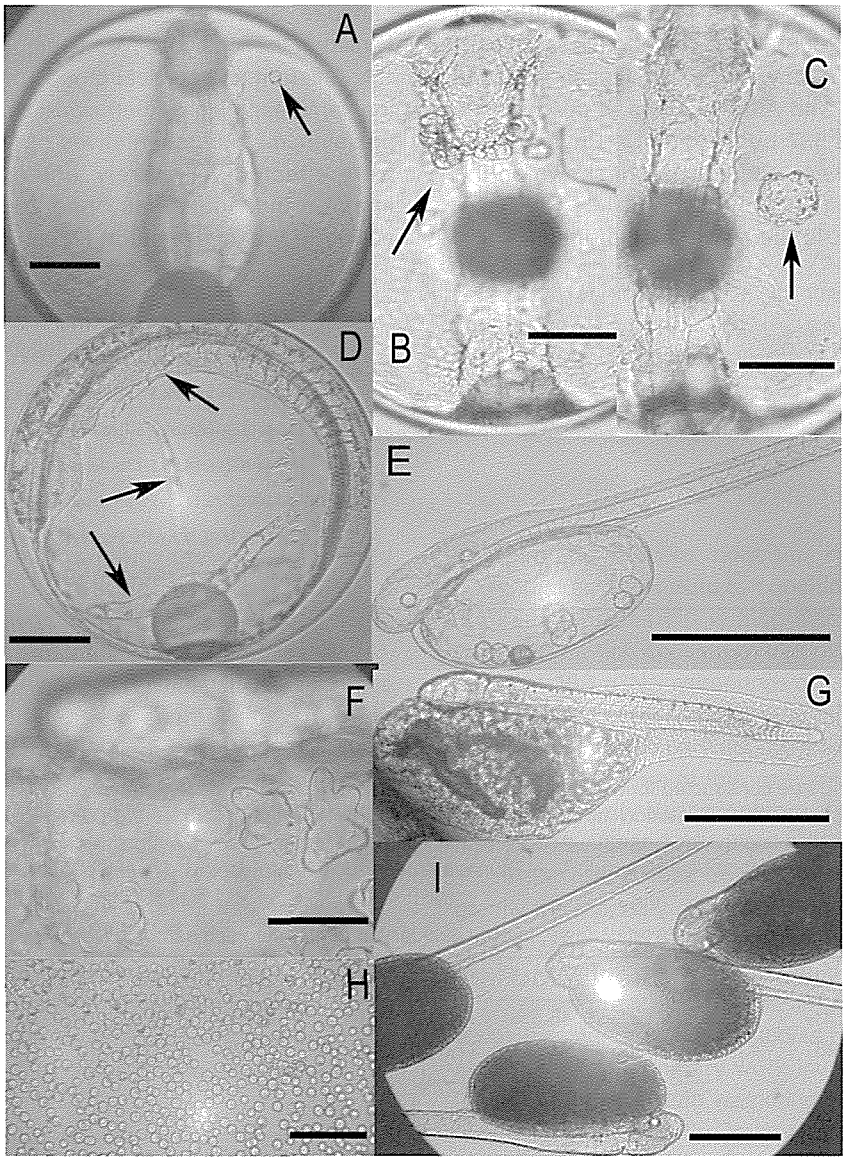

Fig. 1. Fish eggs and larvae infected with different stages of *Ichthyodinium* sp. A; egg of a leiognathid fish with primordial schizont (arrow) in the yolk sac, Bar = 100 μm, B; egg of a leiognathid fish with more than 24 primordial schizonts (arrow) in the yolk sac, Bar = 50 μm, C; beginning of first division of parasite cell (arrow) of a leiognathid fish, Bar = 100 μm, D; growing primordial schizonts (arrows) of an uncommon form, Bar = 100 μm, E; clupeid larva with primordial schizonts, Bar = 100 μm, F; mullid larva with primordial schizonts, Bar = 100 μm, G; leiognathid larva with schizonts in the yolk sac, Bar = 100 μm, H; nonmotile zoospores just released from the yolk sac, Bar = 100 μm, I; clupeid larvae at later stage of infection, Bar = 100 μm.

Sequences of the amplified 18S rRNA fragments were identical in all of the infected yolk sac larvae samples, irrespective of the year of collection. Sequence was submitted to GenBank (accession number HM363146). The results of the BLAST analyses indicated the 100% identity between 18S rRNA fragment sequences of the parasite of unidentified fish yolk sac larvae collected in the South China Sea (Nha Trang Bay) and those of *Ichthyodinium* sp. (GenBank access AB276368, submitted by Mori *et al.* in 2006) and *I. chabelardi* (GenBank access AB264776, submitted by Yuasa *et al.* in 2006), from *Plectropomus leopardus* in Japan and *Thunnus albacares* in Indonesia, respectively. Identity between our parasite sequences and sequences of *I. chabelardi* from *Boops boops* and *Sardina pilchardus* (GenBank access numbers EJ440623–EJ440627, submitted by Skovgaard *et al.* in 2008) in the Atlantic (coast of Portugal) were 98%. There were 29 substitution positions between the Pacific and Atlantic forms of this parasite for the 18S rRNA 1,537 bp fragment. Phylogenetic relations between these sequences are shown on the neighbor-joining tree (Fig. 2).

From 2002 to 2010 the prevalence of the infection varied from 11.8% to 57.0% in a winter-spring and from 6.3% to 35.8% in autumn (Table 2). The prevalence of infection of the samples could vary strongly within one season and even within several days: the difference in the prevalence could be up to 90% among the samples obtained in 2–3 days. Infected eggs and larvae were detected in the majority of the samples, although in February and March of 2006 and 2008 no infection was recorded for about 25–30 days.

Taxonomic diversity of the pelagic fish eggs in the investigated region was very high, and identification of many groups was rather complicated. However, we were able to identify some of them confidently and we can say with certainty that infected representatives of families Leiognathidae, Engraulidae, Clupeidae, Mugilidae, Labridae, Scaridae, Soleidae and Serranidae, were presented in the ichthyoplankton samples regularly.

Different taxonomical groups of fishes presented in the same samples differed significantly by their infection prevalence. Representatives of several other groups were infected very rarely, and in some of them no infected individual was observed during the whole period of the investigation (Table 3). The prevalence

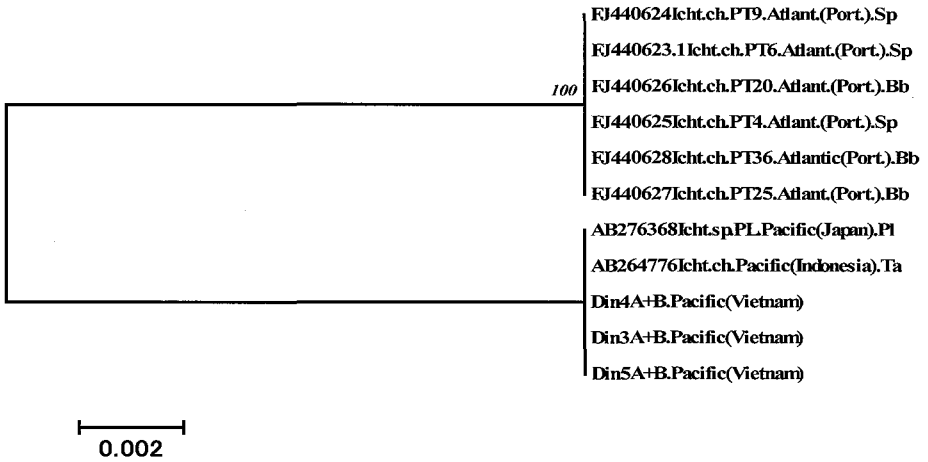

Fig. 2. Neighbor-joining tree constructed for sequences of two fragments of 18S rRNA gene (total length 1,537 bp) of parasite, using the Kimura 2-parameter substitution model. At the nodes – Bootstrap probabilities (1,000 replicates). Each sequence includes the location and fish host name. Abbreviations: Atlant. – Atlantic, Port. – Portugal; Bb – *Boops boops*, Sp – *Sardina pilchardus*, Pl – *Plectropomus leopardus*, Ta – *Thunnus albacares;* Icht. sp.-*Ichthyodinium* sp., Icht. ch.-*I. chabelardi*.

Table 2. Infection prevalences of *Ichthyodinium* sp. in eggs and larvae of unsorted fish sampled at each season of 2001 to 2010

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Winter to Spring	52.3%	16.7%	21.2%	48.8%	48.1%	39.9%	41.8%	11.8%	–	57.0%	42.9%
	498*	4,536	3,965	10,837	31,504	12,285	17,625	6,228		14,198	10,1178
Summer	–	–	–	23.5%	11.7%	–	–	–	–	–	13.3%
				2,492	16,333						18,825
Autumn	–	–	–	15.0%	–	35.8%	6.3%	8.5%	11.4%	–	19.5%
				1,823		3,548	573	620	4,356		10,920
Total	52.3%	16.7%	21.2%	40.6%	35.7%	38.9%	40.7%	11.5%	11.4%	57.0%	36.5%
	498	4,536	3,965	15,152	47,837	15,833	18,198	6,848	4,356	14,198	131,421

* Numbers of fish sampled

Table 3. The infection prevalences of *Ichthyodinium* sp. in eggs/larvae in some fish taxons

Taxon of fish	Infection prevalence of the parasites / Number of examined eggs or larvae										
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Soleidae	–	–	–	–	–	–	–	–	–	83%	83%
	–	–	–	–	–	–	–	–	–	78	78
Leiognathidae	–	–	–	–	–	78.8%	–	–	–	–	78.8%
	–	–	–	–	–	400	–	–	–	–	400
Unsorted*	52.3%	16.7%	21.2%	40.6%	35.7%	38.9%	40.7%	11.5%	11.4%	57.0%	36.5%
	498	4,536	3,965	15,152	47,837	15,833	18,198	6,848	4,356	14,198	131,421
Anguilliformes	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	17	19	56	70	127	72	115	53	32	87	648
Synodontidae (Aulopiformes)	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	31	45	144	213	302	190	210	166	45	329	1,675
Fistulariidae	–	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(Gasterosteiformes)	0	3	8	22	16	7	33	10	5	29	133
Uranoscopidae (Perciformes)	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	5	0	4	7	5	3	5	4	0	17	50
Trichiuridae (Perciformes)	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	3	0	9	11	7	7	9	5	4	27	82
Ostraciidae	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
(Tetraodontiformes)	3	2	15	8	10	5	3	6	2	8	62
Engraulidae											
Encrasicholina punctifer	–	–	–	–	15.8%	18.6%	12.8%	15.4%	6.7%	8.8%	13.4%
					1,232	614	1,395	2,026	104	1,872	7,243
Encrasicholina heteroloba	–	–	–	–	0%	0%	0%	0%	0%	0%	0%
					683	1,065	389	230	27	0	2,394
											12,765**

* Different taxonomic groups of fishes included Leiognathidae, Engraulidae, Clupeidae, Mugilidae, Labridae, Scaridae, Soleidae and Serranidae

** Total number of sorted fish in this study

was very different even among the closely related species. For example, during the whole period of study, sometimes even within the same sample, no infected egg or larva was found in *Encrasicholina heteroloba* (Engraulidae), while in *E. punctifer* infected eggs and larvae were constantly present (Table 3). The infection prevalence of the same families strongly varied in different periods. Leiognathidae, which usually demonstrated high infection rates, sometimes did not contain a single infected individual even during mass spawning.

Discussion

Morphological characteristics of the parasite from Vietnam were identical to the species from Atlantic (Hollande and Cachon, 1953; Stratoudakis *et al.*, 2000; Meneses *et al.*, 2003) and Pacific oceans (Yuasa *et al.*, 2007).

Complete identity between 18S rRNA sequences (1,537 bp) of parasite infecting yolk sac larvae of unidentified fish, collected in coastal waters of Vietnam (South China Sea, Nha Trang Bay) and those of *Ichthyodinium* sp. (Mori *et al.*, 2007) and *I. chabelardi* (Yuasa *et al.*, 2007), infecting *Plectropomus leopardus* in Japan and *Thunnus albacares* in Indonesia, respectively, makes it possible to conclude that we deal with the same species. It is also evidence of wide spectrum of host fish species of this parasite. Differences between 18S rRNA sequences of *I. chabelardi* from the Atlantic and Pacific Oceans may reflect high geographical diversity of this parasite, as well as the existence of two forms or races of this species. Future investigations may clarify the taxonomic status of this parasite from the Pacific and Atlantic.

This infection is obviously a factor influencing greatly the mortality of eggs and early larvae of pelagic-spawning fishes. The investigation of the ichthyoplankton in Nha Trang Bay started in 1993. From 1993 up to 2000 the prevalence increased slightly but generally it was low, and no focused studies have been conducted so far. The preliminary work investigating the prevalence of *Ichthyodinium* was conducted in 2001, when it was discovered that the level of prevalence was rather high, so this fact became the reason for launching the study in the following years. On the basis of the data collected in 2002–2010, the prevalence tended to increase in 2002–2004, keeping high in 2005–2007, but in 2008–2009 it showed its decrease, and once again in

2010 a significant increase was observed (Table 2).

Seasonal prevalence fluctuations were not clearly expressed. Most likely it is related to general stability of the tropical environment. The dry season (February–March) is usually favorable for most hydrobionts. The rainy season, which starts in October, is characterized by the decrease of water temperature and the increase of precipitations causing certain desalination of water, and also by storms. Obviously, the living conditions in coastal ecosystems change dramatically only in autumn. At the same time no significant seasonal variation in the infection prevalence was found. In autumn, because of strong storms and desalination, significant decrease of spawning activity is usually registered in most dominant species. These factors may reduce the number of circulating parasites in the environment and consequently cause quantity reduction of infected eggs and larvae or their absence for a long period in February and March, as it was observed in 2006 and 2008. However, the average infection rate of summer material was quite low. This indicates first and foremost the complicated dynamics of the process itself. In order to gain a more complete understanding of its mechanisms all-season studies should be conducted. The obtained results undoubtedly show that the phenomenon under investigation is an important factor strongly affecting fish mortality at the early stages of development for every season.

The resistance of different taxonomic fish groups to the infection is different. Fishes can be divided into two main groups by the degree of resistance to the infection – "infected" and "uninfected" by *Ichthyodinium* sp. Both groups contain species with different life history strategies, but among the "infected" group the pelagic fishes dominate, while among the "uninfected" group the bottom dwelling fishes mainly occur. The mechanism of selectivity of parasites may be related to its ecological features. However, it seems that much more complicated processes are responsible for the mechanisms of selectivity, as the resistance to the infection varys significantly even among some closely related species with a very similar biology (e.g. *E. punctifer* and *E. heteroloba*). It is quite probable that taxonomic selectivity of *Ichthyodinium* sp. could be revealed only if the main details concerning the penetration mechanism of this parasite into the host are thoroughly investigated.

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ベトナム・ニャチャン湾の卵・仔魚に内部寄生する渦鞭毛虫 *Ichthyodinium* sp. 感染の長期変動

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ベトナム・ニャチャン湾の卵・仔魚に内部寄生する *Ichthyodinium* sp. の感染を季節ごとに長期に渡り調査した。寄生虫の同定は形態と18S rRNA 塩基配列によって行った。塩基配列は沖縄の種苗生産スジアラから報告されたものと一致した。1993年に初めて検出された後，感染率は2004年までは増加し続け，その後，2007年まで高位で推移し，2008－2009年で減少したものの，2010年に再び急増した。感染率は，異なる分類群の魚種間で差異が見られた他，近縁な魚種間でも大きな違いが見られた。

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種苗を放流した河川での冷水病の発生状況調査

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放流前の検査で冷水病菌陰性の人工アユ種苗を放流しているにもかかわらず，毎年本病が発生している河川の感染源を検討した。2006，2007年に放流前の検査で本菌が分離されなかった種苗を冷水病菌フリー用水で成熟期まで継続飼育した結果，両年とも陽性であったことから，放流魚が保菌していたと考えられる。これらの放流後，天然魚と放流魚における本病の発生状況を調査した結果，両年とも6～8月に冷水病が発生したが，発病源は06年が放流魚，07年が天然魚と放流魚であった。成熟期には90％以上のアユから本菌が検出された。

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PCR によるスクーチカ症の原因繊毛虫 *Miamiensis avidus* の同定および検出

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形態観察による同定が難しいスクーチカ症原因繊毛虫 *Miamiensis avidus* を特異的に検出するため，SSU rRNA 遺伝子を標的とした PCR 法を確立した。本 PCR の検出限界は DNA 量にして 125 fg であり，他のスクーチカ繊毛虫3種の DNA を増幅しなかった。感染したヒラメ群を PCR 検査した結果，発症魚の90％，未発症魚の50％が陽性となり，特に脳での検出率が高かった。以上より，本 PCR は *M. avidus* の同定および魚体からの迅速検出に有効であることが明らかとなった。

魚病研究，45（3），130–132（2010）

ベタにみられた腎芽腫

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米国でベタ *Betta splendens* の成魚に腎芽腫がみられた（2症例）。いずれの症例も，腫瘍塊は腎臓組織の90％以上を占めていた。病理組織学的にはこれらの腫瘍は，芽球細胞，未熟な間葉系組織および糸球体様構造を形成する上皮性細胞より構成されていた。また。最初の事例では嚢胞性の腫瘍塊が体腔の2/3をおおい，他の事例では白色の腫瘍塊が頭蓋から背びれにかけて広がっていた。Wilms' Tumor-1 診断用抗体との反応性は不明瞭であった。本論文はベタにおける腎芽腫の初報告である。

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Acremonium sp. に対する各種抗真菌剤の *in vitro* および *in vivo* での効果

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罹病シャコより分離された *Acremonium* sp. は，*in vitro* で抗真菌剤，ボリコナゾール，アンホテリシン B および塩酸テルビナフィンに感受性を示した。*Acremonium* sp. に人為感染させたクルマエビにボリコナゾールを経口および筋肉内投与した結果，肉眼所見，死亡率および病理組織学的所見より，本薬剤は *Acremonium* sp. に対して有効な抗真菌剤であることが示された。

魚病研究，45（3），109–114（2010）

ヒラメ白血球包囲化反応における顆粒球およびケモカインの活性

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Ichthyophonus hoferi の多核球状体に対するヒラメ白血球の *in vitro* 包囲化反応では，ペルオキシダーゼ陽性の顆粒球が多数を占めていた。顆粒球は *I. hoferi* に接着し，その周囲を取り囲んでいた。包囲化の過程において，3種類の CC ケモカインおよび1種類の CXC ケモカインの mRNA 発現上昇が確認された。特に，CC-CLM および IL-8 は顆粒球により産生され，異物包囲化の初期反応における顆粒球の重要性が示唆された。

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シャコより分離された *Plectosporium oratosquillae* および *Acremonium* sp. のクルマエビに対する病原性

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シャコより分離された不完全菌 *Plectosporium oratosquillae* NJM 0662 と *Acremonium* sp. NJM 0672 の分生子浮遊液をクルマエビに筋肉注射した結果，いずれの菌もクルマエビを死亡させた。病クルマエビの鰓には多数の黒色点が出現し，病理組織学的に鰓弁内および接種部周囲に菌糸が観察され，それらは血球によって取り囲まれていた。供試した菌はクルマエビに病原性を有することが示された。

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16S rDNA を標的とした *Piscirickettsia salmonis* 検出用 PCR の改良

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ピシリケッチア症の診断法として 16S rDNA を標的とする PCR による原因菌の検出が報告されているが，そのプライマー領域の塩基配列に変異を生じている菌株が存在する。そこで，新たに特異的なプライマーを設計した結果，特異性と検出感度が向上した。さらに，本 PCR により人為感染魚の肝臓と腎臓から *P. salmonis* が検出された。従って，本 PCR はピシリケッチア症の診断に利用できると考えられた。

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