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Ichthyobodo necator (Kinetoplastida) — a complex of sibling species

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ABSTRACT: *Ichthyobodo necator* is a parasitic flagellate that attacks fishes, causing disease problems in freshwater worldwide. Findings of similar flagellates in strictly marine fishes have indicated that ichthyobodiosis may be caused by more than 1 flagellate species. We obtained partial small subunit rDNA (ssu rDNA) sequences of 14 *Ichthyobodo* isolates originating from fishes in Norway, Japan, Singapore, South Africa and Brazil, and identified 8 strains or species, including 2 species infecting cultured salmon in Norway. An *Ichthyobodo* species isolated from the skin of Atlantic salmon parr in freshwater is suggested to represent *I. necator* sensu stricto, while another species, showing particular affinity for the gills, infects salmon in both fresh- and seawater. Atlantic cod is infected with a marine *Ichthyobodo* species unrelated to those infecting salmonids; 2 cyprinids originating from different parts of the world had related *Ichthyobodo* strains/species, and 2 isolates from unrelated North and South American fishes were also closely related. The phylogenetic relationships of the *Ichthyobodo* isolates is described, and the implications of the molecular findings on past and future morphological studies of *Ichthyobodo* spp. are discussed.

KEY WORDS: *Ichthyobodo necator* · ssu rDNA 18s · Kinetoplastida · Species complex · Parasitic flagellates

INTRODUCTION

Ichthyobodo necator is an ectoparasitic flagellate that infects the skin and gills of fishes. Flagellates identified as *I. necator* have repeatedly been implicated in diseases and mortalities among ornamental and farmed fishes (Woo 1994). The genus *Ichthyobodo* Pinto, 1928 at present comprises 1 valid species, *I. necator* (Henneguy, 1883), which has been considered cosmopolitan in distribution, with records from both fresh- and seawater (Robertson 1985, Lom & Dykova 1992).

Ichthyobodo necator was originally described from diseased brown trout *Salmo trutta* alevins cultured in tanks at the College de France, Paris (Henneguy 1883, 1884). The flagellate is now one of the principal disease agents in salmonid hatcheries, typically causing epizootics the first few weeks after start-feeding (Plehn 1924, Robertson 1985, Urawa 1993, 1995, 1996, Rintamäki-Kinnunen & Valtonen 1997).

The flagellate has a very wide temperature and pH tolerance (Woo 1994). In Japan, it has been recorded in salmonid hatcheries at temperatures between 2 and 14°C (Urawa 1992). In cyprinids, epizootics of the flagellate have been observed in hibernating carp *Cyprinus carpio* at 2°C and in thermal effluents at temperatures exceeding 30°C (Schäperclaus 1929, 1986, Benisch 1936). In tropical ornamental fishes (poecilids

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and tilapia) *Ichthyobodo necator* may be active at temperatures as high as 38°C (Tavolga & Nigrelli 1947).

Ichthyobodo necator infections in seawater were first detected in freshwater salmonids transferred to the sea, suggesting that the parasite originates in freshwater, but is able to survive and proliferate in the marine environment (Wood 1968, Ellis & Wootten 1978, Needham & Wootten 1978). This assumption has been verified in studies on Japanese salmonids (Urawa & Kusakari 1990, Urawa et al. 1998), while the situation in Europe is apparently more complex. In freshwater, the parasite is more common on the skin than on the gills, as opposed to occuring almost exclusively on the gills in seawater (Poppe & Håstein 1982, Poppe 1990). In Scottish salmonids, *Ichthyobodo* on the gills of seawater-reared fish is significantly smaller than specimens from freshwater fish (Bruno 1992), while in Japan the flagellate is the same size in both environments (Urawa & Kusakari 1990). Ultrastructurally, there are also differences in the attachment discs of *I. necator* infecting Scottish salmonids in freshwater and seawater. The former has ridge-like structures on the attachment disc that extend along the cytostome process, while in the latter these structures are smooth (Roubal & Bullock 1987).

Following detection of *Ichthyobodo* infections on seawater-reared salmonids, flagellates resembling *I. necator* were detected on a wide range of strictly marine fish species and also on invertebrates (reviewed by Urawa et al. 1998). This has led several researchers to suspect the existence of a distinct marine *Ichthyobodo* form or species (Morrison & Cone 1986, Diamant 1987). Strong support for this was provided by Urawa & Kusakari (1990), who performed cross-infection cohabitation experiments, and found that *Ichthyobodo necator* from seawater-acclimatized chum salmon *Oncorhynchus keta* did not infect Japanese flounder *Paralichthys olivaceus*, and that *Ichthyobodo* sp. from the flounder did not infect the salmon.

Several lines of evidence thus suggest that *Ichthyobodo necator* in fact comprises various strains or species with varying host and environmental preferences. In this study, we sequenced the small subunit (ssu) rRNA gene of *Ichthyobodo* isolated from freshwater and marine hosts, and revealed a genetic heterogeneity that exceeds recent suggestions that *Ichthyobodo* comprise 2 cosmopolitan species (Woo 1994, Urawa et al. 1998).

MATERIALS AND METHODS

Ichthyobodo isolates were collected from different hosts in both fresh- and seawater from several different geographical regions (Table 1). The salmonids were hatchery-reared or seawater-farmed fishes. The Fusa salmon *Salmo salar* (10 to 50 g) were from a hatchery receiving water from a reservoir with no anadromous fish, the sticklebacks *Gasterosteus aculeatus* were from the reservoir itself. The Masfjorden-I salmon (50 g) came from a hatchery with seawater addition to the inlet water, while the salmon from Ytre Sogn (1000 g) and Masfjorden-II were postsmolts pen-reared in seawater (35 and 10 wk after transfer to seawater). The Japanese masu salmon *Oncorhynchus masou* (Salmonidae) isolate was from a heavily infected juvenile (ca. 1 g) reared at the Chitose Hatchery, western Hokkaido. Cultured cod *Gadus morhua* (Gadidae) comprised pen-reared juvenile fish (a heavily infected 30 g fish from Tromsø and a lightly infected 39 g fish from Parisvatnet). Diseased cichlids *Apistogramma* sp. (*A. pertensis* or *A. uaupesi*) (ca. 4 cm) were

Table 1. *Ichthyobodo*. Hosts and locality data on isolates used in this study. Sk: skin; Gi: gills; Fi: fins, FW: freshwater; BW: brackish-water (or freshwater with added seawater); SW: seawater; Collection dates given as d/mo/yr; –: no data

Code	Host	Site	Water	Collection date	Locality	Accession No.
*Ss*1	*Salmo salar*	Sk	FW	18.08.00	Fusa, W Norway	AY224691
*Ss*2	*Salmo salar*	Sk	FW	14.09.01	Oslo, E Norway	AY224691
*Ss*3	*Salmo salar*	Gi	FW	27.11.02	Tromsø, N Norway	AY229972
*Ss*4	*Salmo salar*	Sk	BW	07.02.02	Masfjorden-I, W Norway	AY224686
*Ss*5	*Salmo salar*	Gi	SW	17.01.02	Ytre Sogn, W Norway	AY224685
*Ss*6	*Salmo salar*	Gi	SW	04.09.02	Masfjorden-II, W Norway	AY229973
Ga	*Gasterosteus aculeatus*	Sk	FW	14.09.01	Fusa, W Norway	AY224691
*Gm*1	*Gadus morhua*	Gi	SW	02.11.00	Parisvatnet, W Norway	AY224690
*Gm*2	*Gadus morhua*	Gi	SW	05.02.03	Tromsø, N Norway	AY255800
Om	*Oncorhynchus masou*	Fi	FW	06.06.01	Hokkaido, Japan	AY224689
Asp	*Apistogramma* sp.	Sk	FW	11.11.02	Rio Negro, Brazil	AY224692
Cc	*Cyprinus carpio*	Sk	FW	28.02.02	South Africa	AY224688
Ca	*Carassius auratus*	Sk	FW	30.08.02	Singapore	AY224687
*M*h	*Morone* hybrid	Sk/Gi	FW	–	North Carolina, USA (Callahan et al. 2002)	AY028448

provided by a local dealer in ornamental fishes. These fish had been kept in a tank with healthy *Pterophyllum altum* (Cichlidae), originating from the same area (lower Rio Negro, Brazil). The South African *Ichthyobodo* isolate was from pond-reared koi-carp *Cyprinus carpio* (Cyprinidae) fry, while the goldfish *Carassius auratus* (Cyprinidae) isolate was from diseased individuals imported from Singapore.

Scrapings from the body surface and gills containing *Ichthyobodo* were stored in 100% ethanol at 4°C. The flagellates were studied by light microscopy and identified as *Ichthyobodo* by morphological characteristics and their appearance in hematoxylin or Diff-Quick™-stained smears.

DNA was extracted from the ethanol-conserved samples using the DNeasy protocol for animal tissues (Qiagen). DNA was eluted in 50 µl AE buffer supplied with the Qiagen-kit and stored at −20°C before use in PCR.

PCR primers were constructed based on ssu rDNA sequences from other kinetoplastids: *Bodo caudatus* (Accession No. AY028450), *B. saltans* (AF208889), *Dimastigella mimosa* (AF208882), *D. trypaniformis* (X76494), *Rhynchomonas nasuta* (AF174378), *Trypanoplasma bullocki* (AF080224) and *T. salmositica* (AF080225). The primers targeted conserved areas of the kinetoplastid ssu rDNA, and at the same time avoided areas that showed close similarities to salmon ssu rDNA (Table 2). In addition, a primer (KinSSUF1) described by Callahan et al. (2002), targeting kinetoplastid ssu rDNA, was used to increase the length of the partial ssu rDNA sequences for the different *Ichthyobodo* isolates.

The PCR products were purified on Qia-quick PCR purification columns (Qiagen) and then sequenced using the BigDye terminator sequencing kit. The sequencing was performed using the amplification primers BF4 and BR3, in addition to other upstream and downstream primers (Table 2).

The sequence data were assembled with the help of Vector NTI software (InforMax), and GenBank searches were done with BLAST (2.0). The vector NTI Suite software package was used for multiple alignments of partial sequences. To perform pairwise comparisons between the different isolates, the multiple-sequence alignment-editor GeneDoc (available at www.psc.edu/biomed/genedoc) was used. The following sequences from the EMBL nucleotide database were included in the analyses: *Blastocrithidia gerricola* (AF153036), *Bodo caudatus* (AY028450, X53910), *B. designis* (AF209856), *B. edax* (AY028451), *B. saliens* (AF174379), *B. saltans* (AF208889), *Bodo* cf. *uncinatus* (AF208884), *Crithidia fasciculata* (Y00055), *Cryptobia helicis* (AF208880), *Diplonema papillatum* (AF119811), *Diplonema* sp. (AF119812), *Dimastigella trypaniformis* (AY028447, X76494), *Euglena gracilis* (AY029409), *E. stellata* (AF283310), *Herpetomonas muscarum* (L18872), *Ichthyobodo* sp. (AY028448), *Leishmania tarentolae* (X53916), *Leptomonas peterhoffi* (AF153039), *Parabodo nitrophilus* (AF208886), *Procryptobia* (*'Bodo'*) *sorokini* (AF208888), *'Rhynchobodo'* (U67183), *Rhynchomonas nasuta* (AF174377, AF174378), *Trypanoplasma* (*'Cryptobia'*) *bullocki* (AF080224), *T.* (*'C.'*) *salmositica* (AF080225), *T.* (*'C.'*) *catostomi* (AF080226), *T. borreli* (L14840), *Trypanosoma avium* (AF416563), *T. boissoni* (U39580), *T. brucei gambiense* (AJ009141), *T. brucei rhodesiense* (AJ009142), *T. cobitis* (AJ009143), *T. cruzi* (AF303660), *T. ranarum* (AF119810), *T. rotatorium* (AJ009161), *T. triglae* (U39584), *Wallaceina inconstans* (AF153044).

Phylogenetic analysis of the data sets were performed using the PAUP* 4.0 version (Swofford 1998) and Puzzle (Version 4.0.2). Phylogenetic trees were constructed from molecular sequence data by the maximum likelihood method. Phylogenetic trees were drawn using TreeView (Page 1996).

Table 2. Primers. 'Position' refers to the small-subunit (ssu) rDNA of *Ichthyobodo* sp. Accession No: AY028448 (Callahan et al. 2002)

Name	Sequence	Position
BF3	5′-AAGGACTAAGCCATGCATGC-3′	34–54
BF4	5′-CATTAAAACAGAGATAATCTACCGGGG-3′	95–120
BF5	5′-AGAGAAATAGCGACCCAGACCTTC-3′	500–523
BF6	5′-GCGAAGGCATTTTCCAAGTATACC-3′	1074–1097
BF8	5′-GGATAACTTCGCTAACGCGAAGC-3′	147–169
BF10	5′-AAACGTATCTGAGCGAGAGAGGTG-3′	1019–1042
BFX	5′-GGGAGCCTGAGAAACAGCTACC-3′	418–439
BR3	5′-GCAGGTTCACCTACAGCTACCTTG-3′	1998–1975
BR4	5′-TTAAATACCACTCATTCCGATC-3′	553–532
BR5	5′-GTCAATCCTTGCGTTTTCCTGTAC-3′	1396–1373
BR7	5′-TGCTTCCTCTACTAGTTAAACATGGGG-3′	1964–1938
BR10	5′-AAATTAAACCGCACACTCCACG-3′	1348–1327

RESULTS

Partial ssu rDNA sequences were obtained from all *Ichthyobodo* isolates by PCR using BF4 and BR3 as primer set. The primer KinSSUF1 was used to add nucleotides at the 5′-end; the other primers were used for sequencing the different *Ichthyobodo* isolates.

The sequence similarities between the various *Ichthyobodo* isolates are given in Table 3. The isolate from the skin of Atlantic salmon parr *Salmo salar* from a freshwater hatchery in Fusa (W Norway) and from freshwater in Oslo (E Norway) (Isolates *Ss*1 and

Table 3. *Ichthyobodo*. Small-subunit (ssu) sequence similarities between different isolates. Above diagonal: percent similarity; below diagonal: number of substitutions per alignment (bp). Sp.: species or strain (I–VIII); *Ss*: *Salmo salar*; *Ga*: *Gasterosteus aculeatus*; *Om*: *Oncorhynchus masou*; *Gm*: *Gadus morhua*; *Mh*: *Morone* hybrid; *Asp*: *Apistogramma* sp.; *Cc*: *Cyprinus carpio*; *Ca*: *Carassius auratus*

Sp.		*Ss*1	*Ss*2	*Ga*	*Ss*3	*Ss*4	*Ss*5	*Ss*6	*Om*	*Gm*1	*Gm*2	*Mh*	*Asp*	*Cc*	*Ca*
I	*Ss*1		100.0	100.0	92.2	92.2	92.2	92.2	91.2	93.3	91.9	92.7	92.2	91.8	91.6
I	*Ss*2	0/1958		100.0	92.2	92.2	92.2	92.2	91.2	93.3	91.9	92.7	92.2	91.8	91.6
I	*Ga*	0/1958	0/1958		92.2	92.2	92.2	92.2	91.2	93.3	91.9	92.7	92.2	91.8	91.6
II	*Ss*3	149/1913	149/1913	149/1913		99.7	99.7	99.7	98.5	94.3	92.2	92.7	93.1	93.4	93.5
II	*Ss*4	150/1929	150/1929	150/1929	5/1937		100.0	100.0	98.4	94.2	92.1	92.8	93.2	93.6	93.7
II	*Ss*5	150/1929	150/1929	150/1929	5/1937	0/1955		100.0	98.4	94.2	92.1	92.8	93.2	93.6	93.7
II	*Ss*6	150/1929	150/1929	150/1929	5/1937	0/1943	0/1940		98.4	94.2	92.1	92.8	93.2	93.6	93.7
III	*Om*	169/1924	169/1924	169/1924	29/1897	30/1918	30/1918	30/1918		93.8	93.8	91.9	92.1	92.7	92.4
IV	*Gm*1	125/1870	125/1870	125/1870	107/1872	108/1872	108/1872	108/1872	118/1898		100.0	94.0	94.0	94.6	94.3
IV	*Gm*2	158/1958	158/1958	158/1958	151/1943	155/1968	155/1968	155/1968	120/1928	1/1907		92.1	92.0	92.6	92.5
V	*Mh*	143/1958	143/1958	143/1958	141/1937	141/1959	141/1959	141/1959	154/1924	115/1907	156/1978		98.7	93.9	94.0
VI	*Asp*	154/1931	154/1931	154/1931	133/1931	131/1930	131/1930	131/1930	150/1903	112/1877	156/1948	25/1938		94.2	94.1
VII	*Cc*	159/1949	159/1949	159/1949	128/1951	126/1974	126/1974	126/1974	141/1931	103/1899	145/1970	121/1977	113/1959		97.3
VIII	*Ca*	163/1950	163/1950	163/1950	125/1931	123/1954	123/1954	123/1954	146/1931	109/1900	147/1956	118/1956	114/1937	51/1916	

*Ss*2), and the isolate from the skin of stickleback *Gasterosteus aculeatus* (*Ga*) from the lake in Fusa had identical ssu rDNA sequences. The freshwater isolate from the gills of salmon parr (*Ss*3) from Tromsø (N Norway) showed 92% sequence similarity (1884 nucleotides) with the other freshwater isolates from salmon parr.

The *Ichthyobodo* isolates collected from salmon in marine farms (*Ss*5 and *Ss*6) and from parr (*Ss*4) in a hatchery where seawater was added were identical. These isolates came from different regions in western Norway, and their ssu sequences were also identical (99.7%) to the isolate (*Ss*3) from the gills of Atlantic salmon parr reared in freshwater in Tromsø. These 'marine' isolates (*Ss*3 to 6) from Norwegian salmon were also similar (98.4 to 98.5%) to the *Ichthyobodo* isolate from masu salmon in Japan.

The Norwegian isolates of *Ichthyobodo* from a strictly marine fish, Atlantic cod *Gadus morhua*, showed 92 to 94% similarity to the freshwater (*Ss*1 and 2) and seawater (*Ss*3 to 6) isolates from Norwegian salmon.

The 2 American *Ichthyobodo* isolates, originating from hybrid striped bass (*Morone saxatilis* × *M. chrysops*) and *Apistogramma* sp., grouped with 98.7% sequence-similarity. The 2 *Ichthyobodo* isolates from the cyprinids *Cyprinus carpio* and *Carassius auratus* also showed the highest ssu sequence similarity with each other (97.3%).

Based on ssu sequences, Fig. 1 shows the phylogenetic relationships between the different *Ichthyobodo* isolates and other kinetoplastids. The *Ichthyobodo* isolates comprise a sister group to the other kinetoplastids. The topology of an *Ichthyobodo* tree rooted with other kinetoplastids (Fig. 2) shows the Norwegian freshwater isolates from the skin of salmon and stickleback (*Ss*1, *Ss*2 and *Ga*) as a sister group to the other *Ichthyobodo* isolates. The isolate from *Oncorhynchus masou* groups closely with the seawater isolates from Norwegian salmon *Salmo salar*. The 'warm water' isolates (*Apistogramma* sp., *Cyprinus carpio*, *Carassius auratus*, *Morone* hybrid), all from freshwater fishes, group together with good support (support value, SV = 85). The position of *Ichthyobodo* from the strictly marine fish *Gadus morhua* (*Gm*) is poorly resolved (SV = 59). A distance tree (data not shown) showed the exact same topology as that in Fig. 2.

DISCUSSION

The present study of the ssu rDNA from different isolates of *Ichthyobodo* indicates that this genus comprises several species. Callahan et al. (2002) adopted an ssu rDNA sequence divergence of 1.3% between 4 unambiguous *Trypanoplasma* species (see Doležel et al. 2000, Maslov et al. 2001) to indicate separate spe-

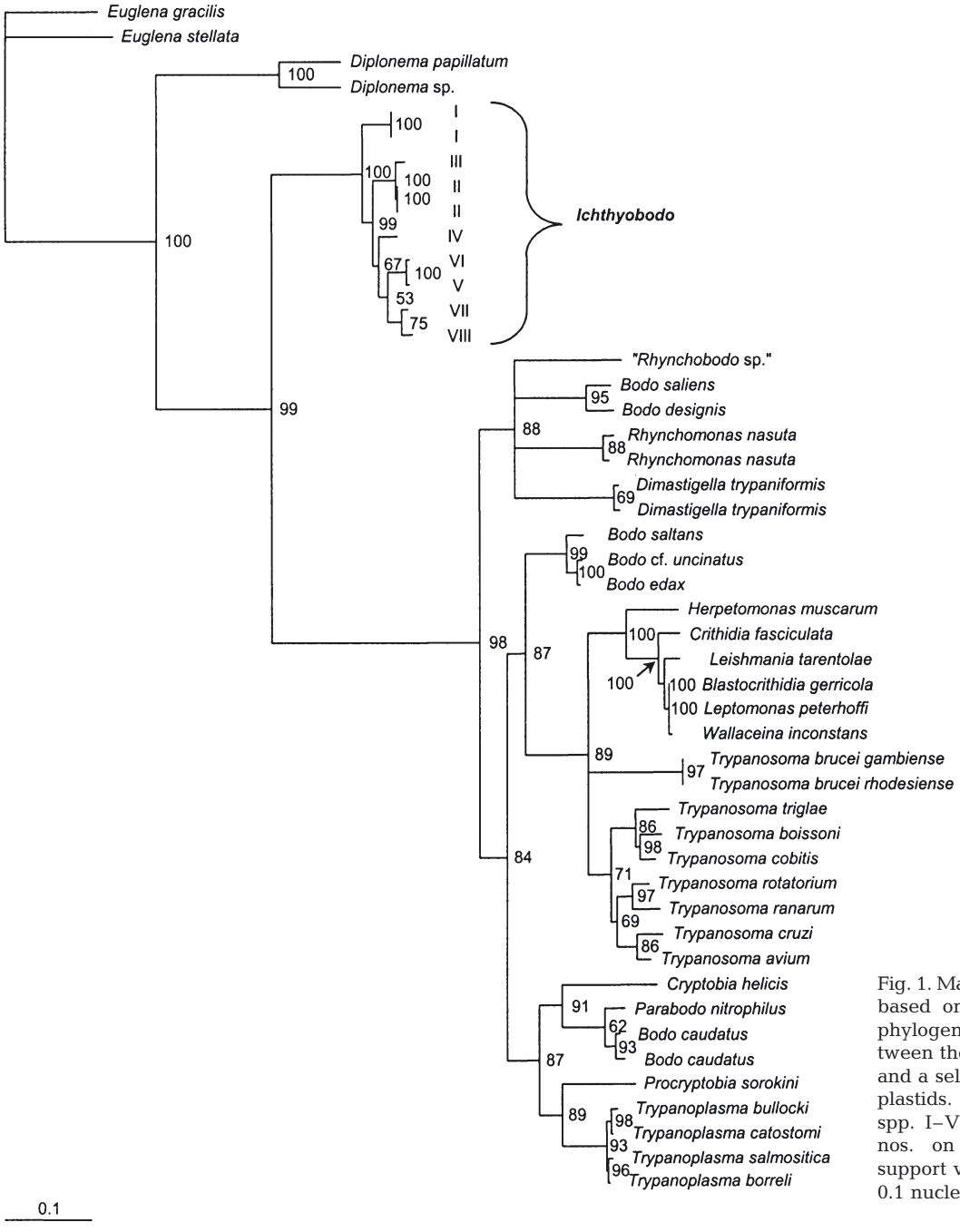

Fig. 1. Maximum-likelihood tree based on ssu rDNA, showing phylogenetic relationships between the *Ichthyobodo* isolates and a selection of other kinetoplastids. Rooted with *Euglena* spp. I–VIII: strains or species; nos. on branches: bootstrap support values (%); scale bar = 0.1 nucleotide substitutions per site

cies. Applying the same criterion to the present *Ichthyobodo* isolates and to the isolate of Callahan et al. (2002) from *Morone* hybrids suggests that 8 *Ichthyobodo* species may be represented in our study.

The ssu sequences of *Ichthyobodo* isolates from the skin of *Salmo salar* in freshwater (from E and W Norway) and from *Gasterosteus aculeatus* in freshwater (W Norway) were identical, and hence these are probably isolates of a single species (Species I). *Ichthyobodo* sp. I represents a lineage (Lineage A) that appears to be a sister group to the other *Ichthyobodo* isolates (Lineage B). *Ichthyobodo* isolates from the gills of Atlantic salmon reared in fresh-, brackish and seawater were nearly 100% similar, and represent a second species (Species II). *Ichthyobodo* from Japanese masu salmon was most similar to *Ichthyobodo* sp. II, but a sequence divergence of 1.5 to 1.6% suggests that it represents a distinct strain or species (III). The masu isolate grouped with Species II, so that these salmonid *Ichthyobodo* strains appear to represent a distinct sublineage (Sublineage B1).

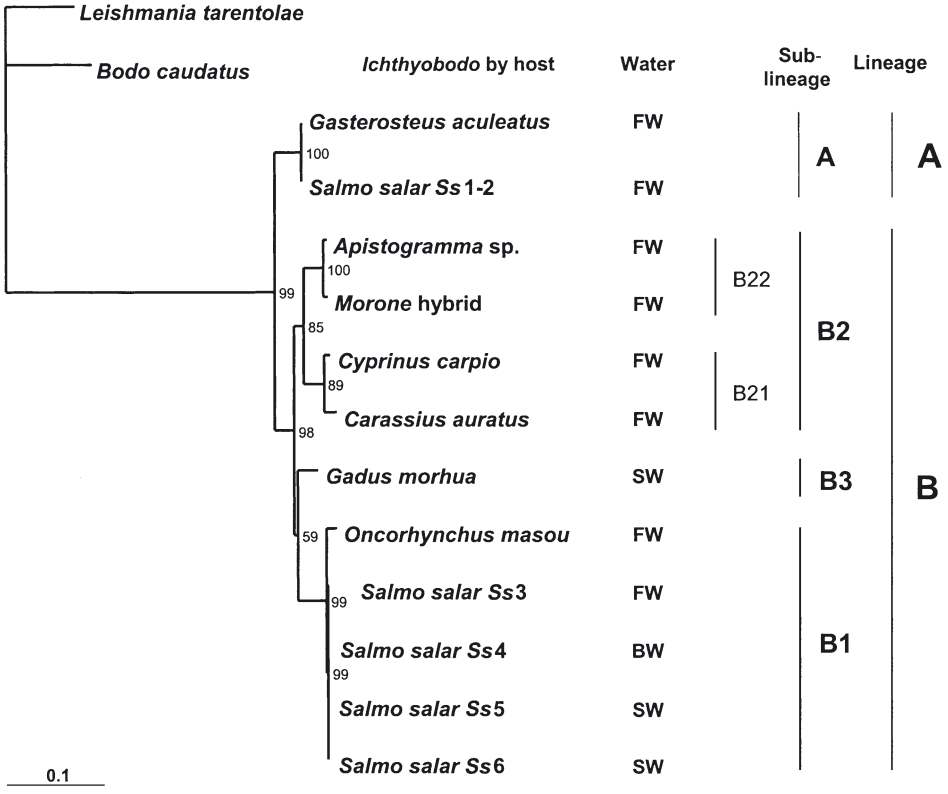

Fig. 2. Maximum-likelihood tree showing phylogenetic relationships among *Ichthyobodo* isolates based on ssu rDNA. Rooted with other kinetoplastids, showing 2 major lineages and 4 sublineages. FW: freshwater; BW: brackish water; SW: seawater; nos. on branches: bootstrap support values (%); scale bar = 0.1 nucleotide substitutions per site

The other 5 *Ichthyobodo* isolates diverged from each other by 1.3 to 8.0%, suggesting that all may represent different species (for reference purposes designated Species IV to VIII in Table 3). *Ichthyobodo* from the cyprinids were most similar to each other and may represent a distinct branch (B21) in a second sublineage (B2). The American isolates from a Brazilian cichlid and a North American moronid diverged by 1.3%, and together constituted a second branch (B22). There was good bootstrap support (85%) grouping these *Ichthyobodo* isolates together in a sublineage (B2), which appears to comprise *Ichthyobodo* from strictly freshwater fishes (Fig. 2). Finally, the *Ichthyobodo* isolate from a marine fish (*Gadus morhua*) constituted a third (B3) sublineage, with unresolved affinities to B1 and B2.

The increasing numbers of reports on *Ichthyobodo* infections in strictly marine fishes have led to a consensus that a distinct marine species exists (reviewed by Urawa et al. 1998). The present study confirms this, but also reveals a significant heterogeneity among freshwater *Ichthyobodo*, showing that *I. necator* in traditional sense is a species complex. This finding actually holds promise for many marine *Ichthyobodo* species.

We have shown that 2 *Ichthyobodo* species infect farmed Atlantic salmon in Norway. *Ichthyobodo* sp. I is predominantly a skin parasite, but also occurs on the gills. The 3-spined stickleback can apparently act as a reservoir host for Species I in lakes that supply hatcheries with water.

Ichthyobodo sp. II is predominantly a gill parasite in Atlantic salmon, and our detection of the flagellate in both hatchery-reared parr and in salmon reared in seawater pens for several months suggest that *Ichthyobodo* sp. II is euryhaline. Interestingly, Species II showed highest sequence similarity with *Ichthyobodo* from *Oncorhynchus* in Japan. Urawa & Kusakari (1990) showed that *Ichthyobodo* on *Oncorhynchus keta* smolts survive and proliferate following transfer to the sea. It is likely that *O. keta* and *O. masou* are infected by the same *Ichthyobodo* species; hence, Sublineage B1 appears to consist of flagellates adapted to anadromous salmonid hosts.

In Norway, salt or seawater has, to some extent, been used to control *Ichthyobodo* infections in farmed salmon (Bristow 1990). The seawater tolerance of *Ichthyobodo* sp. I is presently unknown, but as seawater treatment has been observed to cure skin infections, this suggests that it is restricted to freshwater.

Depending on the extent of nucleotide difference separating the species, the present material may include as many as 7 freshwater *Ichthyobodo* species. Evidently, morphological studies of *I. necator* from various hosts may have addressed different species, which would account for some of the reported differences.

Among the tasks ahead is molecular and morphological characterization of *Ichthyobodo necator* in a strict sense. This flagellate was originally described from the skin of diseased brown trout *Salmo trutta* alevins cultured in tanks at the College de France, Paris (Henneguy 1883, 1884). The host, site and relatively large size of Henneguy's flagellates suggests that our *Ichthyobodo* sp. I may be *I. necator* (Henneguy, 1883) sensu stricto. The morphology of *Ichthyobodo* sp. I in the present study was outlined in a preliminary account by Isaksen et al. (2002) (as *I. necator*).

Our results also suggest that some *Ichthyobodo* species reduced to synonymy with *I. necator* s.l. may prove to be valid, e.g. *I. nitschei* (Weltner, 1894) (syn. *Tetramitus nitschei*) from goldfish, provided the species can be recognized.

There appear to be few clear morphological characteristics to differentiate the strains or species which can be discerned from nucleotide sequences. At the light-microscope level, variations in the dimensions (e.g. Bruno 1992) and number of vacuoles have been reported (Diamant 1987, Urawa & Kusakari 1990). Ultrastructural examination of the attachment disc and cytostome process of *Ichthyobodo* from Scottish freshwater salmonids has revealed ridge-like processes on these structures (Roubal & Bullock 1987), whereas such ridges are absent from *Ichthyobodo* from Scottish seawater salmonids (Roubal & Bullock 1987, Lamas & Bruno 1992). Such ridges likewise do not occur in *Ichthyobodo* from the marine fishes *Limanda limanda*, *Paralichthys olivaceus* and *Takifugu rubripes* (Diamant 1987, Urawa et al. 1991, 1998), or in other freshwater fishes such as goldfish and swordtails (Schubert 1966), carp *Cyprinus carpio* alevins (Joyon & Lom 1966, 1969; host identity communicated by J. Lom) or Japanese salmonids (Urawa et al. 1998). The *Ichthyobodo* species infecting the Scottish freshwater salmonids is most probably identical to our Species I (Lineage A), while the species with the smooth attachment disc and cytostome would appear to belong to Lineage B (those from cyprinids, Japanese *Oncorhynchus* spp., marine fishes). If confirmed, this character may prove to be of generic value. The realization that several *Ichthyobodo* species exist will presumably instigate morphometrical and ultrastructural studies of molecularly characterized infections, which will probably reveal additional, previously unrecognized, characteristics.

Our phylogenetic analysis of ssu rDNA sequences suggests that the *Ichthyobodo* isolates represent a sister group to all other kinetoplastids, as also reported by Callahan et al. (2002). This result removes the genus *Ichthyobodo* from the family Bodonidae, and necessitates the erection of a new taxon to encompass these flagellates.

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