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Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia

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Summary

The genetic identification and distribution of *Anisakis* larvae in Indonesia is described. 110 *Auxis rochei rochei* and 45 *Decapterus russellii* were sampled from fish markets in North (Anturan) and South (Kedonganan) Bali. Nematode larvae from *A. rochei rochei*, *Caesio cuning* and *Epinephelus areolatus* from Kedonganan and from *Coryphaena hippurus* from Pelabuhan Ratu, South Java, were identified using sequence analysis of the internal transcribed spacers (ITS-1, ITS-2) and 5.8S region of rDNA. The larvae belonged to *Anisakis typica* with an identical sequence to this species from the spinner dolphin (*Stenella longirostris*) from Brazil, and to 2 further genotypes that differed from that sequence by 0.24 – 0.47 %. *A. typica* occurred in the migratory *A. rochei rochei* and *C. hippurus*, while *Anisakis* sp. 1 and 2 were isolated from the same fish species and the non-migratory *C. cuning* and *E. areolatus*. The latter genotype is distinguishable by 4 positions in the ITS-1 region (1.1 %), a genetic distance that indicates the presence of an Indonesian *A. typica* sibling species. The musculature infection in *A. rochei rochei* was low (2.5 %), indicating no major risk for the fish consumers. The much higher *A. typica* infection of fish intermediate hosts in the northern Bali coast is suggested to be dependent on the large dolphin population (nematode final hosts) in the waters off Lovina Beach (North Bali).

Keywords: *Anisakis typica*; Bali; Java; Indonesia; molecular genotyping; sibling species; zoogeography

Introduction

In Indonesia, as a maritime country, fisheries and fish products play an important role in the economic development, both of local communities and as a highly valuable export commodity. Fish food is also of major importance for the Indonesian tourism industry. As such, fish health and fish hygiene are of high significance for consumers, fisheries and the local fish processing industry. Zoonoses mainly originate from helminths, that utilize finfish as intermediate and marine mammals as final hosts. They are able to survive inside the human digestive tract. A spectrum of disease causing zoonotic fish worms is known. The most recognized and widely distributed are nematodes of the genus *Anisakis* (Anisakidae, Ascaridoidea). This cosmopolitan genus is known to cause the human anisakiasis, a painful inflammation of the gastro-intestinal tract (Ishikura & Namiki, 1989; Ishikura & Kikuchi, 1990). The most common in tropical waters is *Anisakis typica*, a species that has been recently genetically identified from the South West Atlantic (Brazil, Nadler *et al.*, 2005), North West Atlantic (Florida, Mattiucci *et al.*, 2005) and the Mediterranean (North Africa, Farjallah *et al.*, 2007).

Marine fish parasitology in Indonesia is widely undeveloped, and the local literature is not recognized outside the country. The first comprehensive fish parasitological examinations of marine finfish started with the discovery of several new species in South Sulawesi waters (Yamaguti, 1952, 1953a, b, 1954a, b, c, d). Hadidjaja *et al.* (1977), Hutomo *et al.* (1978) and Ilahude *et al.* (1978) studied anisakid nematodes from East Sumatra and the North Java coast, and Burhanuddin and Djamali (1978) used anisakid nematodes for stock separation in the roundscad *Decapterus russelli* in the Java Sea. Ilahude *et al.* (1978) and Burhanuddin and Djamali (1983) recorded 23 different fish species from the northern Java coast that were infected with anisakid nematodes, among these 4 different *Epinephelus* species. However, no infection was recorded from the fish musculature, most probably due to the study of fish preserved in 10 % formalin. Palm (2000, 2004) mainly focused on commercially important fish parasitic cestodes belonging to the Trypanorhyncha (Cestoda) from South Java. The author reported that Indonesia is at the centre of the diversity of this group of fish worms, and predatory fish especially can be highly infected. Lester *et*

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al. (2001), Moore *et al.* (2003) and Latama (2006) used fish parasites to distinguish between Indonesian and Australian stocks of the narrow-barred Spanish mackerel *Scomberomorus commerson*, and Jakob and Palm (2006) studied 5 commercially important fish species off the south-western Java coast for fish parasites. The latter recorded 38 different parasite species, among these was *Anisakis* sp., reaching a prevalence of 100 %. Yuniar *et al.* (2007) studied 8 commercially important fish parasites in the only remaining large mangrove ecosystem in southern central Java, and recorded 23 ectoparasitic crustaceans and 27 endoparasitic metazoans. More than 400 different parasite species have been recorded so far from Indonesian marine waters (Jakob & Palm, 2006; Rückert, 2006; Yuniar *et al.*, 2007).

Fish parasitological studies in Bali are scarce. Because of the importance of parasites in finfish mariculture, information is available on grouper (e.g. *Cromileptes altivelis*) parasites from net cages at the Gondol Research Station in North Bali (Yuasa *et al.*, 1998; Zafran *et al.*, 2000). Koesharyani *et al.* (2001) presented the first record of anisakid nematodes from *C. altivelis* and *Plectopomus leopardus* from Bali. Besides mariculture, the Balinese economy is heavily dependent on international tourism. The Balinese people themselves and the tourists appreciate the freshness and high diversity of marine food fish. However, some dishes such as Sushimi or Surimi from Japan and Kinilaw from the Philippines (Petersen *et al.*, 1993), if practiced in Bali, bear the risk of transmitting zoonotic fish parasites to the consumer. Similarly, the traditional North Sulawesi dish 'gohu cakalang' of raw fish, spiced with citrus or lemon juice, might bear risks for the fish consumers. A wide variety of different fish dishes is a speciality in the vicinity of the Kedonganan fish market at the southern Bali coast.

If known to doctors as a potential threat, anisakiasis can be easily detected and is no major human health problem due to proper treatment. However, so far, no data do exist on the occurrence of the zoonotic *Anisakis* in Balinese waters, and no record exists on anisakid musculature infection. The real identity of the *Anisakis* in Indonesian waters is still unknown. The purpose of the present study was 1) to identify a possible human health risk from free living finfish from Bali caused by the marine fish nematode *Anisakis*; 2) a molecular genotyping of *Anisakis* from Balinese and adjacent waters; 3) to analyse two pelagic marine fish species of similar size and trophic level from public fish markets in South (Kedonganan) and North Bali (Anturan) for prevalence and intensity of infection with anisakid nematodes.

Material and methods

Parasitological examination

Fish (n = 115) belonging to the scombrid *Auxis rochei rochei* (70 specimens) and the carangid *Decapterus russelli* (45 specimens) were examined for larval *Anisakis* sp. in the body cavity and internal organs in May – December 2005. A second sample of 40 specimens of *Auxis rochei rochei* was studied in October – November 2006 for infection with *Anisakis* in the body cavity, internal organs and musculature to detect annual variation. The fish were obtained fresh from the local fish markets in Kedonganan (South Bali, 35 *A. rochei rochei* and 35 *D. russelli* in 2005, and 40 *A. rochei rochei* in 2006) and Anturan (North Bali, 35 *A. rochei rochei* and 10 *D. russelli* in 2005), and transported on ice to the laboratory of the Faculty of Veterinary Sciences, Parasitology Laboratory, Udayana University, Denpasar, for further investigation. In the laboratory, the standard length to the nearest cm was recorded for each fish before examination. The body cavity was opened and studied by naked eye, the internal organs were separated and all parasites were removed. The musculature was sliced into 0.5 – 1 cm thick filets, pressed between 2 petridishes until they became translucent, and studied against a strong light source. The living *Anisakis* sp. were easily identifiable by the large white ventricle behind the oesophagus and the lack of any appendices. The ecological parameters were taken according to Bush et al. (1997). The fish was screened for the stomach contents after the parasitological examination.

Isolation of genomic DNA, PCR amplification and sequencing of ITS-1, 5.8S and ITS-2

Nematodes isolated from the examined fish were identified morphologically by existing keys and descriptions as belonging to the genus *Anisakis* (as described in section 2.1, Anderson 2000). They were then fixed and stored in 100 % ethanol. Eight specimens of *A. typica* from *A. rochei rochei* from Kedonganan fish market collected in 2006 were used for molecular identification. They were compared with 9 specimens from *Caesio cuning* and 3 specimens from *Epinephelus areolatus* from the same market and sampling period in November 2006. Seven *Anisakis* larvae were collected in June 2006 from *Coryphaena hippurus* from Pelabuhan Ratu, south-western Java coast.

Genomic DNA was isolated and purified from individual larvae by using a genomic DNA extraction kit (Peqlab Biotechnology GmbH, Erlangen, Germany) according to the instructions of the manufacturer. The rDNA region comprising the ITS-1, 5.8S, ITS-2 and flanking sequences (=ITS+) was amplified by using the previously described primers NC5 (5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3') and NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') (Zhu *et al.*, 2000). PCR-reactions (26 µl) included 13µl Master-Mix (Peqlab Biotechnology GmbH, Erlangen, Germany) containing dNTP, $MgCl_2$, Buffer and Taq-Polymerase, 3 µl of each primer, 2 µl water and 5 µl genomic DNA. Each PCR reaction was performed in a thermocycler (Biometra, Germany) under the following conditions: after initial denaturation at 95 ºC for 15 min, 30 cycles of 94 ºC for 1 min (denaturation), 55 ºC for 1 min (annealing), 72 ºC for 1 min (extension), followed by a final extension at 72 ºC for 5 min. Samples without DNA were included in each PCR run. PCR products were examined on 1 % agarose gels. A 100 bp ladder marker (Peqlab Biotechnology

GmbH, Erlangen, Germany) was used to estimate the size of the PCR products. To identify the anisakid nematodes, the PCR products were purified with E.Z.N.A. Cycle-Pure Kit (Peqlab Biotechnology GmbH, Erlangen, Germany). Afterwards a total volume of 7 µl, including 2 µl primer (individually) and 5 µl of the PCR product (~250 ng/µl) were sequenced by Seqlab (Goettingen GmbH, Germany). Both spacers and the 5.8S gene were sequenced in both directions from each PCR product, using primers NC5, NC13 (forward; 5'-ATC GAT GAA GAA CGC AGC-3'), NC13R (reverse; 5'-GCT GCG TTC TTC ATC GAT-3'), XZ1R (reverse; 5'-GGA ATG AAC CCG ATG GCG CAA T-3') and NC2.

Alignment

The sequences (forward and reverse) of the ITS-1, 5.8S and ITS-2 region were assembled and edited using Bio Edit Sequence Alignment Editor (V 7.0.5.3). They were compared manually with the original chromatograms, identified via GenBank and aligned with a previously characterized sequence (GenBank) of *Anisakis typica* from the spinner dolphin (*Stenella longirostris*) from Brazil (AY826724, see Nadler et al. 2005), using CLUSTAL W (1.83) Multiple Sequence Alignments (Thompson et al. 1994). Nucleotide sequence data are available in the GenBank database under the accession numbers EU346091-3. They were then aligned with sequences from other *Anisakis* species from Genbank; *A. brevispiculata* (AY826719) from *Kogia breviceps* in Florida, *A. pegreffii* (AB277823) from a mackerel and *A. simplex* (AB277822) from the arabesque greenling in Japan, *A. simplex* 'C' (AY821739) from *Mirounga angustirostris* in California, and *A. ziphidarum* (AY826725) from *Ziphius cavirostris* in South Africa. Genetic distances were determined and a phylogenetic tree was calculated with the optimality criterion set to distance and the UPGMA algorithm using PAUP* (Swofford 2001).

Results

Genetic identification

The informative gene products for larval *Anisakis* identification from Indonesia were 844 base pairs long and comprised most part of the ITS-1 (Fig. 1), 5.8 S and partial ITS-2. They were 0.00 – 0.47 % different in sequence to adult *A. typica* from the spinner dolphin (*S. longirostris*) from Brazil, with a 0.84 – 2.0 % difference in the partial ITS-1 region (355 informative bp) and a 0.35 – 0.83 % difference (3 – 7 bp) in the complete gene product (844 bp) including 3 deletions. Genetic distances among different *Anisakis* species compared to the Indonesian *Anisakis typica* by using 903 informative base pairs resulted in the same genetic distance of the sibling species *Anisakis simplex* (*s.s*) and *A. simplex* C (4 bp) for the whole gene product than that observed for *A typica* from Brazil/Indonesia and *Anisakis* sp. 2 in the ITS1-region (Table 1, Fig. 2). No other *Anisakis* species could be identified.

Two most frequent genotypes were identified as *A. typica* and *Anisakis sp. 2* (Figs 1, 2). *A. typica* occurred in the migratory fish *A. rochei rochei* (4 from 8 specimens) and in *C. hippurus* (one specimen infected). It differed in 3 positions (solely deletions) in the ITS-1 from the Brazilian material (Fig. 1), the rest of the sequences of the 5.8S and ITS-2 were identical. The predominant genotype *Anisakis* sp. 2 (21 of 27 studied *Anisakis*) was found in the non-migratory *C. cuning* and *E. areolatus* (all studied *Anisakis* in these fish belonged to this type) and also in *A. rochei rochei* and *C. hippurus* (Fig. 1). It differed in 7 positions in the ITS-1 region from the sequence of the spinner dolphin, including the 3 deletions that were also observed in the other specimens. A third genotype (*Anisakis* sp. 1) was isolated in a single case from *A. rochei rochei*. It had an intermediate position, and differed in 5 positions in the ITS-1 from *A. typica* from Brazil and in 2 positions from both other genotypes, respectively.

The 5.8S of all specimens was identical with the sequence of *A. typica* from Brazil, and minor variation was found in 2 positions (781 G vs R, 811 T vs C) of the ITS-2 in 3 specimens of *A. typica* from Indonesia. No further variation was observed.

Parasite infection: Data on *A. typica* and *Anisakis* sp. 1 and 2 infection in fish from Indonesia are given in tables 2 – 4. Because it is not possible to morphologically distinguish between these genotypes, the tables refer to a mixed genotype infection (see discussion).

Both studied fish species were infected with *Anisakis* spp. in the body cavity and internal organs. The number of larval *Anisakis* increased with fish standard length, with no difference in the intensity of infection in male and female fish. Most *Anisakis* specimens were collected from the *A. rochei rochei* sampled in the North (Anturan) compared to the South (Kedonganan) of Bali (see Tab. 2). In contrast, the less infected *Decapterus russelli* had a higher prevalence and intensity in the South compared to the North of Bali (Table 2). The study of *A. rochei rochei* in 2006 revealed a lower infection compared to 2005.

Within both years, *Anisakis* spp. appeared to be evenly distributed within the organs. The most heavy infection occurred in the mesentery and attached to the intestinal wall (Tab. 3). The gonads, liver and body cavity were also infected with a prevalence higher than 25 %. This picture was similar between the fish hosts and sampled years. The musculature of *A. rochei rochei* harboured only a single specimen of *A. typica* in one out of 40 sampled hosts (2.5 %) (investigated only in 2006).

Other observation: In addition to *A. typica* and 2 related genotypes, three other fish parasite species were isolated from *A. rochei rochei* and *D. russelli*. Most abundant was the acanthocephalan *Rhadinorhynchus* sp. (cf *celebesensis* Yamaguti 1954b) in the intestine of *A. rochei rochei*. Undeveloped larval stages of an unidentified cestode were collected from the body cavity of *A. rochei rochei*, and a single larval specimen of the raphidascarid nematode

Table 1. Genetic distances of nucleotide sequences in the ITS-1, 5.8S and ITS-2 ribosomal DNA regions (consensus sequence) among different *Anisakis* species compared to the Indonesian *Anisakis typica*

	A. z.	*A. b.*	*A. p.*	*A. s.(ss)*	*A. s. C*	*A.* sp. 2	*A.* sp. 1	*A. typ.*	*A. typ.*
A. ziphidarum.	-								
A. brevispicul.	0.12498	-							
A. pegreffii	0.06030	0.13323	-						
A. simplex (*ss*)	0.06277	0.13322	0.00239	-					
A. simplex C	0.06153	0.13189	0.00478	0.00478	-				
Anisakis sp. 2	0.14961	0.16092	0.16838	0.16713	0.16713	-			
Anisakis sp. 1	0.14701	0.15815	0.16581	0.16455	0.16456	0.00237	-		
A. typica Indon.	0.14697	0.15811	0.16578	0.16452	0.16453	0.00474	0.00237	-	
A. typica Brazil	0.14699	0.15813	0.16580	0.16454	0.16454	0.00474	0.00237	0.00000	-

Note that the genetic distance of the sibling species *Anisakis simplex* (*s.s*) and *A. simplex* C is the same (4 Bp) distance that is observed for the Indonesian *A. typica* and *Anisakis* sp. 2 in the ITS-1 region. The grey fields indicate low genetic distance among *Anisakis* sibling species.

Table 2. Data on fish hosts *Auxis rochei rochei* and *Decapterus russellii* and their parasites *Anisakis* spp. from South (Kedonganan) and North (Anturan) Bali fish markets

Fish market	Kedonganan			Anturan		
Fish hosts/year	n	cm	P, I, min-max	n	cm	P, I, min-max
Auxis rochei rochei	35	19.2 – 31.7	48.6 %	35	25.5 – 29.0	74.3 %
2005			3.6 (1 – 8)			4.8 (1 – 20)
Auxis rochei rochei	40	24.5 – 31.8	20.0 %	-		-
2006			2.9 (1 – 6)			-
Decapterus russellii	35	14.0 – 23.8	28.6 %	10	26.9 – 29.5	2.9 %
			1.9 (1 – 5)			1

P-prevalence (%), I-mean intensity of infection, min-max-minumum and maximum number of parasites per host

Table 3. Prevalence, mean intensity of infection and min-max number of *Anisakis* spp. in *Auxis rochei rochei* and *Decapterus rusellii* according to fish organs

Site of Infection	n	Body cavity	Mesentery, intest. wall	Stomach wall	Liver	Gonads
Auxis rochei rochei	70	25.7 %	37.1 %	11.4 %	25.7 %	31.4 %
2005		1.7 (1 – 5)	2.5 (1 – 13)	1.3 (1 – 2)	1.6 (1 – 4)	2.4 (1 – 8)
Auxis rochei rochei	40	2.5 %	22.5 %	2.5 %	2.5 %	7.5 %
2006		2	1.4 (1 – 4)	1	1	1.7 (1 – 3)
Decapterus rusellii	45	4.4 %	11.1 %	2.2 %	0	13.3 %
		1	1.8 (1 – 4)	1	0	1.3 (1 – 3)

A single *Anisakis* larva was found in the musculature of 40 *A. rochei rochei* dissected exclusively in 2006.

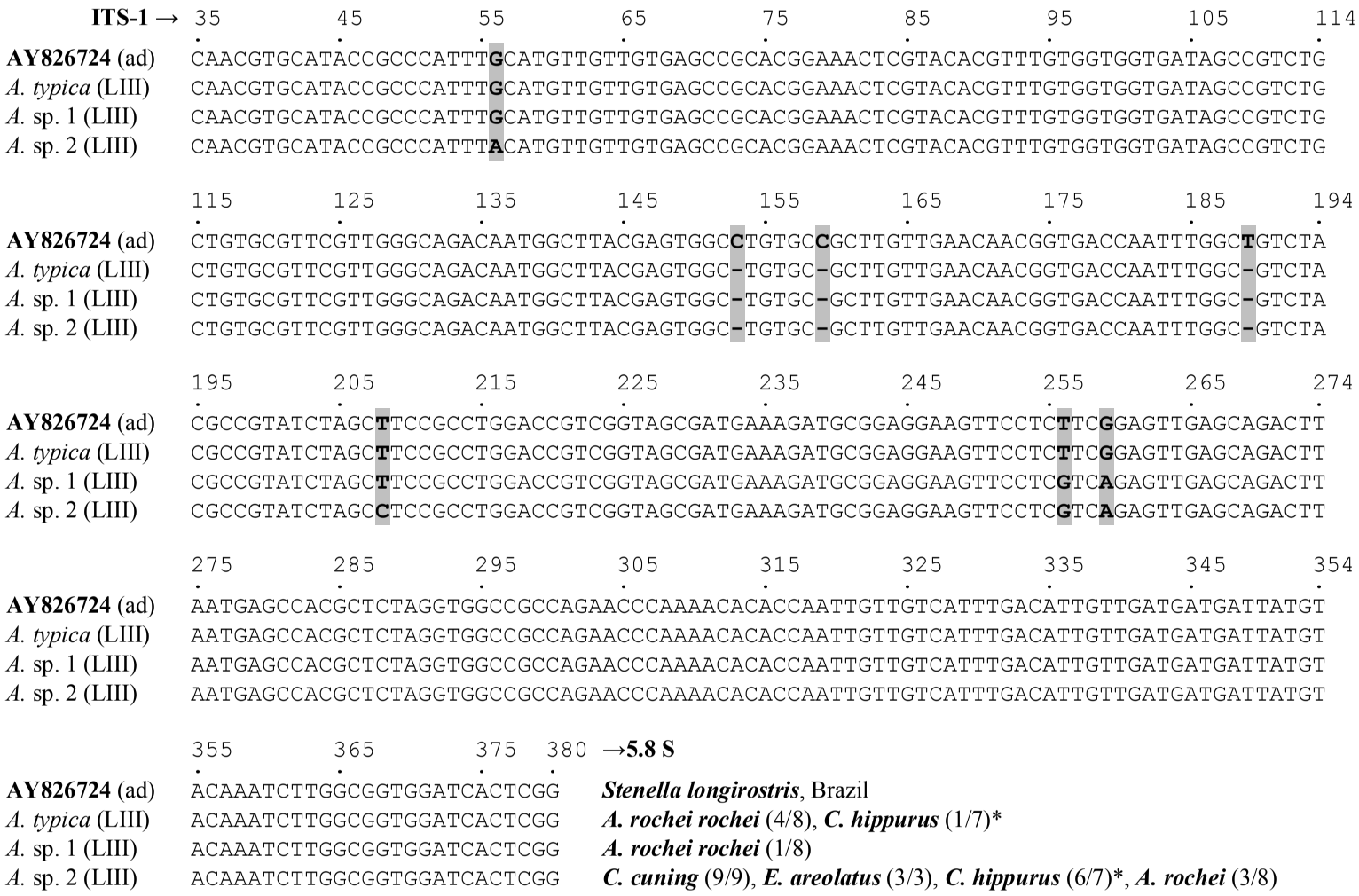

Fig. 1. ITS-1 from larval Indonesian *Anisakis typica* compared with an adult specimen from *Stenella longirostris* from Brazil.
The Indonesian genotype *Anisakis* sp. 2 differed in 7 positions (2%) from the Brazilian material and was represented in 21 from 27 (78%) studied specimens and in all studied fish species from the southern Bali and Java (*) coast.

Raphidascaris sp. was found in the body cavity of *D. russelli* (also see Yamaguti 1954a). Both studied fish species had their stomachs filled with small crustaceans, and only *A. rochei rochei* harboured small fish with a maximum length of appr. 3 cm. A single specimen of the digenean *Hirudinella ventricosa* (Baird, 1853) was isolated from the stomach of *Auxis thazard thazard* (Lacepède, 1800), also from Kedonganan fish market.

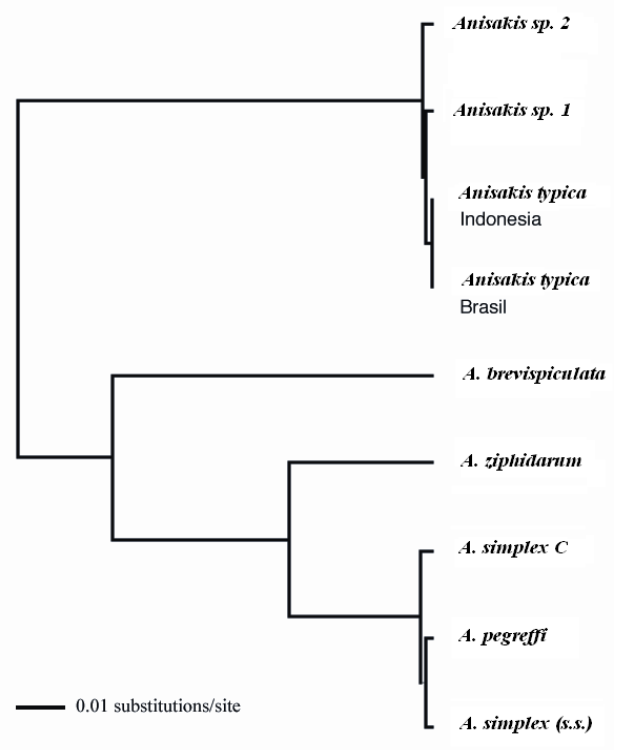

Fig. 2. Phenogram depicting the genetic differences (upon pairwise comparison) among the Indonesian *Anisakis typica* and different *Anisakis* species for the ITS-1, 5.8S and ITS-2 rDNA sequence.

Discussion

The present study provides the first molecular identification of anisakid fish nematodes from Balinese waters, Indonesia. The identified specimens belonged to *Anisakis typica* and to two closely related genotypes (here named *Anisakis* sp. 1 and 2). *A. typica* is a common parasite of various dolphin species from warmer temperate and tropical waters, belonging to the families Delphinidae, Phocoenidae and Pontoporidae (see Mattiucci *et al.*, 2002). Mattiucci *et al.* (2002) recorded larval *A. typica* from *Auxis thazard thazard* and *Thunnus thynnus* from the Brazilian coast (SW Atlantic), *Scomber japonicus* and *Trachurus picturatus* from the NE Atlantic off Madeira, *Euthynnus affinis, Scomberomorus commerson, Sarda orientalis* and *Coryphaena hippurus* from the West Indian Ocean off Somalia, and from *Merluccius merluccius* from the Eastern Mediterranean Sea. Further records were provided by Mattiucci *et al.* (2005) from Florida (*Stenella attenuata*, *Globicephala macrorhynchus*, *Mesoplodon* sp. as final hosts) and Farjallah *et al.* (2007) from the Mediterranean coast of North Africa (*Scomber scombrus*, *M. merluccius*, *Phycis phycis* as intermediate hosts).

The present study adds new locality records from the southern Balinese and Javanese coast, East Indian Ocean (Table 4), and a new host record for *Auxis rochei rochei*. A new genotype that might represent a sibling species of *A. typica* was identified from *A. rochei rochei, C. cuning*, *C. hippurus* and *E. areolatus.* The records of *Anisakis* sp. from the gempylids *Gempylus serpens* and *Thyrsitoides marleyi*, the trichiurid *Trichiurus lepturus* and the bramid *Brama dussumieri* by Jakob and Palm (2006) came from the same locality than the specimens isolated from *C. hippurus* in the present study, and might also represent these genotypes. Thus, larval *A. typica* or closely related siblings (see below) infest a wide range of clupeiform, perciform and also gadiform fish in warmer waters, and seem to be the most common anisakid in the Indonesian region. A total of 21 fish species are known to harbour *Anisakis* spp. or *A. typica* in Indonesia, with 34 fish species known to be infected with anisakid nematodes (Table 4).

Three different genotypes of anisakid nematodes could be identified, differing in 3 – 7 base pairs in the ITS-1 region from an adult specimen from the spinner dolphin (*S. longirostris*) from Brazil. While the migratory fish *C. hippurus* and *A. rochei rochei* harboured specimens of *A. typica* that was very similar compared with the Brazilian material (differed in 3 deletions in the ITS-1), most isolated specimens (78 %) and all those from the non-migratory *C. cuning* and *E. areolatus* belonged to an Indonesian genotype (*Anisakis* sp. 2) that consistently differed in 4 base pairs of the ITS-1. Within the former type, only 3 specimens varied by a single base pair in the ITS-2 regions, indicating a high homogeneity of all genetically identified *Anisakis* specimens from Indonesia. Mattiucci *et al.*, (2002) already stated a high similarity of her studied specimens, despite being geographically quite distant. Kellermanns *et al.* (2007) suggested that a constant gene flow in different anisakid nematodes is caused by a) extensive final host migration in the case of *A. simplex* (*s.s.*), b) an overlapping distribution of final host populations along the continental shelves for *Pseudoterranova decipiens* (*s.s.*), and c) a low host specificity and large population size in the intermediate and final hosts for *Hysterothylacium aduncum*. Palm *et al.* (2007) suggested d) extensive final as well as intermediate host migration as being responsible for a high gene flow in a cosmopolitan fish cestode, the trypanorhynch *Tentacularia coryphaenae*. *A. typica*, as a tropical anisakid, seems to follow the latter dispersal mechanism, being more similar to the trypanorhynch than to the congener *A. simplex* (*s.s.*), as can be seen in highly overlapping fish intermediate hosts in the tropics (Table 4 vs Palm, 2004). More extensive studies from other fish hosts and Indonesian localities and possibly other gene regions are needed in order to clarify the occurrence of other *Anisakis* species in Indonesian waters, and to determine that the observed differences in the ITS-1 represent normal intraspecific variation or are a characteristic of a possibly existing Indonesian *A. typica* sibling species. The 4 bp difference is of the same range than re-

corded for the siblings *A. simplex* (*s.s.*) and *A. simplex* C as well as *A. pegreffii* (Fig. 2).

The life cycle of *A. typica* involves dolphins as final hosts that harbour the 4th stage larva and adult (Mattiucci *et al.*, 2002). The nematode eggs of the congener *A. simplex* (s.s.) leave their hosts with the faeces and embryonate in sea-water (e.g. Klimpel *et al.*, 2004; Kellermanns *et al.*, 2007). Larvae hatch as free living third-stage larvae (L3), still surrounded by the sheath of the second-stage larvae (L2), and get eaten by small crustaceans (copepods, euphausiids)

Table 4: Host records of *Anisakis* from Indonesia. *Anisakis* sp. 1-2 might represent a local variation of *A. typica* or a sibling species in Indonesian waters (explanation see text)

Fish species	Family	Parasite	Locality	Reference
Atule mate	Carangidae	***Anisakis* sp.**	N Java	1, 2
Caranx crumenophthalmus	"	***Anisakis* sp.**	"	1, 2
Brama dussumieri	Bramidae	***Anisakis* sp.**	S Java	3
Decapterus kurroides	"	Anisakidae	N Java	4
D. lajang	"	***Anisakis* sp.**	"	4, 5
D. russelli	"	***Anisakis* sp. (*typica*, 1-2?)**	Bali, Java	4, 5, 6, 7, 8 present study
Caesio cuning	Caesionidae	***Anisakis* sp. 2**	Bali	Present study
Amblygaster sirm	Clupeidae	***Anisakis* sp**	N Java, Sunda Strait	4, 5, 7, 8
Sardinella fimbriata	"	Anisakidae	"	4
S. gibbosa (=jussieui)	"	***Anisakis* sp.**	"	1, 2
Coryphaena hippurus	Coryphaenidae	***A. typica* #, *Anisakis* sp. 2**	S Java	Present study
Gempylus serpens	Gempylidae	***Anisakis* sp.**	"	3
Thyrsitoides marleyi	"	***Anisakis* sp.**	"	3
Leiognathus dussumieri	Leiognathidae	***Anisakis* sp.**	Sulawesi	9
Lutjanus kasmira	Lutjanidae	Anisakidae	N Java	2
Auxis thazard thazard	Scombridae	Anisakidae #	"	4
A. rochei rochei	"	***A. typica*, *Anisakis* sp. 1-2**	Bali	Present study
Euthynnus affinis	"	***Anisakis* sp. #**	N Java	1, 4
Rastrelliger brachysoma	"	***Anisakis* sp.**	"	1, 2
R. kanagurta	"	***Anisakis* sp.**	"	4, 5, 7, 8
Scomberomorus commerson	"	Anisakidae **#***, ***A. simplex* ***	Kupang	4, 10
Cromileptes altivelis	Serranidae	Anisakidae	Bali	11
Epinephelus areolatus	"	***Anisakis* sp. 2**	"	Present study
E. fuscoguttatus	"	***Anisakis* sp.**	N Java, Sulawesi	4, 12
E. maculatus	"	Anisakidae	"	4
E. quoianus (=megachir)	"	"	"	4
E. summana	"	"	"	4
Plectopomus leopardus	"	***Anisakis* sp.**	Bali, Sulawesi	11, 12
Siganus guttatus	Siganidae	Anisakidae	"	4
Saurida isarankurai	Synodontidae	"	"	4
S. longimanus	"	"	"	4
S. micropectoralis	"	"	"	4
S. undosquamis	"	"	"	4
Trichiurus lepturus	Trichiuridae	***Anisakis* sp.**	S Java	3

1 - Ilahude *et al.*, 1978; 2 – Ilahude, 1980; 3 - Jakob & Palm, 2006; 4 - Burhanuddin & Djamali, 1983; 5 – Martosewojo, 1980; 6 - Burhanuddin & Djamali, 1978; 7 - Hutomo *et al.*, 1978; 8 - Hadidjaja *et al.*, 1978; 9 – Yamaguti, 1954a; 10 - Lester *et al.*, 2001; 11 – Koesharyani, 2001; 12 - Asmanelli *et al.*, 1993

A. typica in intermediate hosts from other localities (Mattiucci *et al.*, 2002)

* Lester *et al.* (2001) recorded *A. simplex* from Kupang on a morphological basis (must be confirmed)

as first intermediate hosts. The L3 develops inside the first intermediate host, and larger invertebrates, cephalopods and various fish species serve as transport hosts that acquire the nematodes through the food chain. If small fish and cephalopods are preyed upon by larger fish, the larvae are capable of re-infecting the latter without moulting. Larger fish thus may accumulate enormous numbers of larvae as paratenic or transport hosts (see Jakob & Palm 2006). The life-cycle is completed when the definitive host preys upon infected crustaceans, cephalopods or fish (Kellermanns *et al.*, 2007). The stomach of the studied *A. rochei rochei* was filled with small crustaceans and only few small sized fish, and *D. russelli* preyed upon small crustaceans as well. According to Froese and Pauly (2007), the main prey items of both fish species are planktonic crustaceans and small fish, particularly anchovies in the case of the former species. A low intensity of *Anisakis* in both sampled species together with the observed prey items suggest that the *Anisakis* larvae uptake originates directly from the crustacean first intermediate hosts (see Palm, 1999), while larger predatory fish ingest them through the clupeid or carangid schooling fish intermediate hosts (Table 4). In all cases, mainly pelagic fish species are infected, suggesting a pelagic life cycle for the tropical *A. typica*.

The observed prevalence and the intensity of infection of *A. typica* were higher in the scombrid *A. rochei rochei* compared to the carangid *D. russelli*, assuming that the former species is a better intermediate host for the nematode in Balinese waters. With the former fish being infected during 2005 and 2006, *A. typica* was readily available throughout the years. The infection level for both fish is much lower than observed for *D. russelli* from northern Javanese waters (80 – 100 % prevalence), however, very similar to the infection level of 14.2 % during March and 18.9 % in October 1978 in the Bali Strait (Burhanuddin & Djamali, 1978). Most other records of anisakids from Indonesian waters are quite old (Table 4), and also recorded a much higher infection rate of *D. russelli* from different places in the Java Sea during the '80s. It cannot be decided at present whether this difference is real and reflects a decrease of *Anisakis* in Indonesian fish populations that depends on the sampling site or the presence of suitable intermediate or final hosts. Possible reasons for a drop in the fish anisakid load can be seen in a drop of final host numbers, dolphins in the case of *A. typica*, in the region. Further studies are needed to compare the present situation with that of nearly 30 years ago.

The comparison of the infection between the northern and southern Balinese coast demonstrated a higher rate in northern Bali waters compared to the South in 2005. By using anisakid fish parasites as a biological indicator for the final host abundance, the northern *A. rochei rochei* was infected in higher level than the southern fish. In marine anisakids there is a well known relationship between the abundance of the final host population and the infection rates in the fish intermediate hosts (e.g. Palm, 1999). This might also indicate that the dolphin population as the *Anisakis typica* final hosts is higher in northern Balinese waters compared to the South. This is not astonishing since dolphin watching is highly popular in front of Lovina Beach in North Bali, where high local dolphin populations occur.

The present study is the first scientific record of *Anisakis* from the musculature of a commercial fish from the tourist island Bali. However, the recorded infection in the muscle was low, indicating no major risk for the consumers from the studied fish. Also earlier studies from the Java Sea revealed no significant musculature infection in different Indonesian fish species (Burhanuddin & Djamali, 1978, 1983; Ilahude *et al.*, 1978), though this might be also a consequence of the sampling procedure. The typical site of infection for Indonesian *A. typica* is the body cavity and mesentery, followed by specimens in the gonads and the liver. In contrast to the related species *Anisakis simplex* (*s.s.*) that also infests the fish musculature as a common site in temperate waters (e.g. Strømnes & Andersen, 1998, 2003), this seems to be not the case in *A. typica*. Thus, besides the life cycle and dispersal mechanism, a typical site of infection might be another distinguishing feature among the different *Anisakis* species or even among sibling species within the *Anisakis simplex* complex, explaining why *A. typica* to date has not been recorded to cause human anisakiasis.

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