Anisakis (Nematoda: Ascaridoidea) from Indonesia

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Anisakis (Nematoda: Ascaridoidea) from Indonesia

H. W. Palm[1,2,*], S. Theisen[1,*,**], I. M. Damriyasa[2], E. S. Kusmintarsih[3], I. B. M. Oka[2], E. A. Setyowati[3], N. A. Suratma[2], S. Wibowo[4], S. Kleinertz[1]

[1]Faculty of Agricultural and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany

[2]Centre for Studies in Animal Diseases, Faculty of Veterinary Medicine, Udayana University Bali, 80363 Badung Denpasar, Bali, Indonesia

[3]Institute of Parasitology and Entomology, Faculty of Biology, Jenderal Soedirman University, 53122 Purwokerto, Java, Indonesia

[4]Ministry of Marine Affairs and Fisheries, Research and Development Centre for Marine and Fisheries Product Processing and Biotechnology, 10260 Jakarta, Java, Indonesia

ABSTRACT: Despite Indonesia's high marine biodiversity, there is a lack of information regarding fish parasites in Indonesian waters. During a sampling of 136 teleost species from Indonesian waters, 22 of them were infected with larvae of *Anisakis* Dujardin, 1845, a genus with zoonotic potential. We genetically identified 118 worms, provide a revision of all available sequences of the ITS-1–5.8S–ITS-2 marker from Indonesian *Anisakis* in GenBank (n = 125), and establish 16 new host records. So far, 53 Indonesian teleosts harbour *Anisakis* spp., 32 of them with known sequence data, increasing the worldwide teleosts with genetically identified *Anisakis* from 155 to 177. Sequence analyses of this marker in the 243 *Anisakis* specimens identified 3 *Anisakis* sp. HC-2005 and 39 (16%) *A. typica* (sensu stricto). *A. berlandi* and *A. pegreffii* are reported for the first time from teleosts in the equatorial region and *A. physeteris* from the Pacific Ocean. The latter 3 species were exclusively found in the migratory scombrid *Auxis rochei*. Most common infection sites were the body cavity, with 299 (of 848) worms in the mesenteries surrounding the liver, and 129 unattached. Musculature infection was very low, demonstrating minor risk of anisakiasis for human consumers. A total of 193 worms (~79%) had a distinct genotype distinguished from *A. typica* by 4 positions in the ITS-1 region. This genotype is reported since 2008 as '*A. typica*', 'sibling', '*Anisakis* sp./type 1', 'sp. I', 'sp. 2' or 'sp. II'. To avoid further misleading identification, we hereby apply the subspecific entity *Anisakis typica* var. *indonesiensis* until description of the adults becomes available.

KEY WORDS: Molecular genotyping · *Anisakis typica* (s.s.) · *A. berlandi* · *A. pegreffii* · *A. physeteris* · *A. typica* var. *indonesiensis* · Anisakiasis

INTRODUCTION

The fisheries industry with its highly valuable food products is a driver for the future economic development of the maritime nation Indonesia. Consequently, food safety and food security are important to consumers. Marine fishes can be a source for foodborne, parasitic human diseases (zoonoses), primarily when larval helminths are ingested through the consumption of semi-cooked or uncooked fisheries products (e.g. Petersen et al. 1993). Some worms can survive inside the human gastrointestinal tract. Especially anisakid nematodes of the genus *Anisakis* (Anisakidae, Ascaridoidea) have been reported to cause anisakiasis, an inflammation of the human gastrointestinal tract, in many regions worldwide (Ishikura & Namiki 1989, Ishikura & Kikuchi 1990, Klimpel & Palm 2011, Ivanovic et al. 2015, Lim et al. 2015, Sohn et al. 2015, Nieuwenhuizen 2016).

*Authors contributed equally
**Corresponding author: stefan.theisen@uni-rostock.de

Marine fish parasitology in Indonesia is a widely neglected field, and only a few early studies have been published in an international journal. After the first record of the zoonotic nematode genus *Anisakis* in 1954 (Yamaguti 1954), several Indonesian researchers studied the Anisakidae between 1978 and 2001 based on morphology (Burhanuddin & Djamali 1978, 1983, Hadidjaja et al. 1978, Hutomo et al. 1978, Ilahude et al. 1978, Ilahude 1980, Martosewojo 1980, Asmanelli et al. 1993, Koesharyani et al. 2001, Lester et al. 2001), adding more host fish species. Recent DNA analyses demonstrated that the genus *Anisakis* consist of 2 'clades' and different 'complexes'. Clade one includes *A. typica* (Diesing, 1860), *A. nascettii* Mattiucci et al., 2009 and its sister species *A. ziphidarum* Paggi et al., 1998, and 3 siblings forming the *A. simplex* (sensu lato, s.l.) complex: *A. simplex* (Rudolphi, 1809) (sensu stricto, s.s.), *A. pegreffii* Campana-Rouget et Biocca, 1955, and *A. berlandi* Mattiucci et al., 2014 (formerly known as *A. simplex* C of Nascetti et al., 1986). The second clade consists of 3 species forming the *A. physeteris* (s.l.) complex: *A. paggiae* Mattiucci et al., 2005, *A. brevispiculata* Dollfus, 1966, and *A. physeteris* (Baylis, 1923) (s.s.) (Klimpel & Palm 2011, Kuhn et al. 2011, 2013, Mattiucci et al. 2005, 2009, 2014). Other genotypes such as 'Anisakis sp. HC-2005' have been rarely reported, lack morphological descriptions, and indicate further taxonomic complexity.

A. typica, the most common species in tropical fishes, has been identified from the southwest Atlantic (Brazil; Nadler et al. 2005), west (Florida; Mattiucci et al. 2005) and east Atlantic (Madeira, Portugal; Pontes et al. 2005), the Mediterranean (North Africa; Farjallah et al. 2008), the central Pacific (Hawaii and Moorea; Kuhn et al. 2013, Palm & Bray 2014), and most recently from free-living Indo-Pacific bottlenose dolphins *Tursiops aduncus* off the Hurghada coastline in the northern Red Sea, Egypt (Kleinertz et al. 2014b). Shamsi (2014) summarized the knowledge about Australian *Anisakis* spp., and identified the species *A. brevispiculata*, *A. berlandi*, *A. pegreffii* and *A. typica* in these waters adjacent to Indonesia, mentioning that *A. brevispiculata* and *A. typica* show genetic differences to the original genotypes from other areas.

Palm et al. (2008) genetically identified 3 different genotypes of *A. typica* in Balinese waters. One corresponded with *A. typica* (s.s.) from GenBank with the same sequence as material from Brazil (from the spinner dolphin *Stenella longirostris*). The most frequent genotype was distinguishable by 4 positions in the ITS-1 region, a genetic distance similar to the one between the 2 valid species *A. simplex* (s.s.) and *A. berlandi* (Palm et al. 2008). Since its first record, this genotype was also reported by Koinari et al. (2013), Kuhn et al. (2013), Anshary et al. (2014) and Kleinertz et al. (2014a), under the names *A. typica*, *Anisakis* sp. 1, 2 or I and II. The third detected genotype was scarce, and differed in 2 bp between *A. typica* (s.s.) and the Indonesian sibling.

Palm et al. (2008) provided the first infection rates and organ preferences within the fish hosts as well as a comparison of infections in north Javanese and north and south Balinese waters. Based on no muscular infection observed, the authors suggested a minor risk of *Anisakis*-borne zoonoses in Indonesia. The single published case of anisakiasis in the adjacent Philippines is a wrong citation (cf. Lymbery & Cheah 2007 with Petersen et al. 1993), but Uga et al. (1996) reported 11% of 244 tested East Javanese citizens, especially juveniles, positive for antibodies for *Anisakis* spp. in a seroepidemiological survey. The purpose of the present study was to (1) molecularly genotype the ITS-1–5.8S–ITS-2 region of *Anisakis* spp. in Indonesia throughout a high number of teleost hosts, (2) analyse the site- and host-specificity of the recorded nematodes and (3) estimate the human health risks caused by *Anisakis* spp. from Java and Bali. We hereby apply the subspecific entity *A. typica* var. *indonesiensis* for the genotype so far solely reported from Indonesia in order to avoid future misidentification.

MATERIALS AND METHODS

Parasitological examination

A total of 1801 fishes belonging to 136 species were studied from July 2010 to August 2013. A total of 1531 individuals belonged to 40 teleost species, collected in south Java (Cilacap) and south Bali, Kedonganan (mean of 39 investigated fishes per sample). One species was sampled off north Java (Karimunjawa National Park). According to the salesmen in Cilacap, 2 fish species were originally obtained off Jakarta, northern Java coast. Another sample (270 specimens of 96 species, mean of 3 animals per species) was studied during the First Educational Workshop on Marine Fish Parasites in Bali, July-August 2013. These fishes were collected from fish markets in south Bali (Kedonganan), or were caught alive in southern Balinese waters. Fishes were transferred to the laboratories on ice (or in the case of the workshop, alive in barrels with seawater and sufficient

oxygen supply), and studied at the Institute for Parasitology and Entomology, Jenderal Soedirman University, Java, and the Centre for Studies in Animal Diseases, Udayana University, Bali.

Morphometrical data for each fish species representing the same size class were taken: total and standard length (TL and SL), in mean and range to the nearest 0.1 cm, and total and gutted weight (TW and GW) to the nearest 0.1 g. The body cavity was opened and studied by naked eye. Internal organs were transferred to Petri dishes with NaCl solution (0.9%), and studied for parasites under a Zeiss Stemi DV4 binocular microscope. The musculature was sliced into 0.5 cm thin layers and pressed between a set of glass Petri dishes and then studied against a trans-illuminating light source, allowing isolation of anisakids as well as other flesh parasites.

Parasites were pre-identified under a Novel XSZ-107BN microscope to separate anisakid nematodes; these were transferred to EtOH 99.6% for molecular DNA analysis. Specimens belonging to the genus *Anisakis* are easily distinguished from other nematodes by the distinct ventricle between the oesophagus and intestine, and lack of appendages such as an anterior-directed caecum and posterior-directed appendix (Anderson 2000, Anderson et al. 2009). Ecological parameters for fish infected with *Anisakis* spp. (prevalence, intensity, abundance) were calculated following Bush et al. (1997).

DNA isolation, PCR and sequencing

A total of 118 newly collected specimens of *Anisakis* from 22 teleost species from Javanese and Balinese fish markets were used for molecular identification. Genomic DNA was isolated and purified from individual larvae by using a genomic DNA extraction kit (Peqlab Biotechnology) according to the instructions of the manufacturer. The ribosomal DNA (rDNA) region comprising the ITS-1, 5.8S, ITS-2 and flanking sequences (= ITS+) were amplified by using the previously described primers NC2 (5'-TTA GTT TCT TTT CCT CCG CT-3') and F2662 (~TK1) (5'-GGC AAA AGT CGT AAC AAG GT-3') (Zhu et al. 2000, Ishiwata et al. 2004, Kuhn et al. 2011). PCR reactions (50 µl) included 39 µl Master-Mix (Peqlab Biotechnology), 3 µl of each primer (10 pmol µl^{-1}), and 5 µl genomic DNA. The Master-Mix contained 5 µl ready mixed Peqlab reaction buffer Y (4 mM MgCl$_2$, 40 mM Tris-HCl [pH 8.55], 32 mM (NH$_4$)$_2$SO$_4$, 0.02% Tween 20); 0.25 µl (2.5 U) *Taq* polymerase, 1 µl (0.4 mM) dNTP and 32.75 µl water. Each PCR reaction was performed in a ther-

mocycler (Biozym Scientific) under the following conditions: initial denaturation at 95°C for 1 min; 40 cycles of 94°C for 45 s (denaturation), 55°C for 45 s (annealing), and 72°C for 45 s (extension); followed by a final extension at 72°C for 10 min. Control samples without DNA were included in each PCR run. PCR products were examined on 1% agarose gels. A 100 bp ladder marker (Peqlab Biotechnology) was used to estimate the size of the PCR products. To identify the anisakid nematodes, PCR products were purified with the Cycle-Pure Kit (Peqlab Biotechnology). Afterwards, a total volume of 7 µl, including 2 µl primer (individually) and 5 µl of the PCR product (~250 ng µl^{-1}) were sequenced by Seqlab, Göttingen. Both spacers and the 5.8S gene from each PCR product were sequenced, using the primers TK1 and NC2.

Alignment

Sequences (forward and reverse) of the ITS-1, 5.8S and ITS-2 region were assembled and edited using the BioEdit sequence alignment editor (v. 7.1.3.0). They were compared manually with the original chromatograms, identified via GenBank and aligned with a previously characterized sequence (GenBank) data of *Ansiakis* spp., and with 125 previously identified *Anisakis* sequences from Indonesia (see Palm et al. 2008, Koinari et al. 2013, Kuhn et al. 2013, Anshary et al. 2014, Kleinertz et al. 2014a) using ClustalW (v. 1.83) multiple sequence alignments (settings: full multiple alignment, gap penalties default) (Thompson et al. 1994). They were then aligned with sequences from other *Anisakis* species from GenBank for species and genotype identification as follows: *A. typica* (s.s.) from the spinner dolphin *Stenella longirostris* from Brazil (AY826724; see Nadler et al. 2005), *A. simplex* C, now *A. berlandi* from *Mirounga angustirostris* from California (AY821739; see Nadler et al. 2005), *A. pegreffii* and *A. physeteris* from mackerels from Japan (AB277823 and AB277821 respectively; see Umehara et al. 2008), and *Anisakis* sp. HC-2005 from *Hoplostethus cadenati* from the African shelf (EU718474; see Kijewska et al. 2009). We hereby apply the subspecific entity *Anisakis typica* var. *indonesiensis* for most of the specimens recorded during the present study, and for the specimens from *Auxis rochei* from Indonesia (EU346093; see Palm et al. 2008). Because we do not nominate an available name but apply the subspecific entity, we must not follow the ICZN (2016, their §1.3). New nucleotide sequence data are available in GenBank (accession numbers KY524195–KY524217).

RESULTS

Parasite infection

A total of 6 different genotypes of *Anisakis* were identified, including *Anisakis* sp. HC-2005, *A. berlandi*, *A. pegreffii*, *A. physeteris*, *A. typica* (s.s.), and an Indonesian *A. typica* genotype for which we hereby apply the subspecific entity *A. typica* var. *indonesiensis*. This allows distinction of all thus far analysed *A. typica* from Indonesian waters, formerly named *A. typica*, *Anisakis* sp., sp. 1, sp. 2, I and II into 2 distinct genotypes. *A. berlandi* and *A. pegreffii* are hereby reported for the first time from teleost hosts from a tropical locality, and *A. physeteris* from the Pacific Ocean. During the detailed parasite sampling with at least 20–75 fish specimens per sample (39 in general), 16 of 40 teleost hosts were found to be infected with *Anisakis* spp. in Indonesian waters. Six fish species were found to be infected during the smaller samplings at the Bali workshop in 2013. The infected fish species are given in Tables 1 & 2. The *Anisakis*-negative fish species of the former, detailed sampling, mainly from Cilacap, south Central Java (other origins are mentioned), were Caesionidae: *Caesio cuning* (n = 35, mean TL [mTL] = 21.7 cm), *Pterocaesio diagramma* (n = 20, mTL = 21.9 cm) (both from Kedonganan); Carangidae: *Alepes melanoptera* (n = 20, mTL = 23.0 cm), *Atropus atropos* (n = 35, mTL = 16.4 cm), *Atule mate* (n = 75, mTL = 23.4 cm) (35 from Kedonganan), *Caranx heberi* (n = 35, mTL = 21.2 cm), *Parastromateus niger* (n = 40, mTL = 19.4 cm), *Selar boops* (n = 37, mTL = 17.5 cm); Cichlidae: *Oreochromis mossambicus* (n = 35, mTL = 16.9 cm); Clupeidae: *Amblygaster sirm* (n = 35, mTL = 18.9 cm), *Nematalosa come* (n = 35, mTL = 21.8 cm), *Sardinella lemuru* (n = 35, mTL = 19.4 cm) (the latter 2 from Kedonganan); Cynoglossidae: *Cynoglossus* cf. *arel* (n = 35, mTL = 27.6 cm); Haemulidae: *Pomadasys kaakan* (n = 35, mTL = 19.7 cm); Lutjanidae: *Lutjanus vitta* (n = 35, mTL = 20.1 cm; however, the low sample survey from the workshop was infected); Nemipteridae: *Nemipterus japonicus* (n = 35, mTL = 20.6 cm); Sciaenidae: *Nibea soldado* (n = 35, mTL = 23.3 cm), *Otolithes ruber* (n = 30, mTL = 17.6 cm), *Pennahia macrocephalus* (n = 40, mTL = 17.6 cm); Scombridae: *Rastrelliger kanagurta* (n = 35, mTL = 23.8 cm), *Sarda orientalis* (n = 35, mTL = 29.3 cm) (Kedonganan); Epinephelidae: *Epinephelus fuscoguttatus* (aquaculture, n = 75, mTL = 26.0 cm); Sparidae: *Acan-thopagrus berda* (n = 35, mTL = 21.6 cm); and Stromateidae: *Pampus argenteus* (n = 35, mTL = 22.0 cm).

A total of 22 fish species were found to be infected with *Anisakis* spp., and 16 new host records could be established (Table 2). Revision of 125 available sequence data allowed confirmation of 53 *Anisakis*-infected fish host species from Indonesia, including 32 with genetically confirmed identity (Tables 3 & 4). Table 2 presents the infection rates (prevalence, intensity, abundance) within the macrohabitat (~fish host species) and the site of infection (microhabitat). Because some data go back to earlier studies that allow no molecular identification without further sampling, Table 2 refers in these cases to '*Anisakis* spp.'

Highest infection rates were documented for the scombrid *Auxis rochei* and the carangid *Selar crumenophthalmus* (prevalence of 97.2% and 81.1% respectively). In both fish species, the range of microhabitats was widest (Table 2). We isolated 848 worms, almost all from the body cavity, free or attached to the outer tissues of various organs and/or the mesenteries, with just a single exception: One worm was isolated from the muscle tissue (filet) of a *S. crumenophthalmus*. The preferred sites for attachment were the liver (n = 299), gonads (n = 138), stomach (n = 136), body cavity (freely) (n = 131), intestine (n = 95), pyloric caeca (n = 45). The swim bladder, gill cavity, fins and muscle tissue (filet) were generally uninfected (n = 1 for each of the latter). Infections in gills and fins are unusual for this endoparasite and probably the effect of e.g. everted stomachs or a post-mortem migration (Table 2). The selected size range of the studied fish of a respective species was similar, preventing detailed comparison of different size classes. Male and female fishes had infection levels as follows: (1) *Auxis rochei*: 135 worms were isolated from 12 males, while 532 worms originated from 24 females; (2) *Selar crumenophthalmus*: 18 males harboured 44 worms while 7 females harboured 19 *Anisakis*; (3) *Terapon jarbua*: 8 worms from 17 males and 9 worms from 18 females; and (4) *Priacanthus tayenus*: 7 worms were obtained from 24 males and 8 worms from 11 females. A total of 797 of the 848 worms (~94%) were sampled during the dry season, with the remaining 51 specimens (~6%) during the rainy season. A total of 804 worms (~95%) originated from Kedonganan, Bali, and 44 (~5%) from Cilacap, south Java (although similar fish quantities were analysed from rainy vs. dry seasons respectively in Cilacap vs. Kedonganan; cf. Tables 1 & 2).

Table 1. Fish samples infected by *Anisakis* spp., with locality, season, number of sampled individuals (n_{fish}), total length (TL), total weight (TW), gutted weight (GW) in mean and range, and number of males (M), females (F) and juveniles (J). Additional fish species samples hosting *Anisakis* (workshop, dry season 2013): *Brama* cf. *orcini* (Bramidae) (n = 1), *Epinephelus areolatus* (Epinephelidae) (n = 3), *E. longispinis* (Epinephelidae) (n = 1), *Lutjanus argentimaculatus* (Lutjanidae) (n = 1), *L. vitta* (Lutjanidae) (n = 3), and *Sufflamen fraenatum* (Balistidae) (n = 2)

Fish species and family	Area	Season, year	n_{fish}	TL (cm)	TW (g)	GW (g)	M	F	J
Auxis rochei (Scombridae)	Kedonganan	Dry, 2010	36	30.7 (27.6–33.5)	357.9 (240.2–459.9)	295.5 (191.3–393.8)	12	24	–
A. thazard (Scombridae)	Kedonganan	Dry, 2010	35	28.3 (25.7–31.5)	269.8 (201.2–384.5)	232.2 (167.3–303.3)	–	–	35
Decapterus macrosoma (Carangidae)	Kedonganan	Rainy, 2011	35	20.5 (19.1–22.5)	76.2 (61.4–98.8)	66.3 (50.7–87.3)	14	21	–
D. tabl (Carangidae)	Kedonganan	Dry, 2010	35	18.0 (17.1–19.1)	50.3 (43.2–58.7)	45.6 (39.3–53.0)	–	–	35
Epinephelus ongus (Epinephelidae)	Karimunjawa	Rainy, 2013	35	25.5 (20.5–30.4)	266.3 (108.5–498.0)	238.0 (99.8–463.6)	7	23	5
E. sexfasciatus (Epinephelidae)	Jakarta	Rainy, 2012	41[a]	25.1 (23.1–27.8)	240.5 (189.0–352.4)	225.1 (176.6–325.9)	–	41	–
Megalaspis cordyla (Carangidae)	Kedonganan	Dry, 2010	35[b]	32.5 (29.5–36.0)	303.9 (246.3–403.7)	282.0 (226.7–373.4)	20	15	–
Nemipterus furcosus (Nemipteridae)	Kedonganan	Dry, 2010	35	24.4 (22.7–26.6)	165.8 (136.1–231.1)	151.3 (119.5–210.7)	11	24	–
Opisthopterus tardoore (Pristigasteridae)	Cilacap	Rainy, 2011	35	21.0 (18.6–23.7)	52.9 (36.6–68.1)	50.1 (35.2–63.5)	9	26	–
Pomadasys maculatus (Haemulidae)	Cilacap	Rainy, 2012	35	18.2 (16.5–22.0)	104.3 (71.6–194.5)	94.4 (62.0–169.5)	18	17	–
Priacanthus tayenus (Priacanthidae)	Cilacap	Rainy, 2011	35	26.3 (21.7–31.2)	177.8 (137.0–238.6)	160.6 (121.0–213.7)	24	11	–
Scomberoides tol (Carangidae)	Cilacap	Rainy, 2012	35	27.6 (24.0–31.0)	138.4 (90.0–207.5)	120.3 (80.7–170.0)	9	26	–
Selar crumenophthalmus (Carangidae)	Kedonganan	Dry, 2010	37[c]	18.34 (15.5 – 20.0)	72.5 (46.2–93.4)	61.8 (38.5–81.0)	18	7	12
Selaroides leptolepis (Carangidae)	Cilacap	Dry, 2011	25[d]	15.1 (14.0–16.8)	38.5 (31.6–50.3)	36.4 (29.9–49.0)	15	10	–
Terapon jarbua (Terapontidae)	Cilacap	Rainy, 2011	35	24.2 (20.3–28.9)	201.7 (107.7–310.0)	180.6 (94.8–265.2)	17	18	–
Trichiurus lepturus (Trichiuridae)	Kedonganan	Dry, 2010	35	59.4 (48.2–67.9)	154.2 (95.6–225.1)	145.1 (87.8–205.1)	25	10	–

[a]Sample bought in Cilacap, but originated from Jakarta according to salesman. Another sample of 35 fishes from Cilacap did not harbour any *Anisakis*
[b]A second sample of 40 fishes from Cilacap did not harbour any *Anisakis*
[c]A second sample of 25 fishes from Cilacap did not harbour any *Anisakis*
[d]Additional 8 specimens from Kedonganan (workshop, dry season 2013) without morphometric measurements

Molecular analyses

The ITS rDNA sequence data of 243 Indonesian larvae of *Anisakis* spp. (118 from the present study and 125 from previously sequenced helminths, named in the literature as e.g. real, original, 'sensu stricto' *typica*, '*typica* sibling', or *Anisakis* sp./type 1, sp. 2, sp. I, sp. II) from 32 different bony fishes revealed the presence of 4 valid *Anisakis* species (Tables 3 & 4): *A. physeteris* (n = 4), *A. pegreffii* (n = 1), *A. berlandi* (n = 2) and *A. typica* (s.s.) (n = 39). One further genotype, as defined and illustrated by Palm et al. (2008), was pre-

dominantly found. We hereby apply the subspecific entity *A. typica* var. *indonesiensis* for these specimens. These 193 (~79%) worms showed a 4 bp difference at identical positions in the ITS-1 region to the closely related *A. typica* (s.s.) (for alignment, see Palm et al. 2008). The genetic distance by using ~903 informative bp was the same to the one between 2 valid species, *A. simplex* (s.s.) and *A. berlandi* (4 bp). This genotype was isolated from 28 out of 32 fish species with genetically identified *Anisakis* infection (~88%). On the other hand, *A. typica* (s.s.) (16% of the analysed worms) was documented in only 28% (9/32) of

Table 2. Infected fish species with number of *Anisakis* individuals genetically identified (total $n_{Anisakis}$ DNA-analysed = 118) and infection rates, with total number of specimens ($n_{Anisakis}$ documented), prevalence (P) (proportion of infected hosts among all hosts examined), mean intensity (mI) (mean number of *Anisakis* found in infected hosts), mean abundance (mA) (mean number of *Anisakis* found in all hosts), and infection sites (all data from this study) (bcv: body cavity, gcv: gill cavity; go: gonads; in: intestine; li: liver; mus: muscle tissue; pyl: pylorus; st: stomach; swb: swim bladder). Worms were not isolated from inner lumen of organs, but were located outside, attached to organs or mesenteries. Thus, all worms originated from the body cavity (except the one from muscle), sometimes free-living and sometimes attached to organs

Fish species and family	$n_{Anisakis}$ documented	P (%)	mI (range)	mA	Site of infection	$n_{Anisakis}$ DNA-analysed
Auxis rochei (Scombridae)	667	97.2	19.1 (1–275)	18.53	293 (li), 125 (go), 115 (st), 64 (in), 46 (bcv), 24 (pyl)	24
A. thazard (Scombridae)	10	22.9	1.3 (1–3)	0.29	7 (bcv), 3 (li)	10
Decapterus macrosoma[a] (Carangidae)	8	20.0	1.1 (1–2)	0.23	4 (bcv), 1 (swb), 1 (st), 1 (go), 1 (in)	8
D. tabl[a] (Carangidae)	12	25.7	1.2 (1–2)	0.31	10 (bcv), 2 (st)	12
Epinephelus ongus[a] (Epinephelidae)	3	8.6	1.0 (1)	0.09	1 (go), 1 (in), 1 (pyl)	1
E. sexfasciatus[a] (Epinephelidae)	3	8.6	1.0 (1)	0.09	1 (go), 1 (in), 1 (pyl)	3
Megalaspis cordyla[a] (Carangidae)	1	2.9	1.0 (1)	0.03	1 (li)	1
Nemipterus furcosus[a] (Nemipteridae)	3	5.7	1.5 (1–2)	0.06	2 (go), 1 (st)	3
Opisthopterus tardoore[a] (Pristigasteridae)	2	5.7	1.0 (1)	0.06	1 (pyl), 1 (bcv)	2
Pomadasys maculatus[a] (Haemulidae)	1	2.9	1.0 (1)	0.03	1 (in)	1
Priacanthus tayenus[a] (Priacanthidae)	15	34.3	1.3 (1–2)	0.43	13 (in), 1 (pyl), 1 (bcv)	10
Scomberoides tol[a] (Carangidae)	2	5.7	1.0 (1)	0.06	1 (go), 1 (bcv)	2
Selar crumenophthalmus (Carangidae)	91	81.1	3.0 (1–8)	2.46	49 (bcv), 15 (pyl), 11 (st), 7 (in), 6 (go), 1 (gcv), 1 (fin), 1 (mus)	16
Selaroides leptolepis[a] (Carangidae)	1	4.0	1.0 (1)	0.04	1 (go)	1
Terapon jarbua (Terapontidae)	17	37.1	1.3 (1–3)	0.49	6 (st), 5 (in), 2 (pyl), 2 (li), 2 (bcv)	12
Trichiurus lepturus (Trichiuridae)	1	2.9	1.0 (1)	0.03	1 (in)	1
Additional *Anisakis*-hosting fish species investigated during low-sample spot survey (workshop 2013):						
Brama cf. *orcini*[a] (Bramidae)	1				1 (in)	1
E. areolatus (Epinephelidae)	5				5 (bcv)	5
E. longispinis[a] (Epinephelidae)	1				1 (bcv)	1
Lutjanus argentimaculatus[a] (Lutjanidae)	1				1 (bcv)	1
L. vitta[a] (Lutjanidae)	2				2 (bcv)	2
Sufflamen fraenatum[a] (Balistidae)	1				1 (bcv)	1

[a]New *Anisakis* host record for Indonesia (n = 16)

Table 3. Host records of *Anisakis* spp. from Indonesia. *Anisakis typica* var. *indonesiensis* (*A. t. indonesiensis*) represents the local genotype

Fish host: family and species	Parasite	Locality	Reference	DNA sequenced
Ariommatidae				
Ariomma indicum (= *A. indica*)[a]	*Anisakis* sp.	S. Java	Theisen (2009)	
Balistidae				
Sufflamen fraenatum	*Anisakis* sp. HC-2005	Bali	Present study	Yes
Bramidae				
Brama dussumieri	*Anisakis* sp.	Bali	Jakob & Palm (2006)	
Brama cf. *orcini*	*A. t. indonesiensis*	Bali	Present study	Yes
Caesionidae				
Caesio cuning	*A. t. indonesiensis*	Bali, 'Indonesia'	Palm et al. (2008), Kuhn et al. (2013)	Yes
Caesio sp.	*Anisakis* sp.	S. Java	Setyobudi et al. (2011)	
Carangidae				
Atule mate	*Anisakis* sp.	N. Java	Ilahude et al. (1978), Ilahude (1980)	
Caranx sp.	*A. typica* (s.l.)[b]	Makassar Strait	Anshary et al. (2014), Anshary pers. comm. (Feb 2015)	
Decapterus kurroides	Anisakidae	Makassar Strai	Burhanuddin & Djamali (1983)	
D. macarellus	*A. t. indonesiensis*	Papua New Guinea	Koinari et al. (2013)	Yes
D. macrosoma	*A. t. indonesiensis*	Bali	Present study	Yes
D. russelli (= *D. lajang*)[a]	*Anisakis* sp.	Bali, Java	Burhanuddin & Djamali (1978, 1983), Hadidjaja et al. (1978), Hutomo et al. (1978), Martosewojo (1980), Palm et al. (2008)	
D. tabl	*A. typica* (s.s.)	Bali	Present study	Yes
	A. t. indonesiensis	Bali	Present study	Yes
Megalaspis cordyla	*A. t. indonesiensis*	Bali	Present study	Yes
Scomberoides tol	*A. t. indonesiensis*	S. Java	Present study	Yes
Selar crumenophthalmus (= *Caranx crumenophthalmus*)[a]	*Anisakis* sp.	N. Java	Ilahude et al. (1978), Ilahude (1980)	
	A. typica (s.s.)	Papua New Guinea, Bali	Koinari et al. (2013), present study	Yes
	A. t. indonesiensis	Bali	Present study	Yes
Selaroides leptolepis	*A. t. indonesiensis*	S. Java	Present study	Yes
Clupeidae				
Amblygaster sirm	*Anisakis* sp.	N. Java, Sunda Strait	Hadidjaja et al. (1978), Hutomo et al. (1978), Martosewojo (1980), Burhanuddin & Djamali (1983)	
Sardinella fimbriata	Anisakidae	N. Java, Sunda Strait	Burhanuddin & Djamali (1983)	
S. gibbosa (= *jussieui*)[a]	*Anisakis* sp.	N. Java, Sunda Strait	Ilahude et al. (1978), Ilahude (1980)	
Coryphaenidae				
Coryphaena hippurus	*A. typica* (s.s.)	S. Java	Palm et al. (2008)	Yes
	A. t. indonesiensis	S. Java	Palm et al. (2008)	Yes
Epinephelidae				
Cephalopholis cyanostigma	*A. typica* (s.l.)[b]	Makassar Strait	Anshary et al. (2014), H. Anshary (pers. comm.)	
Cromileptes altivelis	Anisakidae	Bali	Koesharyani et al. (2001)	
Epinephelus areolatus	*A. t. indonesiensis*	S. Java, Bali	Palm et al. (2008), Kleinertz et al. (2014a), present study	Yes
	Anisakis sp. HC-2005	S. Java	Kleinertz et al. (2014a)	Yes
E. fuscoguttatus	*Anisakis* sp.	N. Java, Sulawesi	Burhanuddin & Djamali (1983), Asmanelli et al. (1993)	
E. longispinis	*A. t. indonesiensis*	Bali	Present study	Yes
E. maculatus	Anisakidae	Bali	Burhanuddin & Djamali (1983)	
E. ongus	*A. t. indonesiensis*	N. Java	Present study	Yes
E. quoianus (= *E. megachir*)[a]	Anisakidae	N. Java, Sulawesi	Burhanuddin & Djamali (1983)	
E. sexfasciatus	*A. t. indonesiensis*	N. Java	Present study	Yes
E. summana (~ *E. ongus* or *E. coeruleopunctatus*)[c]	Anisakidae	N. Java, Sulawesi	Burhanuddin & Djamali (1983)	

Table 3 (continued)

Fish host: family and species	Parasite	Locality	Reference	DNA sequenced
Plectropomus leopardus	*Anisakis* sp.	Bali, Sulawesi	Asmanelli et al. (1993), Koesharyani et al. (2001)	
Gempylidae				
Gempylus serpens	*Anisakis* sp.	Bali, Sulawesi	Jakob & Palm (2006)	
Thyrsitoides marleyi	*Anisakis* sp.	Bali, Sulawesi	Jakob & Palm (2006)	
Gerreidae				
Gerres oblongus	*A. t. indonesiensis*	Papua New Guinea	Koinari et al. (2013)	Yes
Haemulidae				
Pomadasys maculatus	*A. t. indonesiensis*	Papua New Guinea	Present study	Yes
Leiognathidae				
Leiognathus dussumieri	*Anisakis* sp.	Sulawesi	Yamaguti (1954)	
Lutjanidae				
Lutjanus argentimaculatus	*A. t. indonesiensis*	Bali	Present study	Yes
L. kasmira	Anisakidae	N. Java	Ilahude (1980)	
L. malabaricus	*Anisakis* sp.	S. Java	Setyobudi et al. (2011)	
L. vitta	*A. t. indonesiensis*	Bali	Present study	Yes
Pinjalo lewisi	*A. t. indonesiensis*	Papua New Guinea	Koinari et al. (2013)	Yes
P. pinjalo	*A. t. indonesiensis*	Papua New Guinea	Koinari et al. (2013)	Yes
Mullidae				
Parupeneus sp.	*Anisakis* sp.	S. Java	Setyobudi et al. (2011)	
Nemipteridae				
Nemipterus furcosus	*A. t. indonesiensis*	Bali	Present study	Yes
Platycephalidae				
Platycephalus endrachtensis (= *P. arenarius*)[a]	*Anisakis* sp.	S. Java	Theisen (2009)	
Priacanthidae				
Priacanthus tayenus	*A. t. indonesiensis*	S. Java	Present study	Yes
Pristigasteridae				
Opisthopterus tardoore	*A. t. indonesiensis*	S. Java	Present study	Yes
Scombridae				
Auxis rochei rochei	*A. typica* (s.s.)	'Indonesia', Bali	Palm et al. (2008), Kuhn et al. (2013), present study	Yes
	A. typica × *A. t. indonesiensis*	Bali	Palm et al. (2008)	Yes
	A. t. indonesiensis	'Indonesia', Bali	Palm et al. (2008), Kuhn et al. (2013), present study	Yes
	A. pegreffii	Bali	Present study	Yes
	A. physeteris	Bali	Present study	Yes
	A. berlandi	Bali	Present study	Yes
A. thazard thazard	*A. typica* (s.s.)	Makassar Strait, Bali	Anshary et al. (2014), H. Anshary (pers. comm.), present study	Yes
	A. t. indonesiensis	'Indonesia', Makassar Strait, Bali	Kuhn et al. (2013), Anshary et al. (2014), H. Anshary (pers. comm.), present study	Yes
Euthynnus affinis	*Anisakis* sp.[d]	N. Java	Ilahude et al. (1978), Burhanuddin & Djamali (1983)	
	A. typica (s.l.)[b]	Makassar Strait	Anshary et al. (2014), H. Anshary (pers. comm.)	
Katsuwonus pelamis	*A. t. indonesiensis*	Makassar Strait	Anshary et al. (2014), H. Anshary (pers. comm.)	Yes
Rastrelliger brachysoma	*Anisakis* sp.	N. Java	Ilahude et al. (1978), Ilahude (1980)	
R. kanagurta	*Anisakis* sp.	N. Java	Hadidjaja et al. (1978), Hutomo et al. (1978), Martosewojo (1980), Burhanuddin & Djamali (1983)	
	A. typica (s.l.)[b]	Makassar Strait	Anshary et al. (2014), H. Anshary (pers. comm.)	
Scomberomorus commerson	Anisakidae[d,e]	Kupang	Burhanuddin & Djamali (1983)	
	A. simplex[e]	Kupang	Lester et al. (2001)	

Table 3 (continued)

Fish host: family and species	Parasite	Locality	Reference	DNA sequenced
S. maculatus (~ *S. queenslandicus* or *S. guttatus*)[f]	*A. typica* (s.s.)	Papua New Guinea	Koinari et al. (2013)	Yes
Thunnus albacares	*A. typica* (s.s.)	Papua New Guinea	Koinari et al. (2013)	Yes
Siganidae				
Siganus guttatus	Anisakidae	Papua New Guinea	Burhanuddin & Djamali (1983)	
Synodontidae				
Saurida isarankurai	Anisakidae	Papua New Guinea	Burhanuddin & Djamali (1983)	
S. longimanus	Anisakidae	Papua New Guinea	Burhanuddin & Djamali (1983)	
S. micropectoralis	Anisakidae	Papua New Guinea	Burhanuddin & Djamali (1983)	
S. undosquamis	Anisakidae	Papua New Guinea	Burhanuddin & Djamali (1983)	
Terapontidae				
Terapon jarbua	*A. t. indonesiensis*	S. Java	Present study	Yes
	Anisakis sp.	S. Java	Setyobudi et al. (2011)	
Trichiuridae				
Lepturacanthus savala	*A. t. indonesiensis*	'Indonesia'	Kuhn et al. (2013)	Yes
	Anisakis sp. HC-2005	'Indonesia'	Kuhn et al. (2013)	Yes
Trichiurus lepturus	*Anisakis* sp.	S. Java	Jakob & Palm (2006), Theisen (2009), Setyobudi et al. (2011)	
	A. t. indonesiensis	'Indonesia'	Kuhn et al. (2013)	Yes
	A. typica (s.s.)	'Indonesia', Bali	Kuhn et al. (2013), present study	Yes

[a]Misapplied/junior/senior synonym name used in the original reference
[b]*A. typica* (s.l.) means that PCR-RFLP resulted in *A. typica*, but could not distinguish *A. typica* (s.s.) from *A. typica* var. *indonesiensis*
[c]*E. summana* is endemic to the Red Sea and not known from Indonesia; *E. ongus* and *E. coeruleopunctatus* (and also *E. corallicola* and *E. macrospilos*) from the Indo-Pacific have often been misidentified as *E. summana* in the past (Fischer & Whitehead 1974, Heemstra & Randall 1993, Froese & Pauly 2016)
[d]*A. typica* documented in the same intermediate fish hosts from other localities (Mattiucci et al. 2002)
[e]Lester et al. (2001) recorded *A. simplex* from Kupang, Timor, on a morphological basis (must be confirmed)
[f]*S. maculatus* is not known from the Pacific but from the Atlantic. Similar species from the region are *S. queenslandicus* (spots and dark dorsal fin) and *S. guttatus* (spots)

the infected fish species. We also documented a single specimen of *Anisakis* sp. HC-2005. Of all 243 genetically identified *Anisakis* from Indonesia since 2008, there was only a single specimen with an intermediate sequence at the same positions, differing from both *A. typica* (s.s.) and the Indonesian genotype in 2 positions in the ITS-1 region each (Palm et al. 2008, their Figs. 1 & 2). It must be stated that analysing deposited sequences from GenBank from different studies and authors without having the original chromatogram resulted in some observed sequence differences. However, they appeared randomly in both *A. typica* and *A. typica* var. *indonesiensis*, and were considered uninformative.

Host range

Both documented *A. berlandi* specimens and the single *A. pegreffii* were isolated from the pelagic,

oceanodromous and migratory scombrid *Auxis rochei*. Also, the 4 specimens of *A. physeteris* were isolated from *A. rochei*, which also hosted the single additional genotype with an intermediate sequence, a mixture of *A. typica* (s.s.) and *A. typica* var. *indonesiensis*, documented by Palm et al. (2008). One of the 3 *Anisakis* sp. HC-2005 from Indonesia is known from the benthopelagic trichiurid *Lepturacanthus savala* (Kuhn et al. 2013), the other from the reef-associated grouper *Epinephelus areolatus* (Kleinertz et al. 2014a) and the third from the reef-associated balistid *Sufflamen fraenatum* (present study). The 2 others, *A. typica* (s.s.) and the Indonesian sibling *A. typica* var. *indonesiensis*, occurred in various fish species: *A. typica* was isolated from 9 teleost hosts with a (bentho)-pelagic, oceanodromous and usually schooling, migratory ecology (in general, scombrids, carangids, trichiurids and 1 coryphaenid). On the other hand, the Indonesian *A. typica* var. *indonesiensis* seems to have no preference of a certain fish host ecology, infecting

Table 4. Molecular identity and distribution of *Anisakis* spp. (in % and absolute numbers) from Indonesian waters with teleost host and ecology (118 of 243 worms from present study, 125 worms from previous studies; see Table 3). Habitat data from Froese & Pauly (2016)

Fish host: family and species	Habitat	*A. berlandi*	*A. pegreffii*	*A. physeteris*	*Anisakis* sp. HC-2005	*A. typica* (s.s.)	*A. typica* (s.s.) × *A. t. indonesiensis*	*A. typica* var. *indonesiensis*
Balistidae								
Sufflamen fraenatum	Reef-associated	0	0	0	100 % (1/1)	0	0	0
Bramidae								
Brama cf. *orcini*	Epi-/benthopelagic	0	0	0	0	0	0	100 % (1/1)
Caesionidae								
Caesio cuning	Reef-associated	0	0	0	0	0	0	100 % (11/11)
Carangidae								
Decapterus macarellus	Pelagic, schooling	0	0	0	0	0	0	100 % (1/1)
D. macrosoma	Pelagic, schooling	0	0	0	0	0	0	100 % (8/8)
D. tabl	Pelagic, schooling	0	0	0	0	50 % (6/12)	0	50 % (6/12)
Megalaspis cordyla	Oceanic, schooling	0	0	0	0	0	0	100 % (1/1)
Scomberoides tol	Reef-associated, near surface, schooling	0	0	0	0	0	0	100 % (2/2)
Selar crumenophthalmus	Pelagic, schooling	0	0	0	0	24 % (4/17)	0	76 % (13/17)
Selaroides leptolepis	Demersal, schooling	0	0	0	0	0	0	100 % (1/1)
Frequency of occurrence in carangids (%)		0	0	0	0	24 % (10/42)	0	76 % (32/42)
Coryphaenidae								
Coryphaena hippurus	Oceanodromous, schooling	0	0	0	0	14 % (1/7)	0	86 % (6/7)
Epinephelidae								
Epinephelus areolatus	Reef-associated	0	0	0	4 % (1/28)	0	0	96 % (27/28)
E. longispinis	Reef-associated	0	0	0	0	0	0	100 % (1/1)
E. ongus	Reef-associated	0	0	0	0	0	0	100 % (1/1)
E. sexfasciatus	Reef-associated	0	0	0	0	0	0	100 % (3/3)
Frequency of occurrence in epinephelids (%)		0	0	0	3 % (1/33)	0	0	97 % (32/33)
Gerreidae								
Gerres oblongus	Reef-associated, benthic, schooling	0	0	0	0	0	0	100 % (1/1)
Haemulidae								
Pomadasys maculatus	Reef-associated, benthic	0	0	0	0	0	0	100 % (1/1)
Lutjanidae								
Lutjanus argentimaculatus	Reef-associated, oceanodromous	0	0	0	0	0	0	100 % (1/1)
L. vitta	Reef-associated	0	0	0	0	0	0	100 % (2/2)
Pinjalo lewisi	Reef-associated, oceanic, schooling	0	0	0	0	0	0	100 % (2/2)
P. pinjalo	Reef-associated, oceanic, schooling	0	0	0	0	0	0	100 % (1/1)
Frequency of occurrence in lutjanids (%)		0	0	0	0	0	0	100 % (6/6)
Nemipteridae								
Nemipterus furcosus	Reef-associated, benthic, neritic, oceanic	0	0	0	0	0	0	100 % (3/3)
Priacanthidae								
Priacanthus tayenus	Reef-associated, benthic, neritic, oceanic	0	0	0	0	0	0	100 % (10/10)

(Table continued on next page)

Table 4 (continued)

Fish host: family and species	Habitat	A. berlandi	A. pegreffii	A. physeteris	Anisakis sp. HC-2005	A. typica (s.s.)	A. typica (s.s.) × A. t. indonesiensis	A. typica var. indonesiensis
Pristigasteridae								
Opisthopterus tardoore	Pelagic, neritic, oceanodromous	0	0	0	0	0	0	100% (2/2)
Scombridae								
Auxis rochei	Pelagic, oceanodromous, schooling	4% (2/46)	2% (1/46)	9% (4/46)	0	24% (11/46)	2% (1/46)	59% (27/46)
A. thazard	Pelagic, oceanodromous, schooling	0	0	0	0	23% (7/30)	0	77% (23/30)
Katsuwonus pelamis	Pelagic, oceanodromous, schooling	0	0	0	0	100% (5/5)	0	0
Scomberomorus maculatus[a]	Pelagic, oceanodromous, schooling	0	0	0	0	100% (1/1)	0	0
Thunnus albacares	Pelagic, oceanodromous, schooling	0	0	0	0	100% (1/1)	0	0
Frequency of occurrence in scombrids (%)		2.4% (2/83)	1.2% (1/83)	4.8% (4/83)	0	30.1% (25/83)	1.2% (1/83)	60.2% (50/83)
Terapontidae								
Terapon jarbua	Demersal, schooling	0	0	0	0	0	0	100% (12/12)
Trichiuridae								
Lepturacanthus savala	Benthopelagic	0	0	0	33% (1/3)	0	0	66% (2/3)
Trichiurus lepturus	Benthopelagic	0	0	0	0	11% (3/27)	0	89% (24/27)
Frequency of occurrence in trichiurids (%)		0	0	0	3% (1/30)	10% (3/30)	0	87% (26/30)
Frequency of occurrence in total (%)		0.8% (2/243)	0.4% (1/243)	1.7% (4/243)	1.2% (3/243)	16.1% (39/243)	0.4% (1/243)	79.4% (193/243)

[a]*S. maculatus* (data/identification from Koinari et al. 2013, see our Table 3) is not known from the Pacific but from the Atlantic, thus a misidentification. Similar species from the region are *S. queenslandicus* (spots and dark dorsal fin) and *S. guttatus* (spots) (Froese & Pauly 2016)

a wide range of (bentho)pelagic, oceanodromous and usually schooling, migratory as well as reef-associated and even demersal fish species. Besides single fish species of the respective families, we also analysed the sequences of pooled *Anisakis* from 7 different carangids (*A. typica*: 23.8%; *A. typica* var. *indonesiensis*: 76.2%), 4 lutjanids (*A. typica* var. *indonesiensis*: 100%), 5 scombrids (*A. typica*: 30.1%; *A. typica* var. *indonesiensis*: 60.2%), 3 groupers (epinephelids) (*A. typica* var. *indonesiensis*: 97%) and 2 trichiurids (*A. typica*: 7.1%; *A. typica* var. *indonesiensis*: 90.5%) (Table 4).

DISCUSSION

The present study provides a comprehensive screening and molecular identification of *Anisakis* spp. from Indonesian waters. Establishing 16 new host records, 53 teleost species are known to be infected with *Anisakis* in Indonesia, demonstrating the low host-specificity and wide distribution of these taxa.

Hosts and life cycle

According to Kuhn et al. (2013), the most common teleosts for *Anisakis* larvae are perciform (57 species) and gadiform (21 species) belonging to the fish families Scombridae (12), Gadidae (10), Carangidae (8) and Clupeidae (7), depending on locality and depth of study in the respective region. Abollo et al. (2001) and Klimpel et al. (2004) reported 200 fish species worldwide to be infected with *Anisakis* larvae, 155 of

them with genetic evidence (Kuhn et al. 2013). Since then, new collections with genetically identified worms have originated from teleosts already stated in Kuhn et al. (2013) (Anshary et al. 2014, Bak et al. 2014, Mladineo & Poljak 2014, Chen & Shih 2015, Cipriani et al. 2015), or from mammalian definitive hosts (i.e. Shamsi 2014, Blažeković et al. 2015). With 53 *Anisakis*-hosting fish species presented in this study, about one-quarter of the worldwide known *Anisakis* fish host species and one-third of the worldwide hosts of genetically identified *Anisakis* genotypes have been reported from Indonesia, even though this country is comparatively understudied, considering its high biodiversity. Sixteen of 40 thoroughly sampled fish species (high-amount fish samples during this study) were infected in this present study, so 40% harboured *Anisakis* spp. Indonesia is one of the most diverse marine regions in the world, with 3600 marine teleost species (Froese & Pauly 2016). It can be expected that many further host records for *Anisakis* spp. will occur in future samplings from the region, because we even recorded *Anisakis* from the low-amount fish samples during the workshop, demonstrating the low host-specificity in the region (see Tables 1–4).

Of the 6 different genetically identified *Anisakis* genotypes, 4 of them were identified to species level. A total of 16.1% of the samples, infecting 9/32 host species, belonged to *A. typica* (s.s.) (Table 4), a species known from many tropical regions throughout the world. Palm et al. (2008) have already reported this species in teleosts from Indonesia, especially in *Coryphaena hippurus* and *Auxis rochei rochei*. The larvae have been found in scombrids and carangids such as *Auxis thazard thazard* and *Thunnus thynnus* from Brazil (SW Atlantic); *Scomber japonicus* and *Trachurus picturatus* from the NE Atlantic off Madeira; *Euthynnus affinis*, *Scomberomorus commerson*, *Sarda orientalis* and *C. hippurus* from the west Indian Ocean off Somalia; and from *Merluccius merluccius* from the eastern Mediterranean Sea (Palm et al. 2008). Further records were provided by Mattiucci et al. (2005) from Florida, Farjallah et al. (2008) from the Mediterranean coast of North Africa (*Scomber scombrus*, *M. merluccius*, *Phycis phycis*), from Australia by Cannon (1977) and more recently by Shamsi (2007, 2014) and Jabbar et al. (2012) from the Great Barrier Reef, and by Chen & Shih (2015) from Taiwan. Kuhn et al. (2013) have already summarized the worldwide known hosts for genetically confirmed worms, naming 26 different teleost species. *A. typica* is a common parasite of various dolphin species from warmer temperate and tropical waters, belonging to

the families Delphinidae, Phocoenidae and Pontoporiidae (see Mattiucci et al. 2002, Kleinertz et al. 2014a). The eggs of the congener *A. simplex* (s.s.) are expelled from their hosts with the faeces and embryonate in seawater (Klimpel & Palm 2011). 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) 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. They can be transferred further into larger transport hosts without moult, acquiring high numbers in these hosts (Jakob & Palm 2006). The life cycle is completed when the definitive hosts preys upon infected crustaceans, cephalopods or fishes (Kellermanns et al. 2007). The stomach of the studied *Auxis rochei rochei* by Palm et al. (2008) was filled with small crustaceans and only a few small-sized fish, and *Decapterus russelli* as a common host preyed upon small crustaceans as well. A low intensity of anisakids in a sampled fish species suggests that the larvae uptake originates directly from the crustacean first intermediate hosts (see Palm 1999), while larger predatory fish also serve as transport host and accumulate the larvae (Table 4). This suggests that several reef fish within the present study, such as *Sufflamen fraenatum*, accidentally aquire the worms, while the heavily infected pelagic scombrids, carangids and coryphaenids either are infected directly through the first intermediate or serve as transport host, suggesting a pelagic life cycle for the tropical *A. typica*, as suggested by Palm et al. (2008) and tentatively by Kuhn et al. (2013).

Anisakis typica var. *indonesiensis*

The predominant recorded genotype within the present study was *A. typica* var. *indonesiensis*, a genotype for the first time reported from *Auxis rochei rochei*, *Caesio cuning*, *Coryphaena hippurus* and *Epinephelus areolatus* from Indonesia by Palm et al. (2008). It was recently described also from Papua New Guinea (Koinari et al. 2013), Sulawesi, Java and Bali, Indonesia (Kuhn et al. 2013, Anshary et al. 2014, Kleinertz et al. 2014a) under different names, having a distinct 4 bp difference (the same range as recorded for the siblings *A. simplex* (s.s.) and *A. berlandi* as well *A. pegreffii*). Multiple marker studies combined with morphological investigation of the adults must test whether this difference distinguishes a dis-

tinct species or is a matter of intraspecific variability. Therefore, we refrain from nominating a distinct taxon, but apply the subspecific entity *A. typica* var. *indonesiensis*. This genotype seems to be more widely spread than known so far, appearing to have no clear host specificity in Indonesian waters, infecting over 28 (of 32 with genetically identified *Anisakis* spp. infection) teleost host species belonging to 14 (of 15) families. In contrast, only 6 of these 32 infected Indonesian fish species harbour both the common *A. typica* (s.s.) as well as this genotype. A single case of an additional genotype reported by Palm et al. (2008) in *Auxis rochei*, possibly a hybrid between the 2 *A. typica* genotypes, suggests that both have the same final hosts in the region and are able to infect the same or a similar intermediate host range. Interestingly, Shamsi (2007) also suggested that the *A. typica* in Australia seems to be genetically different from those reported in other countries, and the cluster analysis of *A. typica* by Iniguez et al. (2011) showed a cluster consisting only of intermediate/paratenic hosts from Asian coasts. Adult specimens must be sampled from the final hosts in the region for a future species description, to morphologically distinguish *A. typica* var. *indonesiensis* from *A. typica* (s.s.).

Distribution of *Anisakis* spp.

A. berlandi and *A. pegreffii* are reported for the first time from an equatorial region (both have been already reported from Australian mammals, see Shamsi et al. 2012), and *A. physeteris* is recorded for the first time from the Pacific Ocean. These 3 species were exclusively found in the pelagic, oceanodromous and migratory carangid *Auxis rochei*. According to Klimpel et al. (2008), Mattiucci & Nascetti (2007, 2008) and Kellermanns (2009), *A. berlandi* and *A. pegreffii* together with *A. simplex* (s.s.) form the *A. simplex* s.l. complex of species that are most common in temperate to cold regions, e.g. from 30–70° N and S latitudes. *A. berlandi* so far has been reported from the North and South Pacific, the Atlantic coast of southern Africa, and from Australia and off New Zealand (Mattiucci et al. 1997, 2014, Mattiucci & Nascetti 2006, 2007, 2008, Klimpel et al. 2008, Kellermanns 2009), and has been found in the migrating pilot whale *Globicephala melas* (Mattiucci et al. 2014) as an adult. *A. pegreffii* infects delphinids, ziphiids, physeterids and neobalaenids as final hosts and has been reported from the waters off Italy, Argentina, Brazil, South Africa and New Zealand

(Klimpel et al. 2008, Mattiucci & Nascetti 2008, Kellermanns 2009), especially in the temperate zones. Recent cases of anisakiasis caused by *A. pegreffii* in Korea (Lim et al. 2015), Italy and Japan (Mattiucci et al. 2013, Lim et al. 2015) demonstrate that besides *A. simplex* (s.s.), *A. pegreffii* can also become human-pathogenic. Interestingly, *A. simplex* (s.s.), commonly found in the northern hemisphere and also in Japan in the North Pacific, could not be identified within the present study. This is of importance because *A. simplex* is the main species causing anisakiasis. Also *A. physeteris*, infecting physeterid whales as adults, has been reported from temperate regions, e.g. the Atlantic and Mediterranean, but not yet from the Pacific Ocean. However, physeterid whales are found worldwide, also in the Pacific. Consequently, the present study sheds some light on the general distribution patterns of *Anisakis* infections in the world oceans. Though only rare findings of the above species from the low latitudes do exist, they also occur as larvae in teleosts of the tropics, and future findings are expected. Because the migrating *Auxis rochei* from Indonesia is widely distributed within the region but does not migrate into temperate zones, they must have acquired the *Anisakis* larvae from the first or second intermediate hosts around Indonesian waters. Whales as final hosts are able to release the eggs while migrating between the northern and southern hemisphere. However, in the case of *A. berlandi*, *A. pegreffii* and *A. physeteris*, the larvae seem to be able to infect also intermediate hosts in tropical waters, explaining their extensive range of distribution. This might be different for *A. simplex* (s.s.) which is restricted to the boreal zone, and especially occurs in the northern hemisphere. The species might have a more restricted first intermediate host regime. Similarly, the genotype *Anisakis* sp. HC-2005 so far has been reported from 3 different teleost hosts in Indonesia (*Sufflamen fraenatum*, present study; *Lepturacanthus savala*, Kuhn et al. 2013; and *Epinephelus areolatus*, Kleinertz et al. 2014a), and from northwest Africa (*Hoplostethus cadenati* from the African shelf, see Kijewska et al. 2009), and seems to be not able to extend the host range into the more northern and southern regions. This underlines the importance of low host-specificity to infect a range of first intermediate hosts to extend the host distribution in the anisakid nematodes, as earlier suggested by Kellermanns et al. (2007). The other factors supporting constant gene flow (see Mattiucci et al. 2002) are extensive final and intermediate host migration, overlapping distribution patterns of different final host populations,

and large population sizes in the intermediate and final hosts (Palm 2004, Kellermanns et al. 2007, Palm et al. 2007).

Infection patterns and site

Analyses of the infected teleost hosts and their ecology reveal a distinct pattern, with highest infection rates in the scombrids and carangids and lowest in some reef-associated fish (cf. Tables 1 & 2). We did not expect differences during the rainy versus dry seasons, because the worms are quite long-living. The fact that 94% of the worms were sampled throughout the dry seasons is a result of uneven distribution in the fishes, because 667 worms (of all 848) were isolated from a sample of 36 *Auxis rochei* during the dry season only (cf. Tables 1 & 2). The observed prevalence and the intensity of infection of *Anisakis* spp. were highest in the pelagic schooling scombrid *Auxis rochei*, also getting infected with 5 of the 6 detected *Anisakis* taxa. This leads to the assumption that this fish species is the most suitable intermediate host for *Anisakis* nematodes in the sampled region. *A. typica* (s.l.) infections of *Auxis rochei rochei* are readily observed throughout the years, especially between 2005 and 2006 (Palm et al. 2008) and 2013 (present study), with a minimum prevalence of 20%. This complies with the presence of the potential final hosts in the region. Other high infection rates within this study were observed in the oceanic/pelagic/schooling *Auxis thazard*, *Selar crumenophthalmus* and *Decapterus tabl*, the benthopelagic *Trichiurus lepturus* and also in the more reef-associated *Epinephelus areolatus* and *Caesio cuning* (cf. Tables 2−4) and *Priacanthus tayenus*. This might refer to the distribution of the dolphin final hosts in the region that migrate throughout the archipelago but also close to coral reef habitats, and may also feed on schooling demersal fish over shallow sandy bottoms (compared to the high prevalence of infection in *Terapon jarbua*). Consequently, the occurrence in reef-associated fish does not refer to a benthic parasite life cycle but to the wide distribution of the suitable first intermediate hosts (offshore to close to the reef ecosystems), low host-specificity concerning the teleost hosts (hundreds of possible intermediate fish host species), and to the mobility of the dolphin final hosts between the different tropical habitats and food webs. This possibly overcomes the limitations of the otherwise highly specialized food web in coral reef habitats, enabling *Anisakis* to infect such a wide host range.

The typical infection site for Indonesian *Anisakis* spp. was the body cavity, with n = 131 free-living worms, and others attached to the mesenteries and outer surfaces of the liver (n = 299), gonads (n = 138), stomach (n = 136) and guts (intestine, n = 95; pyloric caeca, n = 45). Because most of the recorded *Anisakis* belonged to *A. typica* and *A. typica* var. *indonesiensis*, these are the preferred infection sites for these 2 genotypes. Previous studies reported no muscular infections (Burhanuddin & Djamali 1978, 1983, Ilahude et al. 1978), Palm et al. (2008) found only a single case in 110 examined *Auxis rochei*, Anshary et al. (2014) stated possible migration into the musculature, and the present study documented a single specimen from the musculature of *Selar crumenophthalmus*. In contrast, the related *A. simplex* (s.s.) that is known as a main cause of zoonotic anisakiasis often infects fish musculature in temperate waters (e.g. Strømnes & Andersen 1998, 2003). Palm et et al. (2008) suggested that besides the life cycle and dispersal mechanism, a typical site of infection might be another distinguishing feature among the different *Anisakis* taxa. This can explain why *A. typica* (s.l.) so far has not been affiliated with human anisakiasis, and no case of anisakiasis from Bali has been recorded (also see Palm et al. 2008). However, this contradicts Uga et al. (1996), who found *Anisakis* antibodies in a number of Javanese people. The only explanation is that though the most common *A. typica* (s.l.) are regularly taken up and become digested by consumers, they seem to cause no major infection with serious disease symptoms. This suggests a general low risk of Indonesian fisheries consumers to develop anisakiasis. While the most dangerous *A. simplex* (s.s.) could not be recorded from Indonesia, we isolated only a single *A. pegreffii* from an *Auxis rochei*, which is nowadays known to cause anisakiasis (3 cases worldwide). However, it is not clear which *Anisakis* species caused the immunological reaction identified by Uga et al. (1996).

Outlook

There are 3 main outcomes for future studies. First, we recommend sampling adults of *A. typica* var. *indonesiensis* for a morphological species description in future. This can be done either by dissecting stranded marine mammals or via an underwater sampling while diving with dolphins, wild or trained for animal health monitoring purposes. Kleinertz et al. (2014b) demonstrated the functionality of the lat-

ter. Second, until a new species description and taxonomical treatment has been completed, we strongly recommend the name *A. typica* var. *indonesiensis* when dealing with this Indonesian taxon or its genotype, because this genotype has been documented as the predominant one in Indonesia. It also cannot be excluded that it will be reported from other tropical regions outside Indonesia in future. Third, taking into account various *Anisakis* species in a broad range of fish host species, the transfer of northern and southern species via migratory hosts into the region, and positive tests for *Anisakis* antibodies of Indonesian citizens as well as the documentation of the human-pathogenic species *A. pegreffii*, we suggest seroepidemiological or stereoscopical tests of Indonesian citizens. Although presumably at a low risk, additional insights into the real anisakiasis impact in Indonesia, the 4th most populous nation of the world with almost every citizen reliant on ocean and fisheries supplies, but also with a low standard in the medical system, are still required. Consumers should thus be informed that the scombrid *Auxis rochei* bears the highest risk of anisakiasis infection in Indonesia, and should not be used in dishes using raw or semi-cooked fish.

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Anisakis (Nematoda: Ascaridoidea) from Indonesia

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