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SHORT COMMUNICATION

Histopathological changes from parasitic Nematoda infestation in the musculature of some marine teleost fishes from the Algerian coast

Souhila Ramdani, Jean-Paul Trilles, Zouhir Ramdane

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Abstract. Numerous marine fishes from the Algerian coast were examined for nematode parasites. Our study revealed five species of nematodes that were identified according to morphological characteristics: *Anisakis simplex*, *Hysterothylacium aduncum*, *Hysterothylacium reliquens*, *Hysterothylacium fabri*, *Dichelyne pleuronectidis*. *D. pleuronectidis* was newly collected from the study region. Four new host species were recorded for *A. simplex*, six for *H. aduncum*, and three for *H. fabri*. The infection rates observed were higher for *Hysterothylacium* and *Dichelyne* than *Anisakis*. The highest infection rate by Nematoda parasites were recorded for *Trachinus draco* L., *Pagellus acarne* (Risso) and *Mullus barbatus* L. (P = 100%, P = 53.33%, P = 42.5%), respectively. Nematoda larvae were found in body cavity and musculature. Histological changes associated with nematode musculature infestation revealed myodegeneration of fibers with a loss of striated texture and skin damage.

Keywords: teleost fish, nematodes, muscle tissue, histology, Algeria

S. Ramdani, [✉], Z. Ramdane
Laboratoire de la Zoologie Appliquée et d'Ecophysiologie Animale, Faculté Sciences de la Nature et de la Vie, Université Abderrahmane Mira-Bejaia, Algérie.
E-mail: souhila.ramdani@univ-bejaia.dz, sousane.green@gmail.com.

J.-P. Trilles
Université de Montpellier, 34000 Montpellier, France.

Introduction

Fish and fishery products are the most widely traded food commodities worldwide (FAO 2018). Fish provide not only high-value protein, but are also important sources of a wide range of essential micronutrients, minerals, and fatty acids (Wake and Geleto 2019). In studies of fish parasites, nematodes are well-known in marine fishes and squids, and their larvae are found to parasitize various organs and tissues of numerous fishes (Buchmann and Mehrdana 2016). In Algerian waters, nematode larvae have been reported previously in the abdominal cavity, the gonads, the liver, and the intestines (Ichalal et al. 2015, Saadi et al. 2019). The current study describes the first Algerian record of nematode larvae in the musculature of marine fish, which indicates the probable impact of these parasites occurring in the musculature of fishes.

Material and Methods

Ten species of teleost fish were collected from the Gulf of Bejaia off the eastern coast of Algeria and examined for parasites. See Table 1 for the numbers of fishes examined. Fishes caught by local fishers were transported to the laboratory immediately after landing. All parts of the body of each fish were carefully examined for attached parasites with the naked eye and a binocular magnifying glass. Lesions and external changes were noted. Once the parasites had been collected, they were cleaned and immediately preserved in ethanol 70% for subsequent identification. The parasites were identified based on their morphoanatomical characteristics using identification keys.

The skin and muscle around the area of parasite attachment were examined directly and were preserved in 10% formalin for histological studies of possible pathological effects. Samples from infected and uninfected sites were dehydrated in a graded series of alcohols (70 to 95%), embedded in paraffin, cut into 1 μm-thick serial sections, and stained with Mayer's hematoxylin and eosin (H&E). The sections were examined using light microscopy and photographed with a LEICA DM300 microscopy camera. Prevalence, mean intensity, and abundance were calculated according to Bush et al. (1997).

Results

Macroscopic and Microscopic examination

Nematoda larvae were observed easily in the body cavity immediately after the ventral dissection of the fishes. Additionally, some fishes presented larvae embedded in the muscle tissue (Figs. 1). Our light microscopic examination of the Nematoda larvae collected revealed five species: *Anisakis simplex* (Rudolphi), *Hysterothylacium aduncum* (Rudolphi), *Hysterothylacium reliquens* (Norris and Overstreet), *Hyterothylacium fabri* (Rudolphi), *Dichelyne pleuronectidis* (Yamaguti), which were identified according to morphological characteristics.

H. aduncum parasitized the most infected fishes (six species), while *A. simplex* and *H. fabri* infected five host species. *D. pleuronectidis* and *H. reliquens* infected two and one host species, respectively (Table 2). One parasitic nematode species, *D. pleuronectidis*, was newly collected from the study region (Table 3). Three new host species (*Alosa alosa* (L.), *Engraulis encrasicolus* (L.), *P. acarne*) were recorded for the worm *A. simplex*. Six new host species (*Sardinella aurita* Val., *P. acarne*, *M. barbatus*, *Mullus surmuletus* L., *T. draco*, *Xiphias gladius* L.) were recorded for the parasite Nematoda *H. aduncum*, and three new host fish species (*M. barbatus*, *Boops boops* L., *Trachurus trachurus* (L.)) for the parasite *H. fabri* (Table 3). The parasitic nematodes species collected were previously reported by several researchers from various host fish species and different localities from the North African Coast (Table 3); however, four of them were reported from Algeria (Table 3).

Infection rates

Among the host fish species examined, the highest infection rate by Nematoda parasites were recorded for *T. draco* (P = 100%), *P. acarne* (P = 53.33%), and *M. barbatus* (P = 42.5%), whereas, the prevalence of *E. encrasicolus*, *S. aurita*, and *B. boops* was low ranging from 1.5% to 3.81% (Table 1). According to our results, *T. draco*, *T. trahurus*, and *A. alosa* had the highest mean intensity (Mi = 8.63, Mi = 5.38, and Mi = 4.5 parasites per infected host, respectively) (Table 1). The lowest mean abundance was recorded in *E. encrasicolus* (Ma = 0.03 parasites per host examined) (Table 1). Our results showed the highest infection rates in the most frequently consumed pelagic host species, especially *T. draco* (P = 100%) (Table 1). *Hysterothylacium* (Raphidascarididae) and *Dichelyne* (Cucullanidae) exhibited higher infections rates than did *Anisakis* (Anisakidae). Th infection rates are as follows: *H. aduncum*, *H. fabri*, and *D. pleuronectidis* in *T. draco* and *H. reliquens* in *M. barbatus*. However, the lowest infection rates were recorded in *S. aurita* and *B. boops* (Table 2). The infestation rates of the *A. simplex* larvae collected were higher in *P. acarne*, *A. alosa*, and *T. trachurus* (P = 53.33%; Mi = 4.50 parasite per infested fish and Ma = 0.78 parasite per fish examined, respectively). The lowest infection rate was observed for the host species *E. encrasicolus* (P = 1.50%; and Ma = 0.03 parasite per fish examined) (Table 2).

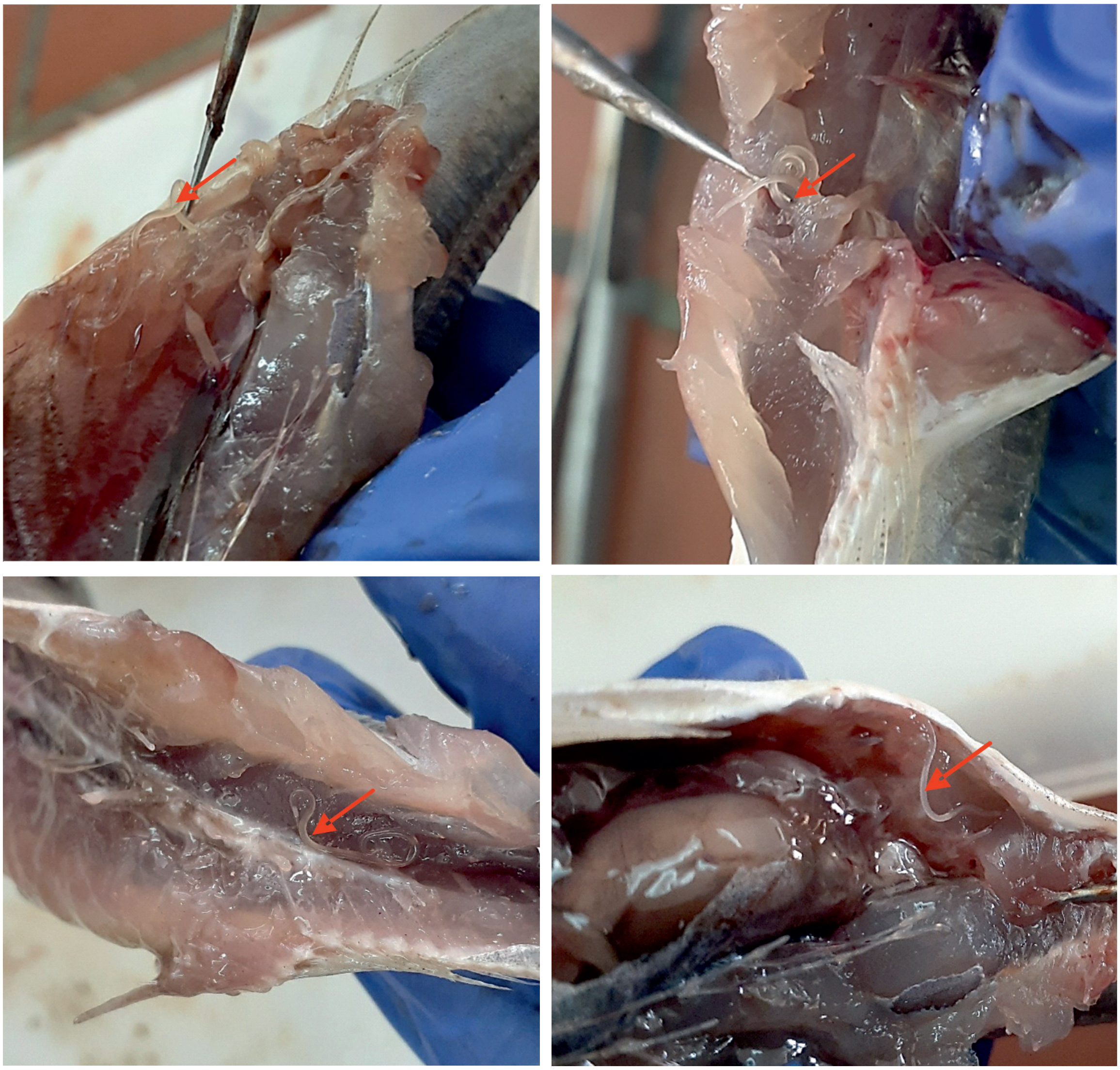

Figure 1. Nematoda parasites embedded in fish musculature (*Trachurus trachurus*). Scale bar: 1 cm.

Table 1

Parasitological indexes of the Nematodes collected per host fish species. NFE – number of fish examined; NIF – number of fish infected; NP – number of parasites collected; P (%) – prevalence (%); Mi – mean intensity; Ma – mean abundance

Host species	NEF	NIF	NP	P (%)	Mi	Ma
Sardinella aurita	525	12	23	2.29	1.92	0.04
Alosa alosa	50	6	27	12.00	4.50	0.54
Engraulis encrasicolus	200	3	6	1.50	2.00	0.03
Trachrus trahurus	50	13	70	26.00	5.38	1.40
Boops boops	105	4	16	3.81	4.00	0.15
Pagellus acarne	60	32	82	53.33	2.56	1.37
Mullus barbatus	120	51	143	42.50	2.80	1.19
Mullus surmuletus	20	5	18	25.00	3.60	0.90
Trachinus draco	30	30	259	100.00	8.63	8.63
Xiphias gladius	5	1	3	20.00	3.00	0.60

Table 2

Parasitological indexes of the Nematode species collected per host fish species. NFE – number of fish examined; NIF – number of fish infected; NP – number of parasites collected; P (%) – prevalence (%); Mi – mean intensity; Ma – mean abundance

Nematoda species	Host species	NEF	NIF	NP	P (%)	Mi	Ma
Anisakis simplex	*Alosa alosa*	50	6	27	12.00	4.50	0.54
	Engraulis encrasicolus	200	3	6	1.50	2.00	0.03
	Trachurus trahurus	50	13	39	26.00	3.00	0.78
	Boops boops	105	4	9	3.81	2.25	0.09
	Pagellus acarne	60	32	27	53.33	0.84	0.45
Hysterothylacium aduncum	*Sardinella aurita*	525	12	23	2.29	1.92	0.04
	Pagellus acarne	60	32	13	53.33	0.41	0.22
	Mullus barbatus	120	51	40	42.50	0.78	0.33
	Mullus surmuletus	20	5	18	25.00	3.60	0.90
	Trachinus draco	30	30	122	100.00	4.07	4.07
	Xiphias gladius	5	1	2	20.00	2.00	0.40
Hyterothylacium fabri	*Pagellus acarne*	30	32	31	106.67	0.97	1.03
	Mullus barbatus	120	51	59	42.50	1.16	0.49
	Trachinus draco	30	30	111	100.00	3.70	3.70
	Boops boops	105	4	5	3.81	1.25	0.05
	Trachurus trachurus	50	13	41	26.00	3.15	0.82
Hysterothylacium reliquens	*Mullus barabatus*	120	51	44	42.50	0.86	0.37
Dichelyne pleuronectidis	*Pagellus acarne*	60	32	29	53.33	0.91	0.48
	Trachinus draco	30	30	26	100.00	0.87	0.87

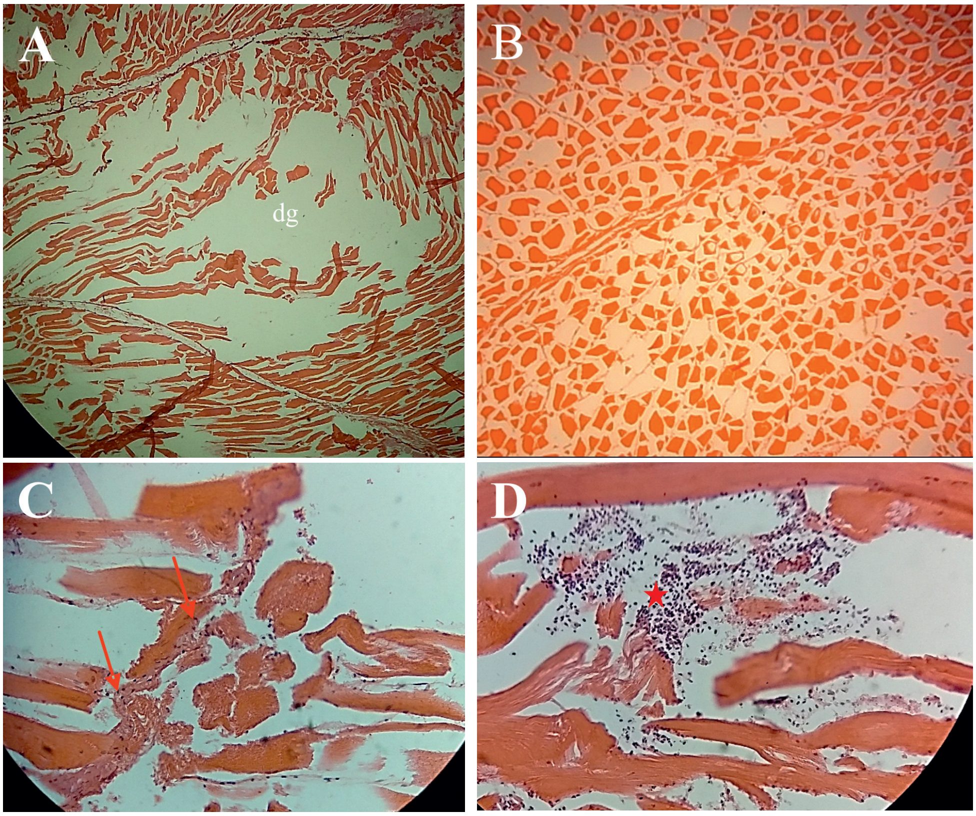

Figure 2. Histological section of Nematoda parasites in the musculature of teleost fishes. A – damaged skeletal muscle, degenerated fibers (dg). B – normal skeletal muscle; C – inflammatory cell infiltrate (see red arrows); D – severe myositis (see red asterisk). Scale bar: A, B, C, D: x10 = 100 μm.

Histological analysis

The histological examination of infected muscle sections revealed myodegeneration of muscle fibers with a loss of striated texture, and the skin components were damaged (Fig. 2a). Infection with Nematoda parasites and inflammation processes often cause myositis (Fig. 2c, d), which was observed clearly in the histological changes associated with nematode infestation of the muscle tissues that were seriously damaged (Fig. 2a, c, d). In the uninfected muscle tissue of the host fishes, the skin components were intact, and there were no defects in muscle fiber cross-sections (Fig. 2b).

Discussion

Five nematode species were identified based on their morphoanatomical characteristics: *A. simplex*, *H. aduncum*, *H. reliquens*, *H. fabri*, *D. pleuronectidis*. The parasitic nematodes *A. simplex*, *H. aduncu*m, *H. reliquens*, and *H. fabri* were collected previously from the region we studied (Petter and Maillard 1988, Marzoug et al. 2012, Hassani and Kerfouf 2014, Hassani 2015, Hassani et al. 2015, Ichalal et al. 2015, Benhamou et al. 2017, Ider et al. 2018, Saadi et al. 2020). We collected one parasitic nematode species, *D. pleuronectidis*, from the study region for the first time, and its characteristics were consistent with the microscopic characteristics Li et al. (2013) reported in the same species collected from the flatfish *Pleuronichthys cornutus* (Temminck & Schlegel) in the East China Sea.

We noted three new host species of *A. simplex* (*A. alosa, E. encrasicolus, P. acarne*), and six new host species (*S. aurita, P. acarne*, *M. s barbatus*, *M. surmuletus, T. draco, X. gladius*) were recorded for the Nematoda parasite *H. aduncum*. Three new host fish species of *H. fabri* were also identified (*M. barbatus*, *B. boops* and *T. trachurus*). Previously in our study region, *A. simplex* was known to infect nine teleost fish species, the nematode *H. aduncum* had been collected from six host fish species, and the parasite *H. fabri* had been noted in seven fish species (Table 3). The host species *T. draco, P. acarne*, and *M. barbatus* were most infected with parasitic nematode larvae. Arthropoda, Mollusca, Nemathelminth, Teleostei species are potential prey of these fish species (Morte et al. 1999, Fehri-Bedoui et al. 2009, Chérif et al. 2011).

In the present study, Nematoda parasites were observed in the body cavity and musculature of various specimens of teleost fishes caught in the Mediterranean Sea (Algerian coast). To our knowledge, this is the first report of nematodes in the musculature and of histopathological changes in muscles from these parasites in our study region. A recent study described the presence of nematodes in the musculature of various fish species. In Denmark, Buchmann and Mehrdana (2016), found the anisakid nematodes *Anisakis simplex* and *Pseudoterranova decipiens* in fish musculature. In Italian waters, Dezfuli et al. (2015) described the infection of *Perca fluviatilis* L. musculature with *Eustrongylides* sp. Silva et al. (2020), Madrid et al. (2016), and Torres et al. (2014) all reported the presence of *Anisakis* spp. and *Pseudoterranova* spp. in the musculature of *Merluccius gayi* (Guichenot), *Merluccius* spp., and *Merluccius australis* (Hutton) in Chilean waters. The presence of nematode eggs of the genus *Huffmanela* were found in the musculature of *Trisopterus luscus* (L.) and in that of *Microchirus azevia* (de Brito Capello) from the Portuguese coast (Esteves et al. 2009, 2016).

It is well known that one of the reactions of hosts to parasites is to form connective tissue capsules to sequester parasites (Dezfuli et al. 2007). In the current study, the histological changes observed with nematode infestations were severe inflammatory reactions with myodegeneration and tissue damage, including marked cellular infiltration. Buchmann and Mehrdana (2016) reported that the larval nematode *P. decipiens* was encapsulated by host cells in *Gadus morhua* L. muscle tissue, and they explained that, as hosts, cod activates a strong cellular reaction when *P. decipiens* larvae penetrate the musculature, and the reaction immobilizes the larva in an encapsulated stage that protects the live worm but also disturbs fillet structure.

Table 3
Previous records of nematodes identified in the study region (Algerian coast). *New parasitic nematode species collected from the study region; **New host for *A. simplex*, *H. aduncum* and *H. fabri*

Parasites species	Host species	References
Anisakis simplex	*Merluccius merluccius*	Petter and Maillard 1988, Saadi et al. 2020
	Scorpaena scrofa	Petter and Maillard 1988
	Trachurus trachurus	Ichalal et al. 2015, Saadi et al. 2020, present study
	Boops boops	Ichalal et al. 2015, Ider et al. 2018, Saadi et al. 2020, present study
	Phycis blennoides	Hassani 2015
	Trachurus mediterraneus	Saadi et al. 2020
	Scomber japonicus	Petter and Maillard 1988
	Mullus surmuletus	Saadi et al. 2020
	Mullus barbatus	Saadi et al. 2020
	*Alosa alosa**	present study
	*Engraulis encrasicolus**	present study
	*Pagellus acarne**	present study
Hysterothylacium aduncum	*Phycis blennoides*	Hassani and Kerfouf 2014
	Phycis phycis	Hassani 2015
	Boops boops	Merzoug et al. 2012, Ichalal et al. 2015, Benhamou et al. 2017, Ider et al. 2018, Saadi et al. 2020
	Trachurus trachurus	Ichalal et al. 2015, Saadi et al. 2020
	Spicara maena	Benhamou et al. 2017
	Merluccius merluccius	Saadi et al. 2020
	*Sardinella aurita**	present study
	*Pagellus acarne**	present study
	*Mullus barbatus**	present study
	*Mullus surmuletus**	present study
	*Trachinus draco**	present study
	*Xiphias gladius**	present study
Hysterothylacium fabri	*Scorpaena notata*	Petter and Maillard 1988
	Scorpaena elongata	Petter and Maillard 1988
	Phycis blennoides	Hassani and Kerfouf 2014
	Phycis phycis	Hassani 2015
	Mullus surmuletus	Hassani et al. 2015, Saadi et al. 2020
	Trachurus trachurus	Saadi et al. 2020
	Pagellus acarne	Saadi et al. 2020, present study
	*Mullus barbatus**	present study
	*Boops boops**	present study
	*Trachurus trachurus**	present study
*Dichelyne pleuronectidis**	*Pagellus acarne*	present study
	Trachinus draco	present study

Several authors have documented the impact of nematode larvae on the physiological state, health, and survival of hosts. Ackman and Gjelstad (1975) demonstrated that excretions from *P. decipiens* contained several pentanols and pentanons, and they suggested that these compounds acted as local anesthetics in cod muscle during worm penetration and that effects on muscle contractility could be expected. Sprengel and Lüchtenberg (1991) and Rohlwing et al. (1998) observed reduced swimming performance in smelt and eel that could lead to increased mortality in the wild of cod infected with parasitic worm infections in their musculature. Decreased swimming speeds were observed in

Paralichthys lethostigma Jordan & Gilbert infested with the nematode *Philometroides paralichthydis* in the muscles of the dorsal and anal fins (Umberger et al. 2013). Fish hosts with impaired swimming abilities were easier prey to catch for marine mammals, including seals, which means this pathogenicity factor optimizes the life cycle of anisakids (Buchmann and Mehrdana 2016).

People's eating habits have changed, with increased consumption of raw marine fishes such as those used to prepare sushi or ceviche (Bao et al. 2017). Data demonstrate the importance of this nematode parasite in public health and the risk of infection from this seafood-borne disease worldwide, which affects many different fish species destined for human consumption such as *M. surmuletus* (Hassani et al. 2015), *Phycis blennoides* (Brünnich) (Hassani and Kerfouf 2014), *T. trachurus* (Ichalal et al. 2015), *P. acarne* (Hadjou et al. 2017), *Anguilla anguilla* (L.) (Tahri et al. 2017), *Pagellus erythrinus* (L.) (Saadi et al. 2019), and *S. aurita* (Ramdani et al. 2020). It is well known that the consumption of raw or undercooked fish increases the risk of zoonoses. Many people have been diagnosed with anisakidosis, some cases of which were caused by ceviche consumption (Madrid et al. 2016). The presence of nematode larvae in the musculature of the fishes examined from Algerian waters could have public health implications in Algeria. It is important to ensure the prompt evisceration of fishes.

Author contributions. S.R. wrote the main manuscript text, prepared all figures, sample collection; J.P.T. contributed to the writing and correction of the manuscript text; Z.D. contributed to the writing and correction of the manuscript text. All authors read and approved the manuscript.

ORCID iD
Souhila Ramdani https://orcid.org/0000-0003-4436-2620

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