

## Research paper

# Resurrection of genus *Phocanema* Myers, 1959, as a genus independent from *Pseudoterranova* Mozgovoï, 1953, for nematode species (Anisakidae) parasitic in pinnipeds and cetaceans, respectively

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## ABSTRACT

Species of the genus *Pseudoterranova*, infect kogiid cetaceans and pinnipeds. However, there is mounting molecular evidence that those from cetaceans and pinnipeds are not congeneric. Here, we provide further evidence of the non-monophyly of members of *Pseudoterranova* from phylogenetic analyses of the conserved nuclear LSU rDNA gene, entire ITS rDNA region and mtDNA *cox2* gene, and identify morphological characters that may be used to distinguish the members of the two clades. We propose the resurrection of the genus *Phocanema*, with *Ph. decipiens* (*sensu stricto*) as the type species, to encompass *Ph. decipiens*, *Ph. azarasi*, *Ph. bulbosa*, *Ph. cattani* and *Ph. krabbei*, all parasites of pinnipeds. We propose to restrict the conception of genus *Pseudoterranova*, which now harbours two species infecting kogiid whales; *Ps. kogiae* (type species) and *Ps. ceticola*. Members of the genera *Phocanema* and *Pseudoterranova* differ by the shape and orientation of the lips, relative tail lengths, adult size, type of final host (pinniped vs. cetacean) and phylogenetic placement based on nuclear rDNA and mtDNA *cox2* sequences.

## 1. Introduction

Some members of the parasitic nematode family Anisakidae are of great public health and socioeconomic importance worldwide [1]. They are responsible of zoonotic disease and can cause economic losses to seafood businesses [1]. They have complex life cycles in the marine environment, where fishes and squids act as second intermediate or paratenic hosts [2]. Humans may become accidental hosts through consumption of improperly cooked seafood containing viable third larval stage (L3) [3]. In this respect, the most important species belong to the genera *Anisakis* Dujardin, 1845, parasites of cetaceans, and *Pseudoterranova* Mozgovoï, 1953, with cetaceans and pinnipeds as definitive hosts [2].

The taxonomy of several anisakid taxa remains to be resolved. The phylogenetic relationships and morphology of *Anisakis* and *Pseudoterranova* species were recently investigated [4–6]. It was proposed to assign *Anisakis brevispiculata* Dollfus, 1968, *A. paggiae* Mattiucci et al., 2005, and *A. physeteris* Baylis, 1923 to genus *Skrjabinisakis* Mozgovoï, 1953 [4,5], and to reassign *Anisakis typica* (Diesing, 1860) to genus *Peritrachelius* Diesing, 1851 [5]. Morphological, genetic and ecological

differences among the *Pseudoterranova* species suggest that this genus also needs revision [4,6].

Adult and larval *Pseudoterranova ceticola* (Deardorff & Overstreet, 1981) from kogiid whales and fishes from the Atlantic, Indian and Pacific Oceans have been morphologically and genetically characterized ([6] and references therein), the main molecular markers being mitochondrial DNA (mtDNA) cytochrome *c* oxidase 2 (*cox2*) and ribosomal DNA (rDNA) internal transcribed spacer (ITS). Herein, we aimed at examining the phylogenetic relationships within the anisakid genera *Anisakis*, *Skrjabinisakis* and *Pseudoterranova* based on partial large subunit (LSU) rDNA, ITS and *cox2* sequences. In addition, we reviewed and compared the morphology between the cetacean vs. pinniped infecting *Pseudoterranova* members to ascertain whether there are any morphological differences which may be used for splitting the genus.

## 2. Materials and methods

## 2.1. Sample collection

The partial LSU rDNA gene of larval *P. ceticola* (N = 17) from meso-

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bathypelagic fish species from off Macaronesia (NW African waters) was sequenced (Table 1, see also [6] for further details). In addition, L3 specimens of *Anisakis ziphidarum* Paggi et al., 1988 (N = 4), *Skrjabinisakis paggiae* (N = 1), a new genotype of a probably novel undescribed species related to *S. paggiae*, i. e. *Skrjabinisakis cf. paggiae* (N = 3), *Pseudoterranova bulbosa* (Cobb, 1989) (N = 3) and *P. krabbei* Paggi et al., 2000 (N = 2), identified based on molecular analysis of the *cox2* and/or ITS gene (unpublished results), were also sequenced (Table 1). The entire ITS and partial *cox2* sequences from 1 L3 of *S. cf. paggiae* were also obtained.

## 2.2. Molecular analyses

DNA was extracted using the DNeasy® Blood & Tissue Kit (QIAGEN® GmbH, Hilden, Germany) according to the manufacturer's instructions with the modification that sample lysis was enhanced by mechanical disruption using a ceramic bead-beating system (Precellys ceramic kit 2.8 MM, VWR® and Precellys® 24 Tissue Homogenizer, Bertin Technologies).

Partial LSU rDNA sequences of the 30 anisakid specimens were amplified using the primers 28SF (5'-AGCGGAGGAAAAGAACTAA-3') and 28SR (5'-ATCCGTGTTTCAAGACGGG-3') [7], following procedures of Li et al. [8]. The entire ITS and partial *cox2* sequences of 1 *S. cf. paggiae* L3 were amplified using the primers NC5F (5' - GTAGGT-GAACCTGCGGAAGGATCATT- 3') and NC2R (5' TTAGTTTCTTTTCTCCCGCT -3') [9], and 211F (5'-TTTCTAGTTATA-TAGATTGRTTTYAT-3') and 210R (5'-CACCAACTCTTAAATTATC-3') [10], respectively, following procedures of Bao et al. [6]. PCR products were sent for purification and sequencing (using the primer 28SF) to Eurofins (Cologne, Germany). The National Center for Biotechnology Information (NCBI) sequence database (henceforth 'GenBank') was searched for similar sequences using BLAST (Basic Local Alignment

Search Tool) (USA). The new sequences obtained in the present study were submitted to GenBank with the accession numbers (28S: OR387329 - OR387358), (ITS: OR378796), (*cox2*: OR371766).

## 2.3. Phylogenetic analyses

LSU sequences were aligned with homologous sequences downloaded from the GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)) from additional *Anisakis*, *Skrjabinisakis* and *Pseudoterranova* species using CLUSTAL W in MEGA 11.0.10 (Table S1) [11]. *Pulchrascaris chiloscyllyi* (Johnston & Mawson, 1951) and *Neoterranova caballeri* (Baruś & Coy Otero, 1966) were used as offshoots of Anisakinae, and *Contracaecum osculatatum* (Rudolphi, 1802) Baylis, 1920 and *Contracaecum rudolphii* Hartwich, 1964 as outgroup taxa (Table S1), as also used in similar phylogenetic analyses studies (see Takano & Sata [4]). Due to indel-induced alignment issues with ITS [6,12,13], only the genetically close *Skrjabinisakis* and *Pseudoterranova* species were aligned together with the new genotype *S. cf. paggiae*, and no outgroup was included. For the *cox2* analysis, the sequence of *S. cf. paggiae* was aligned with deposited sequences previously used for the molecular phylogenetic analysis of *P. ceticola* [6], see also Table S1). The default setting parameters of ClustalW were used. Phylogenetic analyses were performed using the Bayesian inference (BI) method in BEAST v1.10.4. The optimum evolutionary model for the LSU, ITS and *cox2* dataset were estimated using the Bayesian information criterion (BIC) as implemented in MEGA 11.0.10. The BEAST input file was generated in BEAUti with the following characteristics: sites: entering the best substitution model available (i. e. HKY + G (for LSU, ITS datasets), HKY + G + I (for *cox2* dataset)) and otherwise default settings; clock type: strict clock; tree prior: Speciation: Yule process; MCMC: length of chain = 10<sup>7</sup>, echo state to screen every = 1000, log parameters every = 1000. Effective sample size of parameters (i. e. >200) was checked in Tracer v1.7.2. The

**Table 1**

Sampling details of parasite specimens and corresponding GenBank accession numbers for the 28S rRNA sequences generated in the present study.

Parasite species	Sequence isolates §	Host species	Location	28S acc. n.
<i>Pseudoterranova ceticola</i>	ChaDa53T	<i>Chauliodus danae</i>	N Canarias (29.767 N, 16.087 W)*	OR387336
<i>P. ceticola</i>	EuPele13T	<i>Eurypharynx pelecanooides</i>	NE Madeira (33.695 N, 13.232 W)*	OR387337
<i>P. ceticola</i>	DiMo53T	<i>Diaphus mollis</i>	N Canarias (29.767 N, 16.087 W)*	OR387341
<i>P. ceticola</i>	DiMo41T	<i>D. mollis</i>	N Canarias (29.767 N, 16.087 W)*	OR387342
<i>P. ceticola</i>	DiRa23T	<i>Diaphus rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387344
<i>P. ceticola</i>	DiRa37T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387330
<i>P. ceticola</i>	DiRa34-1 T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387331
<i>P. ceticola</i>	DiRa35-2 T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387333
<i>P. ceticola</i>	DiRa49T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387334
<i>P. ceticola</i>	DiRa38T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387335
<i>P. ceticola</i>	DiArg15-13 T	<i>Diretmus argenteus</i>	SW Canarias (26.899 N, 19.232 W)*	OR387329
<i>P. ceticola</i>	DiRa2A	<i>D. rafinesquii</i>	N Cape Verde (17.969 N, 23.956 W)*	OR387340
<i>P. ceticola</i>	DiRa36-3 T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387332
<i>P. ceticola</i>	DiRa26T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387343
<i>P. ceticola</i>	DiRa42-1 T	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387345
<i>P. ceticola</i>	DiMo1A	<i>D. mollis</i>	N Canarias (29.767 N, 16.087 W)*	OR387339
<i>P. ceticola</i>	DiMo20T	<i>D. mollis</i>	SW Canarias (26.899 N, 19.232 W)*	OR387338
<i>Anisakis ziphidarum</i>	DiRa20A	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387346
<i>A. ziphidarum</i>	DiRa28A	<i>D. rafinesquii</i>	SW Canarias (26.899 N, 19.232 W)*	OR387347
<i>A. ziphidarum</i>	EuPele-na-1A	<i>E. pelecanooides</i>	NE Madeira (33.695 N, 13.232 W)*	OR387348
<i>A. ziphidarum</i>	EuPele-na-2 A	<i>E. pelecanooides</i>	NE Madeira (33.695 N, 13.232 W)*	OR387349
<i>Phocanema bulbosa</i>	GMFIB31PL-1	<i>Gadus morhua</i>	Norway (Hjelmsøybanken)**	OR387350
<i>P. bulbosa</i>	GMFIB37PL-1	<i>G. morhua</i>	Norway (Hjelmsøybanken)**	OR387351
<i>P. bulbosa</i>	GMFIB77PL1	<i>G. morhua</i>	Norway (Hjelmsøybanken)**	OR387352
<i>Phocanema krabbei</i>	GMLOB31PP-1	<i>G. morhua</i>	Norway (off Vesterålen)**	OR387353
<i>P. krabbei</i>	GMLOB36PL-2	<i>G. morhua</i>	Norway (off Vesterålen)**	OR387354
<i>Skrjabinisakis paggiae</i>	DiArg2-5-A2-4	<i>D. argenteus</i>	SW Canarias (26.899 N, 19.232 W)*	OR387355
<i>Skrjabinisakis cf. paggiae</i>	DiArg21-8-A2-4	<i>D. argenteus</i>	N Canarias (29.767 N, 16.087 W)*	OR387358
<i>S. cf. paggiae</i>	DiArg21-7-A2-4	<i>D. argenteus</i>	N Canarias (29.767 N, 16.087 W)*	OR387356
<i>S. cf. paggiae</i>	DiArg21-10-A2-4 <sup>^</sup>	<i>D. argenteus</i>	N Canarias (29.767 N, 16.087 W)*	OR387357

\*Fishes from which parasites were extracted were caught in waters off NW Africa from Cape Verde to Northeast (NE) of Madeira during a research cruise on board the Norwegian vessel "RV Kronprins Haakon" during May 2019. \*\*Fishes from which parasites were extracted were caught in northern Norway by commercial fishing vessels in February 2021 (isolates GMLOB31PP-1, GMLOB36PL-2), March 2021 (isolates GMFIB31PL-1, GMFIB37PL-1), June 2021 (isolate GMFIB77PL1). § i. e. template names. ^ GenBank accession numbers for the ITS and *cox2* sequences of this third stage larva are OR378796 and OR371766, respectively.

created tree was drawn in TreeAnnotator v1.10.4 and the burnin as the number of states was specified at  $10^4$ . Figtree v1.4.4 was used to visualize the phylogenetic trees. Bayesian posterior probabilities  $\geq 95\%$  were indicative of strong significant nodal support [14,15]. In addition, the evolutionary genetic distance ( $p$ -distance) between sequences were calculated in MEGA 11.0.10 [11]. Phylogenetic tree reconstructions were also performed by Maximum Likelihood in MEGA 11.0.10 (bootstrap replications set at 1000). The optimum evolutionary model for the LSU (HKY + G + I), ITS (HKY + G) and *cox2* (GTR + G + I) datasets were estimated using the corrected Akaike Information Criterion (AICc) as implemented in MEGA 11.0.10.

### 3. Results and discussion

The LSU rDNA sequences of 17 *P. ceticola* L3 were 100% identical, except for ambiguous positions (i. e. double signals) seen in two sequences. Identical LSU sequences were also found within the species *A. ziphidarum* (N = 4), *S. cf. paggiae* (N = 3), *P. bulbosa* (N = 3) and *P. krabbei* (N = 2). The LSU rDNA sequences of these and of *S. paggiae* (N = 1) were obtained for the first time, as the ITS and *cox2* sequences of *S. cf. paggiae* (N = 1).

In the obtained LSU-based BI phylogenetic tree, two major clades can be observed (Fig. 1). Clade A has two subclades; in which *P. ceticola* is sister to the subclade formed by two highly supported sister groups containing *Pseudoterranova* spp. from pinnipeds and *Skrjabinisakis* spp. (i. e. *S. brevispiculata*, *S. paggiae*, *S. cf. paggiae*, *S. physeteris*) from physeteroid whales. Clade B, similarly, has two subclades, in which *A. typica* is sister to the subclade formed by two highly supported sister groups containing *A. ziphidarum* sister to the group formed by *A. simplex sensu lato* (i. e. *A. berlandi* Mattiucci et al., 2014, *A. pegreffii* Campana-Rouget & Biocca, 1955 and *A. simplex* (Rudolphi, 1809) (*sensu stricto*)). In the unrooted ITS BI tree, two major clades can be observed (Fig. 2). Clade C includes two strongly supported subclades, containing *P. ceticola* sister

to *Skrjabinisakis* spp. Clade D includes *Pseudoterranova* species from pinnipeds. In the *cox2* BI phylogenetic tree, two strongly supported major clades can be observed (Fig. 3). Clade E includes *Anisakis* and pinniped infecting *Pseudoterranova* taxa, but subclades and subgroups within show low nodal support. Clade F has two strongly supported subclades, including *P. ceticola* sister to *Skrjabinisakis* members. Maximum likelihood phylogenetic trees showed similar topology and were congruent in showing *P. ceticola* separated from the monophyletic group formed by *Pseudoterranova* spp. from pinnipeds (LSU, ITS and *cox2* resulting trees provided at supplementary materials, Fig. S1, S2 and S3, respectively).

During the last 30 years, molecular/genetic approaches have revolutionized our understanding of the taxonomy of anisakid nematodes, where traditional morphology-based methods were hampered by the existence of several cryptic and/or sibling species within this family (reviewed by [16,17]). Firstly, multilocus allozyme analyses revealed the existence and allowed identification of distinct morphologically similar species within genera *Anisakis* and *Pseudoterranova* [18–21]. Later, DNA-based methods, including the PCR-RFLP and direct sequencing of the nuclear ITS region of the rDNA as well as the mtDNA *cox2* were increasingly used for species identification and phylogenetic analysis [5,6,22–30]. In addition, the nuclear LSU rDNA has shown utility for inferring Anisakidae phylogeny [4,7,8,10,26,31].

In the present phylogenetic analyses, we confirmed the non-monophyly of *Pseudoterranova* [4,6]. *Pseudoterranova* contain 7 species; i.e. *P. kogiae* (Johnston & Mawson, 1939) and *P. ceticola* from kogiid whales, and *P. azarasi* (Yamaguti & Arima, 1942), *P. bulbosa*, *P. cattani* George-Nascimento & Urrutia, 2000, *P. decipiens* (Krabbe, 1878) (*sensu stricto*) and *P. krabbei* from pinnipeds. In addition, there is also *P. decipiens* sp. E., a pinniped parasite yet to be named and fully described. The cetacean-infecting *P. ceticola* is genetically distant to those *Pseudoterranova* spp. infecting pinnipeds, but close to *Skrjabinisakis* spp. from physeteroid whales on the basis of mitochondrial (*cox1*,

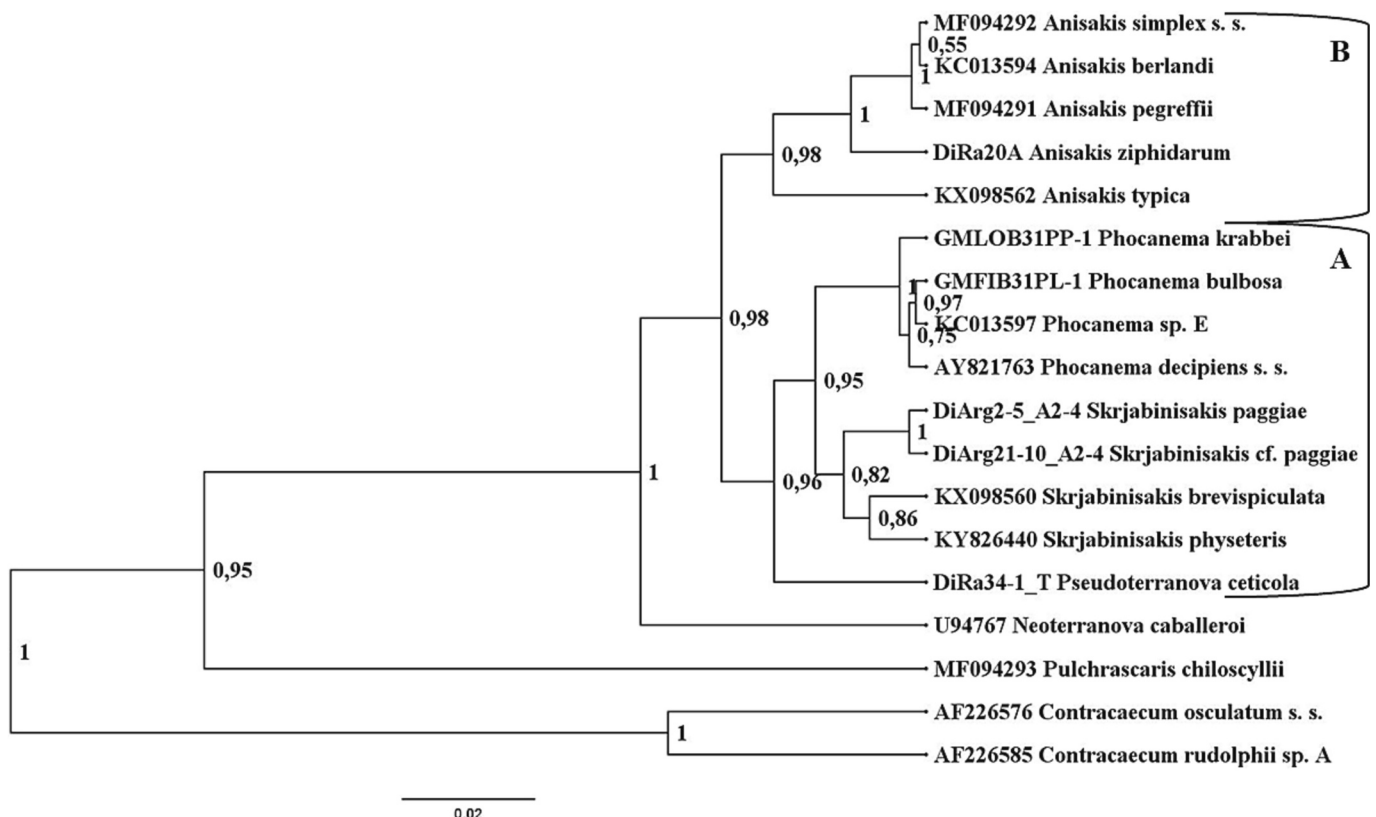


Fig. 1. Phylogenetic tree from Bayesian inference based on partial large subunit (LSU) rDNA sequences. A: clade A, B: clade B.

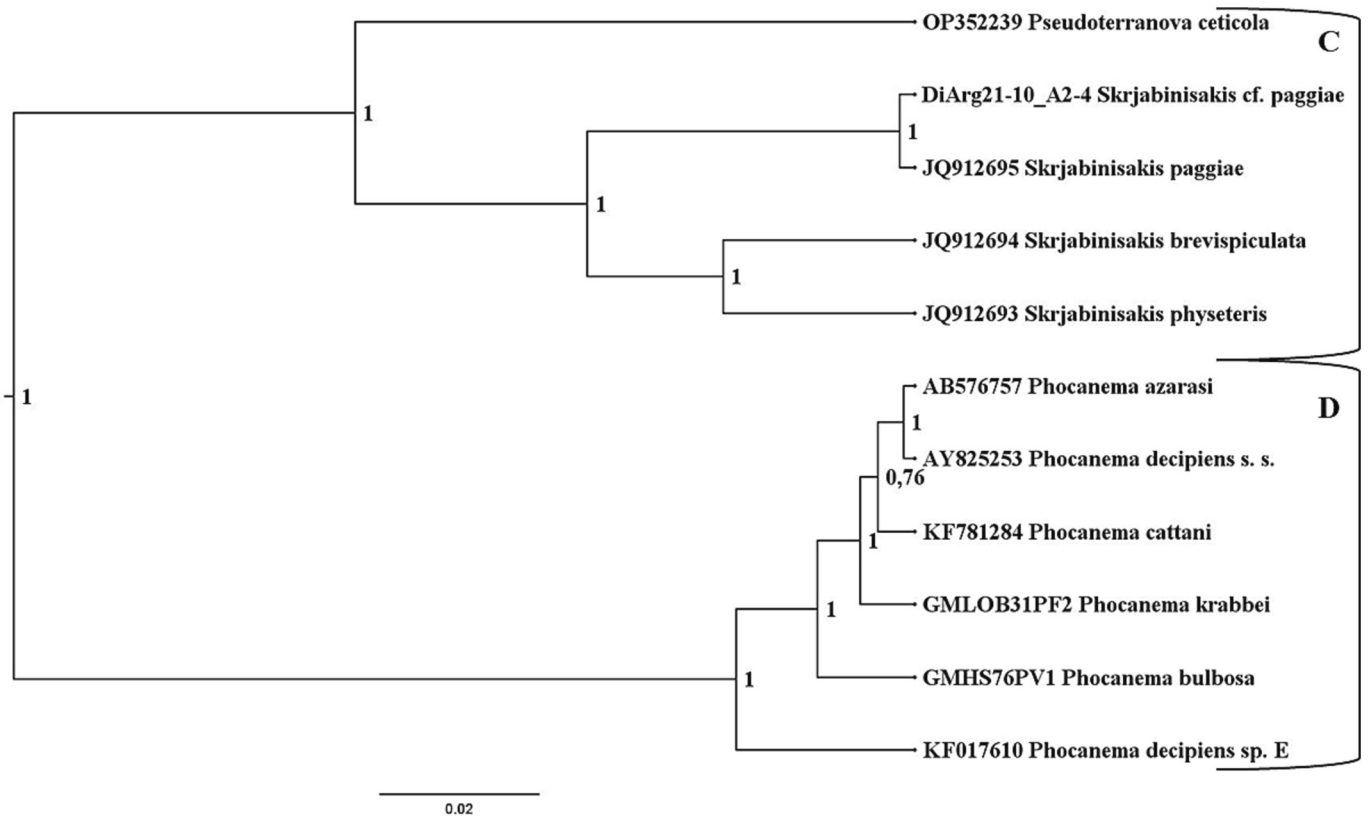


Fig. 2. Unrooted phylogenetic tree from Bayesian inference based on ITS sequences. C: clade C, D: clade D.

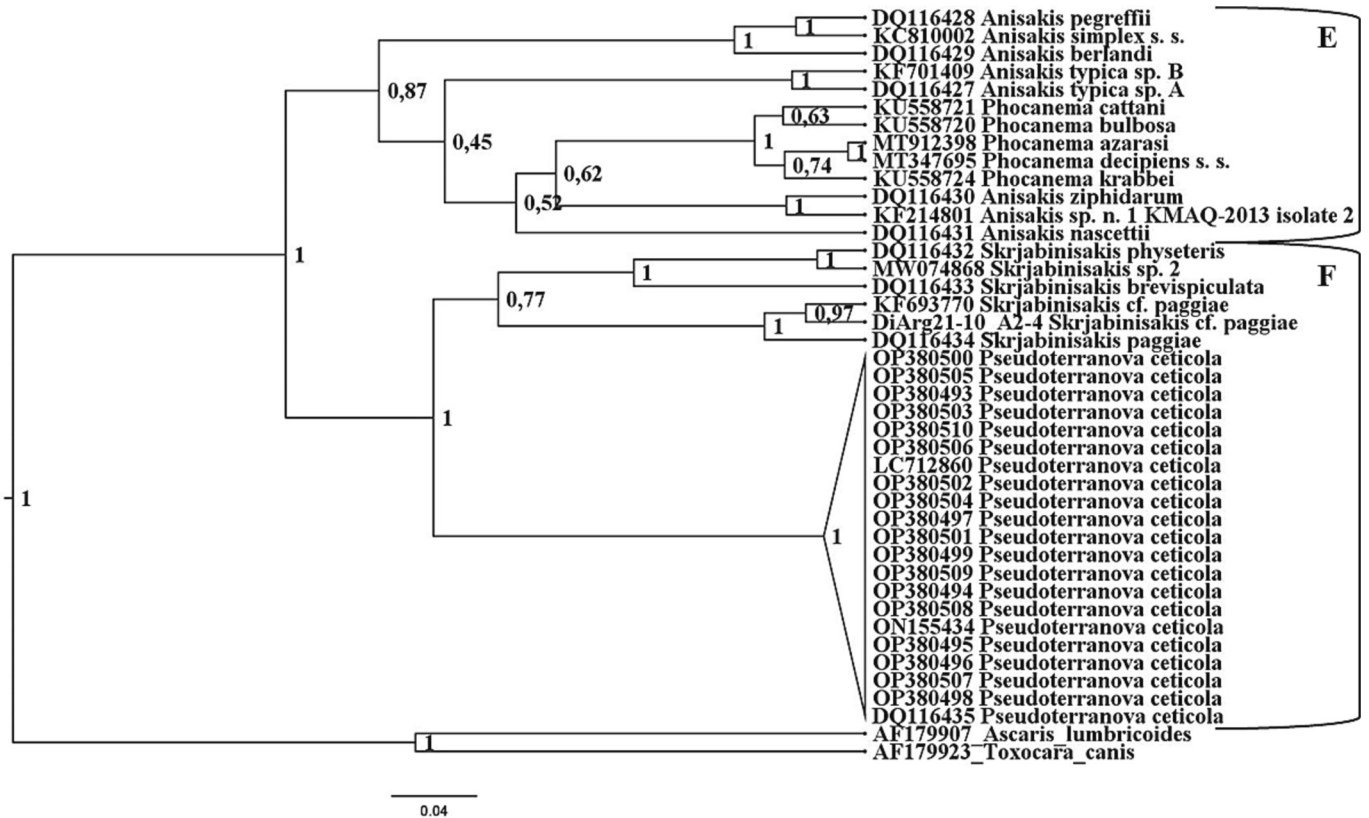


Fig. 3. Phylogenetic tree from Bayesian inference based on cox2 sequences. E: clade E, F: clade F.



cox2 and 12S) markers and ITS sequences as showed in here and previous studies [4,6,25]. This polyphyly of *Pseudoterranova* is here supported on the basis of the more conserved nuclear LSU rDNA gene. The highly conserved nuclear LSU rDNA seems therefore a useful genetic marker for resolving high taxonomic levels (e. g. intergeneric level) within Anisakidae, as previously suggested for molecular systematic studies of helminths [13]. However, the LSU results suggest that *Pseudoterranova* species maturing in pinnipeds are more closely related to *Skrjabinisakis* spp. from physeterids and kogiids than to the species *P. ceticola* from kogiids and larvae from fishes. *Pseudoterranova kogiae*, the type species of the genus *Pseudoterranova*, was described from the kogiid whale *Kogia breviceps* (de Blainville, 1838) [32]. This species has apparently not been recorded since the original description [33]. Genetic information on this parasite is therefore lacking, but it appears to be a species morphologically and ecologically similar to *P. ceticola*. Interestingly, an ITS sequence of an unidentified *Anisakis* sp. recovered from an Australian *K. breviceps* has recently become available [34], which is genetically close to *P. ceticola* [6]. Further research is required to determine if this genotype represents *P. ceticola*, an undescribed *Pseudoterranova* sp. or if it might in fact be *P. kogiae*.

The *p*-distances between the LSU sequences of *P. ceticola* and *Skrjabinisakis* spp., and pinniped infecting *Pseudoterranova* spp. vary in the range 0.018–0.031 and 0.027–0.031, respectively (Supplementary file 2). For ITS sequences, *p*-distances between *P. ceticola* and *Skrjabinisakis* spp., and *Pseudoterranova* spp. from pinnipeds vary in the range 0.091–0.106 and 0.126–0.138, respectively (Supplementary file 3). For *cox2* sequences, intraspecific genetic variation in *P. ceticola* vary between 0.009 and 0.033. Pairwise comparison between *P. ceticola* and *Skrjabinisakis* spp., and *Pseudoterranova* spp. from pinnipeds displayed 0.129–0.152, and 0.116–0.127 nucleotide variability, respectively (Supplementary file 4). The results are congruent in showing a high *p*-distance between *P. ceticola* and *Pseudoterranova* members from pinnipeds (with *Skrjabinisakis* spp. as reference for comparison), therefore providing further evidence that this high genetic divergence among them correspond to the intergeneric level. Interestingly, *p*-distance of mtDNA*cox2*, ITS and LSU between *S. paggiae* and the new genotype *S. cf. paggiae* were 0.051, 0.003 and 0.003, respectively. Interspecific genetic distance between sibling species of the *Anisakis simplex* complex range from 0.045 to 0.061 for mtDNA*cox2* [28], 0.003 to 0.007 for ITS [5], and 0.001 for LSU (see Supplementary file 2). Thus, *p*-distance values seem to correspond to an interspecific level, suggesting that *S. cf. paggiae* would represent an undescribed new sibling species.

Morphologically, adult male *P. ceticola* can be distinguished from adult male *P. kogiae* in the number of precloacal (38–50 vs. 65–70 pairs) and postcloacal papillae (5 vs 6 pairs) and in the absence vs presence, respectively, of three transverse rows of plectanes close to the posterior cloacal lip [32,33,35–37]. However, Abollo and Pascual [36] reported 6 pairs of postcloacal papillae in *P. ceticola* and highlighted the presence of well-developed bulbous anal lips and prominent distal papillae, and the absence of plectanes as important characters of adult *P. ceticola* from Galician *K. breviceps*. In Johnston and Mawson's [32] original description of *P. kogiae* such traits of the male tail (i.e. absence of plectanes) are stated to occur in *Anisakis kogiae* Johnston & Mawson, 1939. There appears to be a problem with the references to the images in Johnston and Mawson [32], i.e. *A. kogiae* and *P. kogiae* (as *Porrocaecum kogiae*) may have been confused. In the images, plectanes are indicated for *P. kogiae* but not for *A. kogiae*, where one might expect these structures to be found, as in other *Anisakis* species [38]. Hence, these two anisakines need to be redescribed based on the types, which are stated to be lodged in the South Australian Museum in Adelaide. If the absence of plectanes is confirmed in *P. kogiae*, then the only difference from *P. ceticola* could be in the number of precloacal papillae, and *P. ceticola* could become a junior synonym.

Adult or maturing *Pseudoterranova kogiae* and *P. ceticola* from kogiid whales are considerably smaller than *Pseudoterranova* spp. maturing in pinnipeds. The total body length of adult males of the former two species

given in the literature was 14.7–30 mm and 11.8–25.5 mm [32,33,35,37], respectively, whereas *P. decipiens* (s. s.) (42.5–54.0 (48.0) mm [39]), *P. krabbei* (31.5–43.0 (35.0) mm [39]), *P. bulbosa* ( $\approx$  50–70 mm [40]; mean  $\pm$  SD = 46.6  $\pm$  5.0 mm [41]), *P. azarasi* (45–80 mm [42]; mean  $\pm$  SD = 49.0  $\pm$  2.6 mm [43]) and *P. cattani* (26.4–61.7 mm [44]; mean  $\pm$  SD = 39.8  $\pm$  9.5 [45]) were reported as considerably larger (see also Table S2). The adult morphology of some of these species is insufficiently known. For instance, the oesophagus length, ventriculus length, distance from the nerve ring to the anterior extremity or distance from the cloaca to the posterior extremity (i.e. tail length) were only measured in a female specimen of *P. kogiae* [32], whilst comparison of morphological features among species are traditionally made on adult males [38,39].

Comparing *Pseudoterranova* spp. from kogiid whales with those from pinnipeds, the following characters, in addition to adult size, distinguish them: i) the relative tail length of *P. kogiae* and *P. ceticola* is clearly greater compared to those species from pinnipeds (i.e. tail length/total body length ratio range 0.012–0.02 vs. 0.002–0.007 (Table S3) and ii) shape, orientation and armament of the lips: two antero-laterally directed, rounded lobes at the tip of lips, projection with dentigerous border in the species from pinnipeds vs. internally-projecting bilobed part of each lip narrow with dentigerous border at the outer margin in the species from kogiids. Other distinguishing characters appears to be iii) the ratio of oesophagus length to total body length (greater in *P. kogiae* and *P. ceticola*), and iv) the ratio of ventriculus length to oesophagus length (smaller in *P. kogiae* and *P. ceticola*); v) the position of the excretory nucleus (posterior to the oesophago-intestinal junction in species from pinnipeds vs. at the level of the oesophago-intestinal junction in those from kogiids [46]), vi) the presence of male gubernaculum (present in *P. kogiae*, absent in *P. decipiens*), vii) the presence of small spined conical process at the male tail tip (present in *P. decipiens* (s. s.), *P. krabbei* and *P. azarasi*, absent in *P. ceticola* and *P. kogiae*), and, likely, viii) the presence of plectanes (absent in *P. ceticola*, present in those species from pinnipeds studied) (Table S3) [32,33,35,36,39,40,42–45,47–49].

The taxonomic history of members of *Pseudoterranova* was reviewed elsewhere [4,30,46,50,51]. *Pseudoterranova decipiens* (*sensu lato*) from pinnipeds was previously allocated to *Phocanema* Myers, 1959 [47,52], which presently is a synonym of *Pseudoterranova*. However, since the species from pinnipeds are morphologically, genetically, and ecologically different (see also [4,6]) from those parasitising cetaceans, we propose that *Phocanema* is resurrected to accommodate them.

*Pseudoterranova* Mozgovoi, 1953 amend.

Small nematodes, with adult males 11.8–30 mm long. The dorsal and the two ventro-lateral lips bearing anteriorly directed, narrow bi-lobed projection at the tip with relatively long teeth in dentigerous borders. Projection width is <50% of lip base width. Interlabia are absent. Excretory pore is located between subventral lips. Excretory nucleus is located at the level of the oesophago-intestinal junction. Ventriculus is present, ventricular appendix absent. Anteriorly projecting intestinal caecum is present. Tails are relatively long and lack ornamentation at the tip, ratio tail length: body length 0.012–0.02. Parasites inhabiting the digestive tract of kogiid cetaceans. Two species, *Pseudoterranova kogiae* (Johnston & Mawson, 1939) Mozgovoi, 1953 (type species) and *Pseudoterranova ceticola* (Deardorff & Overstreet, 1981) Gibson & Colin, 1982.

### 3.1. *Phocanema* Myers, 1959 amend

These are larger nematodes, with adult males 26.4–70 mm in length. One dorsal and two ventral lips bearing antero-laterally directed, rounded bi-lobed projection at the tip with small and numerous teeth in dentigerous borders. Projection width is >60% of base width in ventral lips. Interlabia are absent. Excretory pore is located between subventral lips. Excretory nucleus is located posterior to the oesophago-intestinal junction. Ventriculus is present, ventricular appendix absent.

Anteriorly projecting intestinal caecum is present. Tails are relatively short, ratio tail length: body length 0.002–0.007. Parasites inhabiting the digestive tract of pinnipeds. Five species: *Phocanema decipiens* (Krabbe, 1878) Myers, 1959 (type species), *Phocanema azarasi* (Yamaguti & Arima, 1942) comb. nov., *Phocanema bulbosa* (Cobb, 1889) comb. nov., *Phocanema cattani* (George-Nascimento & Urrutia, 2000) comb. nov. and *Phocanema krabbei* (Paggi et al., 2000) comb. nov.

A consequence of our resurrection of *Phocanema* is that zoonoses due to its members should be referred to as phocanemoses. The valid nominal *Pseudoterranova* species (i. e. *P. ceticola* and *P. kogiae*) have so far not been identified as causative agents of disease, albeit Deardorff et al. [53] demonstrated a zoonotic potential of such larvae.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parint.2023.102794>.

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