

Anisakis spp. as biomarkers of residency of short-finned pilot whales, *Globicephala macrorhynchus* in the Iberian Atlantic coast

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INTRODUCTION

- **Short-finned pilot whales**, *Globicephala macrorhynchus* (SFPWs), occur in **tropical and subtropical** waters worldwide (Fig. A).
- In the **Atlantic Ocean**, its **northern limit** has been established **roughly** at 32°N-40°N (Fig. A).
- **Sporadic records** at higher latitudes (i.e., 43°N-53°N) [1-12] (Fig. B), specially in the last decades, have raised the **question** as to whether SFPWs could be **just transient** or **indeed resident** in those **locations**.
- Several factors **difficult** the **in-field assessment** of the matter:
 - Despite modest differences (Figs. C and D), SFPWs are **morphologically similar** to its sister taxon, the **long-finned pilot whale**, *Globicephala melas* (LFPW) (Fig. C): in areas where the **distribution** of both species **overlaps**, reliable identification is by no means easy.
 - SFPWs and LFPWs appear to **hybridize regularly**.
 - **Photo-ID** of SFPWs have **never** been carried out at its **geographical limits**.
- **Parasites** have been largely used as **markers** to unveil **movement patterns** of cetaceans. Inferences are particularly robust when 'tag' parasites have:
 - **Restricted latitudinal distribution** → delimitation of host's stay areas.
 - **Known lifespan/population structure** → establishment of temporal limits of host's stays.

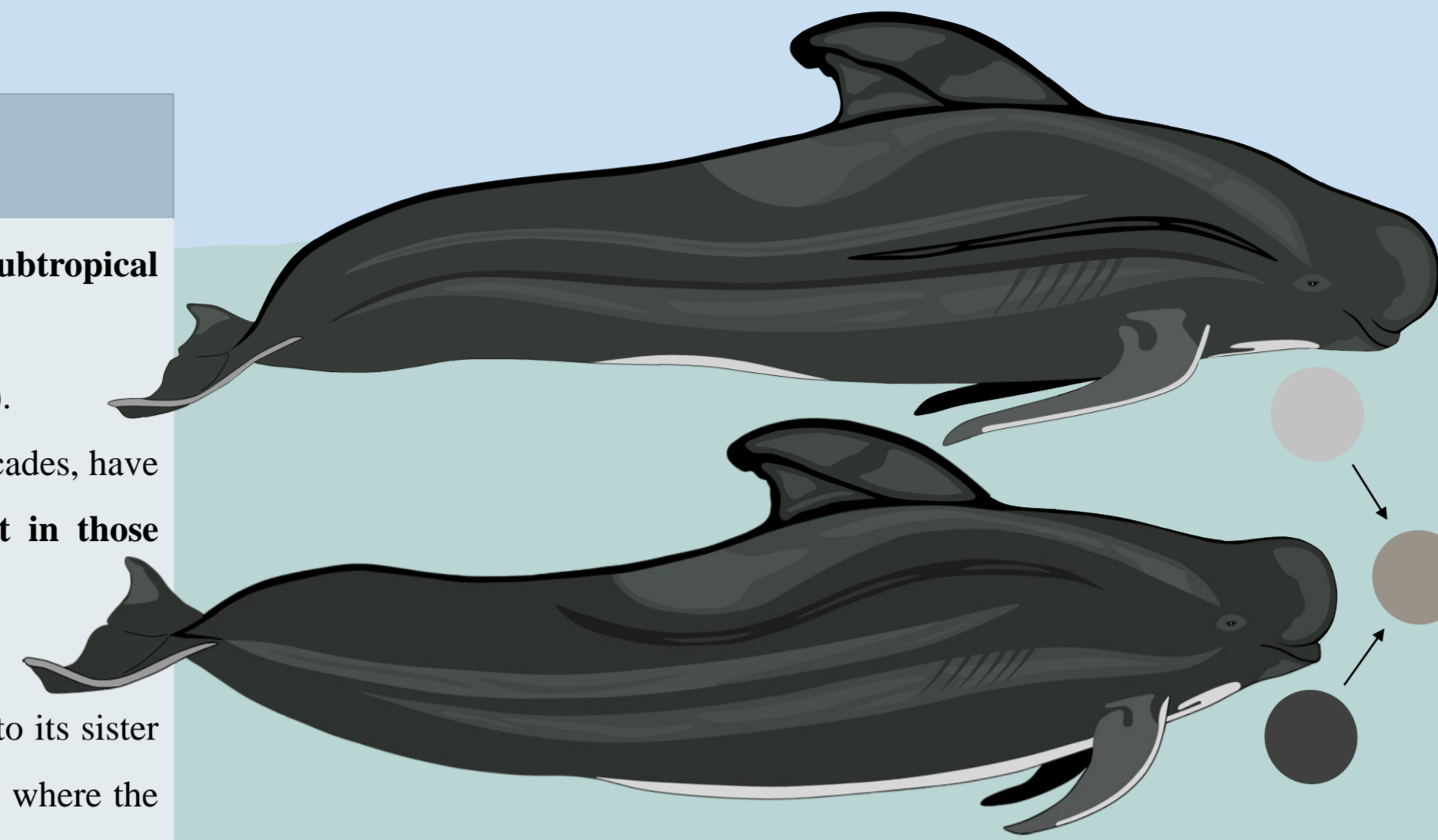


Figure C. Full-body view of long-finned pilot whale, *Globicephala melas* (top) and short-finned pilot whale, *G. macrorhynchus* (bottom). © C. Pons-Bordas.

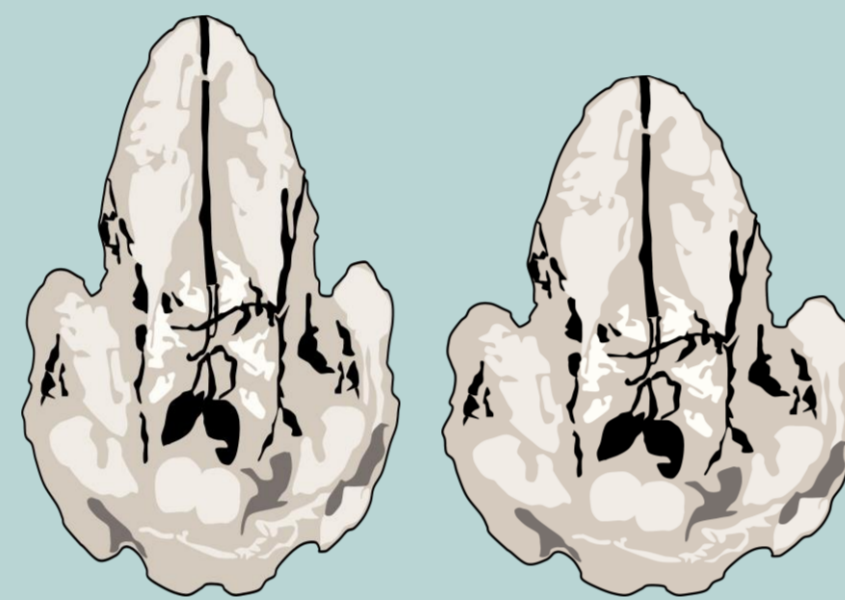


Figure D. Dorsal view of the skull of long-finned pilot whale, *Globicephala melas* (left) and short-finned pilot whale, *G. macrorhynchus* (right). © C. Pons-Bordas.

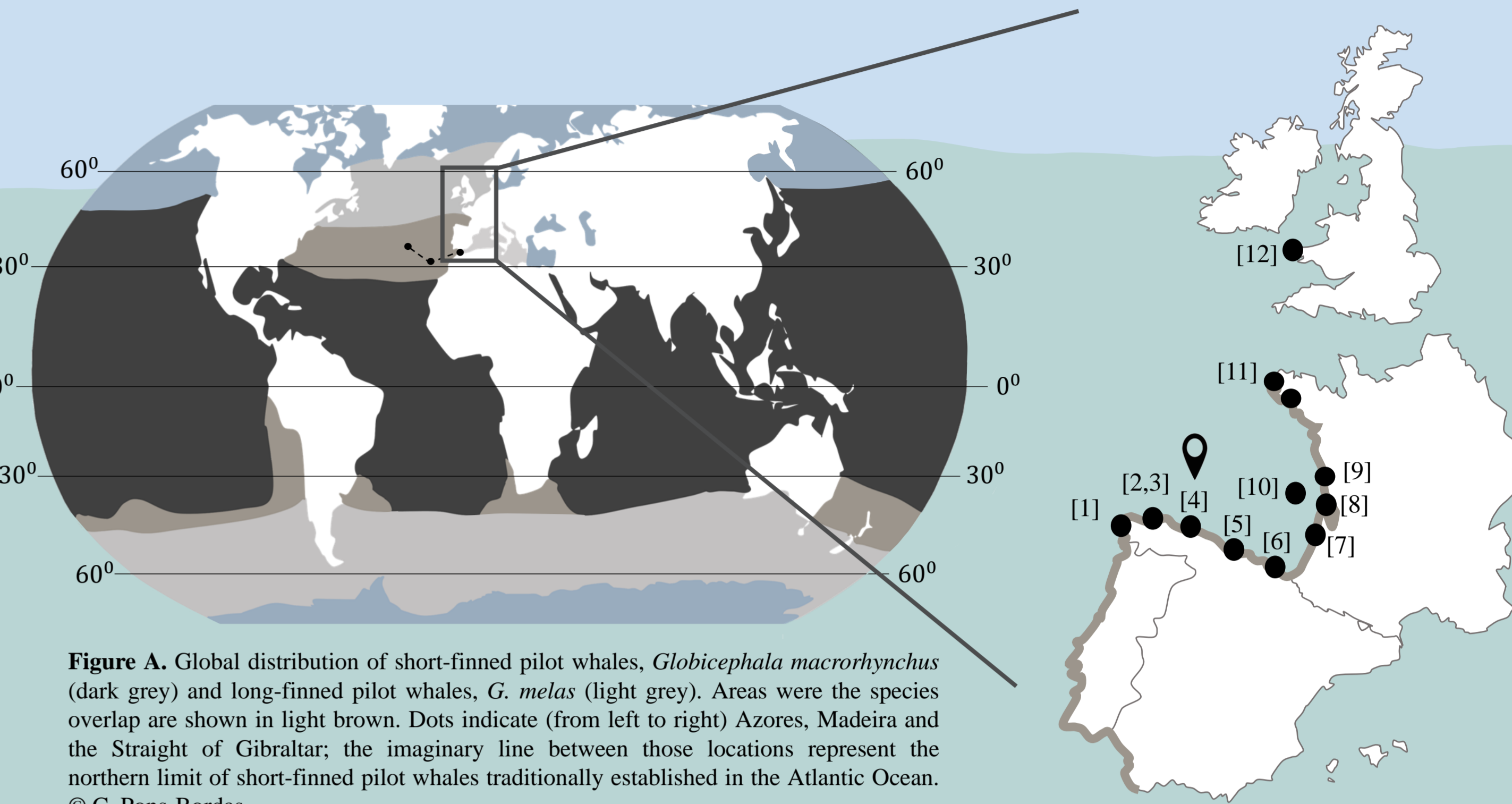


Figure A. Global distribution of short-finned pilot whales, *Globicephala macrorhynchus* (dark grey) and long-finned pilot whales, *G. melas* (light grey). Areas where the species overlap are shown in light brown. Dots indicate (from left to right) Azores, Madeira and the Strait of Gibraltar; the imaginary line between those locations represent the northern limit of short-finned pilot whales traditionally established in the Atlantic Ocean. © C. Pons-Bordas.

Figure B. Northernmost records of short-finned pilot whales, *Globicephala macrorhynchus*, in the Atlantic Ocean. [1] Monteiro *et al.*, 2017; [2] González *et al.*, 2000; [3] López *et al.*, 2002; [4] This study; [5] Nores and Pérez, 1988; [6] Sabatier *et al.*, 2014; [7] Van Canneyt *et al.*, 2012; [8] Culik, 2010; [9] Duguay, 1968; [10] Duguay and Aloncle, 1974; [11] Alfonsi *et al.*, 2013; [12] Kitchener *et al.*, 2022. © C. Pons-Bordas.

STUDY CASE AND CONTEXT

- On September 18th 2020, a **pod of SFPWs stranded** at **atypical northern latitudes** (43°28'27"N, 5°10'35"W) on the beach of Cala Moris, Asturias, **Spanish Atlantic coast** (Fig. B).
- Of 22 animals, 11 eventually died and 8 could be carried out to *ad hoc* facilities and **necropsied**.
- **Large numbers** of trophically-transmitted nematodes, provisionally identified as *Anisakis* spp., were found in the **stomach**.
- In the **North Atlantic**, three *Anisakis* species have been reported to reproduce in **delphinids**, following a clear **latitudinal gradient** (Fig. E):

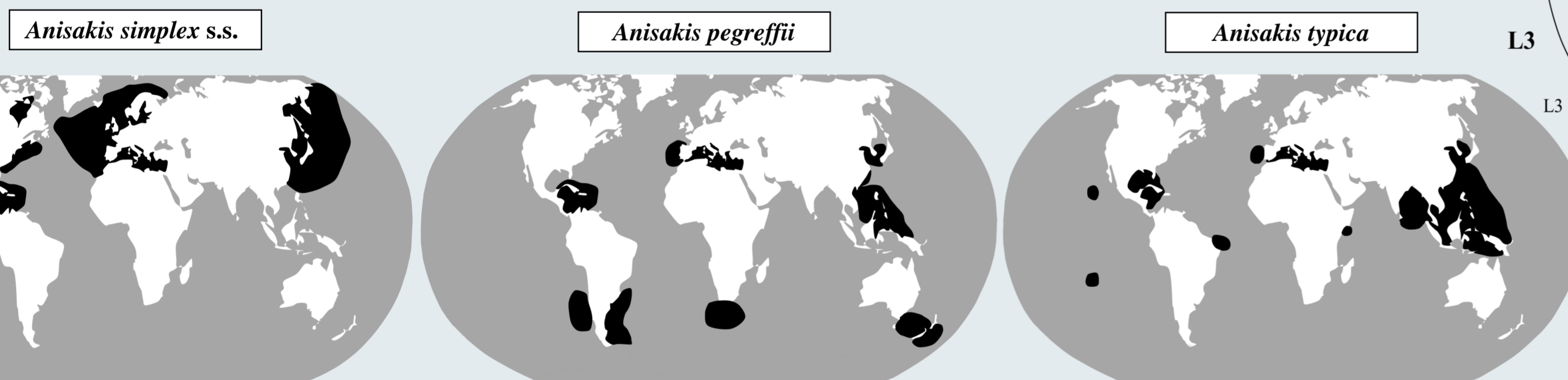


Figure E. Global distribution of *Anisakis simplex* s.s., *A. pegreffii* and *A. typica* in both the intermediate/paratenic and the final hosts [13 and ref. therein, 14-16]. © C. Pons-Bordas.

- **Lifespan** of *Anisakis* spp. has been **estimated** in most of its life stages (Fig. F).
- The **identity** and **population structure** of the *Anisakis* spp. infecting the stranded SFPWs could **shed light** on the **latitudes** that the **hosts** had visited **prior to death**.

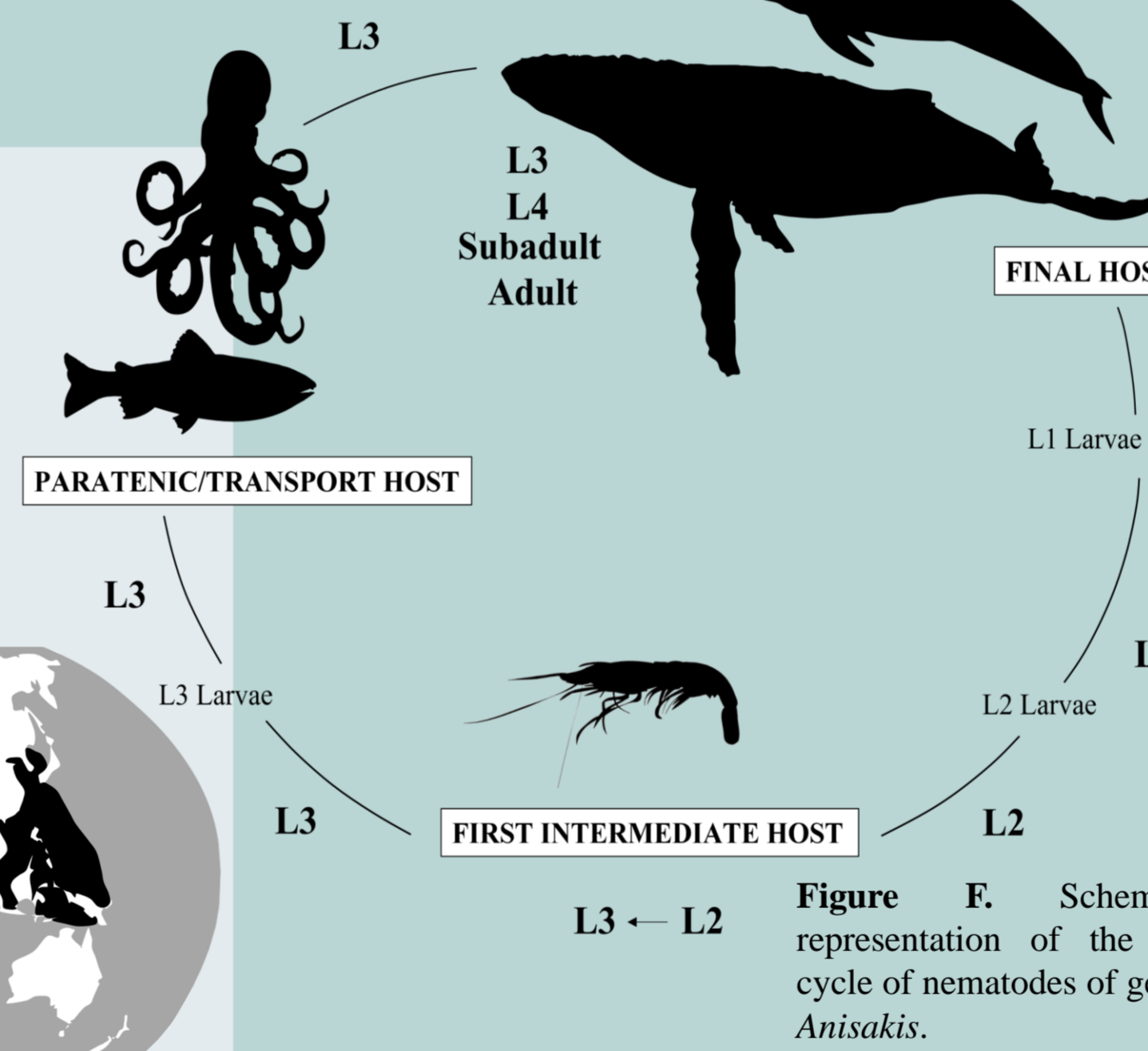


Figure F. Schematic representation of the life cycle of nematodes of genus *Anisakis*.

Table B. Sex ratio estimates of *Anisakis simplex* sensu lato (s.l.) from 6 short-finned pilot whales, *Globicephala macrorhynchus*, mass-stranded in Asturias, Northwest Spain (this study) and from 34 harbor porpoises, *Phocoena phocoena*, off Denmark [18]. X: mean; SD: standard deviation.

Host specie	<i>G. Macrorhynchus</i> (this study)		<i>P. phocoena</i> (Herreras <i>et al.</i> , 2004)	
Life stage	Per host (x ± SD)	Total sample	Per host (x ± SD)	Total sample
Subadult	0.400 ± 0.205	0.297	0.512 ± 0.230	0.504
Adult	0.498 ± 0.194	0.553	0.470 ± 0.194	0.478
Sexables	0.493 ± 0.113	0.458	0.497 ± 0.191	0.482

RESULTS

Table A. Composition by development stage of the population of *Anisakis simplex* sensu lato (s.l.) from 6 short-finned pilot whales, *Globicephala macrorhynchus*, mass-stranded in Asturias, Northwest Spain (this study) and from 34 harbor porpoises, *Phocoena phocoena*, off Denmark [18]. X: mean; SD: standard deviation, N: number of individuals.

Host specie	<i>G. Macrorhynchus</i> (this study)			<i>P. phocoena</i> (Herreras <i>et al.</i> , 2004)		
	Per host	Total sample	N (%)	Per host	Total sample	N (%)
Life stage	x (SD)	% (SD)	N (%)	x (SD)	% (SD)	N (%)
L3	7.3 (9.1)	9.6 (12.7)	44 (4.5)	31.1 (97.9)	23.5 (3.4)	1057 (14.0)
L4	92.2 (124.7)	51.4 (17.3)	553 (56.9)	72.6 (161.5)	44.9 (4.8)	2467 (32.8)
Larvae (L3 + L4)	163.5 (252.9)	76.7 (33.0)	597 (61.4)	103.7 (255.0)	68.5 (3.6)	3524 (46.8)
Subadult male	5.5 (3.9)	6.9 (8.6)	33 (3.4)	9.7 (23.6)	3.7 (6.5)	331 (4.4)
Subadult female	14.2 (11.6)	9.6 (6.6)	85 (8.7)	9.6 (20.7)	4.6 (2.6)	326 (4.3)
Subadult (total)	19.7 (14.1)	16.5 (12.6)	118 (12.1)	19.3 (43.8)	8.3 (17.6)	657 (8.7)
Adult male	19.7 (25.6)	8.9 (6.8)	118 (12.1)	47.1 (132.7)	10.5 (13.2)	1601 (21.3)
Adult female	23.2 (23.3)	13.6 (14.8)	139 (14.3)	51.4 (156.2)	12.7 (19.9)	1747 (23.2)
Adult (total)	42.8 (45.6)	22.5 (19.4)	257 (26.4)	98.5 (288.6)	23.2 (28.5)	3348 (44.5)
Sexable (total)	62.5 (68.3)	40.0 (23.5)	375 (38.6)	117.8 (329.8)	31.5 (33.6)	4005 (53.2)

- **Morphological identification discarded** the presence of *A. typica*, from L4 to adult stages, in all non-degraded worms (i.e., 972).
- The amplification and sequencing of **mtDNA *cox2* gene** from 30 worms revealed the presence of *A. simplex* s.s. (93.3%; n=28) and *A. pegreffii* (6.7%; n=2).
- Species assignment based on the ratio between the length of the left spicule and total body length (**LS/TBL**), **matched molecular identification** in the 14 sequenced males (12 *A. simplex* s.s. and 2 *A. pegreffii*).
- When the **LS/TBL** criterion was applied to the **52 males** that had not been sequenced, **46 (88.5%)** were assigned to *A. simplex* s.s. and **6 (11.5%)** to *A. pegreffii*.

AIM

Here, we provide (1) a **morphological and molecular identification** of a large sample of *Anisakis* spp. collected from the stranded SFPWs, and (2) a detailed account of their **population structure**. The data obtained are used to **reconstruct the latitudinal movements and residence area** of the pod before the stranding.

MATERIAL AND METHODS

- Each individual worm was classified into a **life stage** [17,18].
- Based on **morphological** traits [13 and ref. therein,17,19], we carried out an **identification** of **all specimens** (n = 995) to the lowest taxonomic level.
- Five worms from each infected host were **randomly** collected, and **mtDNA *cox2* gene** was amplified following Pons-Bordas *et al.*, 2020 [20].
- The **molecular analysis** suggested that most (if not all) worms collected could belong to *A. simplex* s.s. or *A. pegreffii* (see the **Results**). *A. simplex* s.s. and *A. pegreffii* show a **non-overlapping ratio** between the **length of left spicule** (Fig. G) and **total body length** (i.e., LS/TBL) [13,21]. Assuming that only these species were present, we used this additional morphological criteria to potentially **assign adult males to species**:
 - We firstly checked this diagnostic criterion using the sample of males identified to species by molecular methods.
 - Then, we applied this criterion to 52 males in good state of preservation, from the remaining sample.

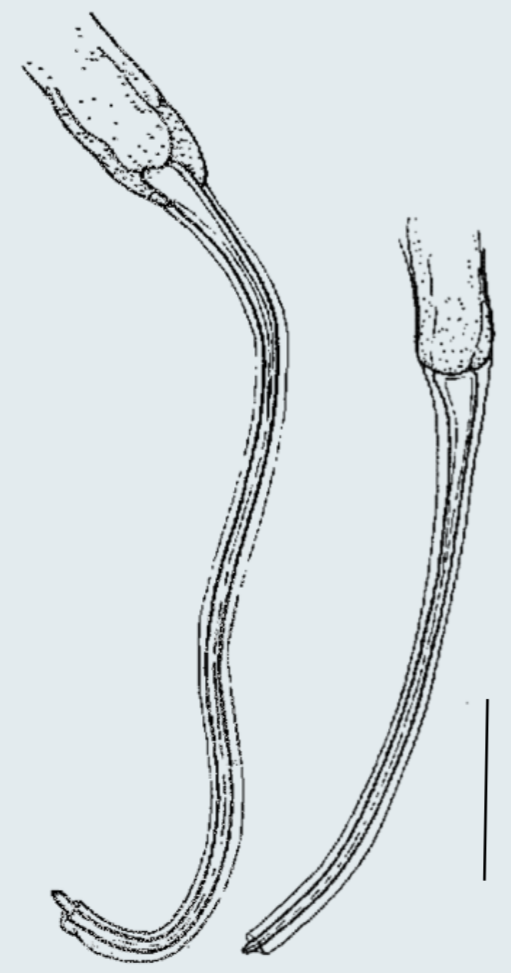


Figure G. Left and right spicules of an adult male of *Anisakis* spp. Scale bar: 0.5 mm. © Grabda, 1976.

DISCUSSION

- **First worldwide report** of *A. pegreffii* infecting SFPWs.
- In the **Atlantic Ocean**, *A. simplex* and *A. pegreffii* coexist without *A. typica* in both the **Norwegian Sea** and the **Iberian Atlantic coast**. Morphological and molecular percentages of *A. simplex* (s.s.) and *A. pegreffii* of this study, **mirror** those reported in **delphinids** from the **Iberian Atlantic coast** [13 and ref. therein, 20, 22] (Fig. H).
- **Population structure** and **sex-ratio** of *Anisakis simplex* s.l. (Tables A and B) **parallels** those of a **resident cetacean specie** (i.e., the harbour porpoise, *Phocoena phocoena*) in the NE Atlantic, revealing a **constant larvae recruitment** and a **consolidated adult population**. Since **most adult specimens** were **found naturally dead**, and considering that (1) **molt to adult** takes **43-71 days** and (2) **adult lifespan** is estimated in **39.2 days** [22,23,24], the *Anisakis* spp. here analysed were infecting the SFPWs **at least during three months prior to the stranding**.
- To our knowledge, we provided the **first feasible evidence** of the **residence of SFPWs at latitudes above 43°N**, at least with some continuity. However, we cannot ensure its permanent establishment in those waters: round-trips of 2000 km and 4.5 months of duration have been described in resident SFPWs from the Macaronesia region [25].
- The possible **ampliation of the geographical distribution** of SFPWs could be mainly related to (1) atmospheric phenomena, such as the **North Atlantic Oscillation** (NAO), and (2) **climate change**, a phenomenon observed in other cetacean species globally, and expected to increase in the coming decades. The septentrional **expansion of SFPWs** may **wider the area of co-occurrence with LFPWs**, increasing the opportunities for **interspecific hybridization** between both species, particularly during the reproductive peaks in summer [26].

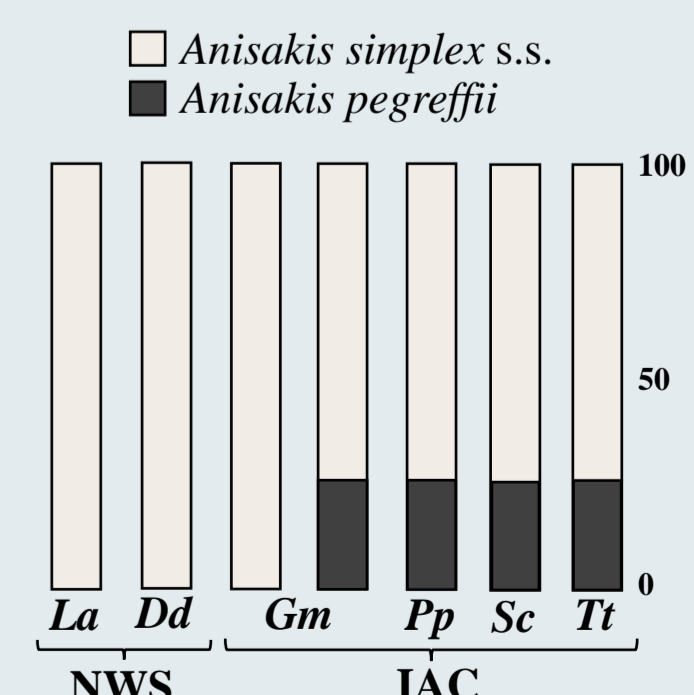


Figure H. Relative proportions of *Anisakis* spp. in some final hosts from the Norwegian Sea (NWS) and the Iberian Atlantic coast (IAC). La: *Lagenorhynchus albirostris*; Dd: *Delphinus delphis*; Gm: *Globicephala melas*; Pp: *Phocoena phocoena*; Sc: *Senella coeruleoalba*; Tr: *Tursiops truncatus* [13 and ref. therein, 20, 22].

