A NEW SPECIES OF THE GENUS CRASSICAUDA
LEIPER ET ATKINSON, 1914 (NEMATODA: SPIRUROIDEA)
FROM THE PENIS OF GLOBICEPHALA MELAS (TRAILL, 1809)
(CETACEA: GLOBICEPHALIDAE)
IN THE WESTERN MEDITERRANEAN SEA
J. A. RAGA, J. A. BALBUENA

SUMMARY

The examination of a male long-finned pilot whale (Globicephala melas) stranded at Cullera (Spanish Mediterranean coast) resulted in the finding of nematodes of the genus Crassicauda parasitising the penis. Pathologies associated with these worms were observed and are described here. The morphometric and morphological study of these helminths revealed that they represent a new species, Crassicauda carbonelli n. sp., which differs from other species of the genus Crassicauda in body size, cephalic morphology and spicule size in the males. Crassicauda sp. described by Dollfus (1968) must be ascribed to C. carbonelli n. sp. The taxonomy, morphometric variability of the eggs, site in the hosts, and pathology of the Crassicauda species are discussed.


Au cours de l'examen d'un globicéphale noir mâle (Globicephala melas), échoué à Cullera (côte méditerranéenne espagnole), on a détecté des Nématodes du genre Crassicauda dans le pénis. Des lésions parasitaires liées à la présence de ces vers ont été observées et ont décrites. L'étude morphométrique et morphologique des helminthes indique qu'ils doivent être considérés comme une espèce nouvelle, Crassicauda carbonelli n. sp. Elle peut être séparée des autres espèces du genre Crassicauda en raison de la longueur du corps, de la morphologie céphalique et de la taille des spicules chez les mâles. Les spécimens nommés Crassicauda sp. par Dollfus (1968) doivent être rapportés à C. carbonelli n. sp. La taxonomie, la variabilité morphométrique des œufs, la localisation dans l'hôte et la pathologie des espèces du genre Crassicauda sont discutées.

INTRODUCTION

Since 1981, cetaceans stranded on the Comunidad Valenciana coasts (Spanish Mediterranean) have been routinely examined for parasites. The necropsy of a 4.55 metre-long male long-finned pilot whale (Globicephala melas (Traill, 1809)) stranded at Cullera (Valencia Province) on March 17, 1987 resulted in the finding of numerous nematodes in the penis. No entire worms could be collected since they were found their cephalic ends deeply embedded in the corpus cavernosum. The morphometric and morphological study of these helminths revealed that they represent a new species of the genus Crassicauda Leiper and Atkinson, 1914, which is described herein. The specimens previously described by Dollfus (1968) designated as Crassicauda sp. seem to correspond to this new species.

The habronematoid nematodes of the genus Crassicauda represent one of the most characteristic groups of marine mammal helminths. They parasitise cetaceans exclusively (Dailey, 1985; Raga, in press). The taxonomy of the genus Crassicauda is controversial. Descriptions of the species of this genus are usually poor. This is due largely to the difficulty in obtaining specimens intact since these nematodes are typically found entwined within the host tissues and usually having an extremely long and delicate body (Baylis, 1922; Geraci et al., 1978; Geraci and St.-Aubin, 1986). In some cases, the poor condition and scarcity of the material only permitted determination at the generic level (Dollfus, 1968; Dailey and Perrin, 1973; Robineau, 1975; Dailey, 1985) and new Crassicauda species have been described on the basis of either cephalic or posterior portions of the parasites (Lambertsen, 1985). The poor characterisation of some of these species made redescriptions and taxonomic revisions necessary (Skrbjabin, 1973; Lambertsen, 1985; Raga, 1987).

Egg size (length × width) has been one of the characters commonly used in the taxonomy of Crassicauda spe-
species (Delyamure, 1955; Skrjabin, 1969; Lambertsen, 1985). However, Dollfus (1965, 1968) pointed out that: i) egg size can vary a great deal even within the same individual; and ii) variations in the ranges of egg length and width of many *Crassicauda* species overlap considerably. This author concluded that egg dimensions should not be used for species discrimination. In order to analyse the value of these metrical variables in the classification of *Crassicauda*, a detailed study of the variability of egg dimensions of our material, as compared with those of Dollfus (1968), was carried out.

The species of the genus *Crassicauda* usually occur in the urinogenital system and mammary glands of Cetacea, although some species have been found in different body muscles, pterygoid sinuses and stomach (Dollfus, 1965; Skrjabin, 1969; Arvy, 1982; Geraci and St.-Aubin, 1986). The life cycles of these helminths are unknown although a heteroxenous cycle has been postulated (Geraci et al., 1978). Evidence indicates, at least in the case of *C. boopis* Baylis, 1920, that somatic migration of larvae from the intestine to the final site of parasitisation may occur (Lambertsen, 1986). *Crassicauda* worms have frequently been associated with pathologies in their hosts. Lesions due to these parasites have been reported in cranial sinuses (Dailey and Perrin, 1973; Robineau, 1975; Raga et al., 1982; Dailey, 1985), kidneys (Arvy, 1973-1974; Lambertsen, 1986), mammary glands (Dailey and Perrin, 1973; Geraci et al., 1978) and penis (Baylis, 1922; Dailey, 1985). Pathogenesis by *Crassicauda* species may be a significant factor in natural mortality or organic weakening of several cetacean populations (Dailey and Perrin, 1973; Geraci et al., 1978; Perrin and Powers, 1980; Lambertsen, 1986). The presence of these worms or their eggs within the host tissue may provoke the formation of calcified cysts, tissue reaction and leukocyte infiltration (Cockrill, 1960; Gibson, 1973; Dailey and Stroud, 1978; Howard et al., 1983; Dailey, 1985; Lambertsen, 1986). This strongly suggests a mutual agression model of host-parasite coevolution (Holmes, 1983).

### MATERIAL AND METHODS

Caudal fragments of 40 specimens (20 male and 20 female) and two cephalic portions were studied and compared with six fragments of *Crassicauda* sp. described by Dollfus (1968) deposited at the Museum National d'Histoire Naturelle, Paris (MNHN, coll. No. 579BC).

Our material we washed in saline solution and fixed and preserved in 70 % ethanol. Chloral-lactophenol, lactic acid or lactophenol were used as clearing agents.

Type material (anterior extremities and caudal fragments of male and female worms) is deposited at the helminthological collections of the Department of Animal Biology, University of Valencia (DAB, UV) (Coll. No. Gm11, March 17, 1987).

In order to study the variability of the egg dimensions, measurements were taken from the eggs in utero of each 14 of the 20 caudal ends of the females studied (Possibly due to manipulation, the uterus of the remaining six females studied was empty or contained too few eggs to allow statistical analyses). Likewise, 20 eggs from a *Crassicauda* sp. female of Dollfus (1968) were measured. Measurements were taken and processed with a computerised image analyser (IBAS 2000, Kontron, Munich). A measurement programme designed by Dr J. F. Pertusa (DAB, UV) allowed the computation of maximum and minimum diameter of the digitalised image. Maximum and minimum diameter of the digitalised image, are assumed to correspond to egg length and width respectively.

Mean eggs length across females was compared by means of an one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981). A Tukey test was used for pairwise comparisons of mean length between individual females. In this case, the harmonic mean of all group sizes was employed as the sample size for all groups (Norusis, 1984).

As for egg width, a preliminary analysis indicated heteroscedasticity across samples (Bartlett-Box test, P < 0.01) (Sokal and Rohlf, 1981). As no simple data transformation could homogenise the sample variances, a rank transformation (RT-1 procedure of Conover and Iman (1981)) was performed to allow the use of parametric statistical analyses. ANOVA and Tukey test were then applied as above to compare mean ranked width of the eggs across and between female worms respectively.

All statistical tests were carried out with an SPSS/PC+ statistical package (SPSS Inc., Chicago, Illinois). Significance was set at the 0.05 probability level.

Samples of lesions were fixed in 10 % formaline. Serial sections 10 µm thick were cut for histopathology, using the classical paraffin embedding and Mayer's haematoxylin-eosin staining techniques.

The terms « site », « location » and « habitat » are used as defined by Margolis et al. (1982).

### RESULTS

#### DESCRIPTION OF Crassicauda carbonelli n. sp.

*Definitive host*: long-finned pilot whale, *Globicephala melas* (Traill, 1809) (Cetacea: *Globicephalidae*).

*Site*: penis.

*Locality*: Cullera, Valencia Province, Spanish Mediterranean.

*Etymology*: named in honour of Pr E. Carbonell, DAB, UV.

*Syn.:* *Crassicauda* sp. sensu Dollfus (1968).

Nematodes with elongate, spiral body. Cuticule opaque, non-striated. Cephalic region elongate, narrow and tightly coiled; cuticular collar absent. Oral aperture surrounded by two pseudolabia, two amphids, four outer submedian cephalic papillae and two less conspicuous inner lateral papillae (*Fig. 1C*). Pharynx chitinoid, 125-130 x 23-30 µm (*Fig. 1A*). Muscular portion of oesophagus 1,442-1,339 x 100-110 µm, opens into larger glandular oesophagus. Nerve ring situated at 288-295 µm from anterior end of body. Excretory pore at 446-457 µm from cephalic extremity distance (*Fig. 1B*).
Fig. 1. — *Crassicauda carbonelli* n. sp. A: Cephalic end in lateral view, detail of the pharynx. B: Cephalic end in lateral view, detail of the muscular oesophagus. C: Cephalic end, apical view. D: Caudal end of male, ventral view. E: Egg. F: Caudal end of male, right lateral view. G: Caudal end of male, left lateral view. H: Caudal end of female, lateral view.
**Morphometric variation of the eggs**

Both the mean egg length and width of Dollfus' (1968) specimen are similar to those of the present material (Fig. 2) and the variation ranges of these measurements reported by this author fall well within those of the worms studied (Table I). The mean length and ranked width of the eggs differed significantly between females ($F = 21.87$, $P < 0.01$ for egg length; $F = 5.80$, $P < 0.01$, for ranked egg width). The results of the Tukey tests for both variables are summarised in Figure 3. The mean length and mean ranked width of the eggs of Dollfus' (1968) specimen did not differ significantly when compared to those of 13 of the 14 female individuals of *C. carbonelli* n. sp. Significant differences in mean values of both variables between some females of *C. carbonelli* n. sp. also occurred (Fig. 3).
Lesions

*C. carbonelli* n. sp. was found with the cephalic region tightly coiled within the corpus cavernosum of the penis and the posterior ends hanging freely in the lumen of the urethra. Macroscopic lesions, consisting of broken capillaries and destruction of the corpus cavernosum tissue, were observed around the cephalic ends of the worms. In some cases, these lesions resulted in necrosis, leukocyte infiltration and hyperplastic proliferation of connective tissue.

DISCUSSION

The morphology and morphometry of the specimens studied indicates that they belong to the genus *Crassicauda*. The taxonomic position of this genus is currently unclear. According to Chabaud's (1975) key, followed by several authors such as Margolis and Arai (1989), these nematodes of the order Spirurida must be ascribed to the superfamily Habronematoidae (Chitwood and Wehr, 1932), family Tetrameridae Travassos, 1914, subfamily Crassicaudinae York and Mapstone, 1926. However, Skrjabin and Andreeva (1934) considered that the genus *Crassicauda* should be included into an independent family of this order, the Crassicaudinae (York and Mapstone, 1926). This view was followed by several other specialists (e.g. Delyamure, 1955; Dollfus, 1968; Dailey, 1985).

Skrjabin (1969) listed 12 species of *Crassicauda* in his revision of the genus but the number of valid species was reduced after Lambertsen (1985) demonstrated that *C. pacifica* Margolis and Pike, 1955 is synonymous with *C. boopis* Baylis, 1920. Another problem involves *C. fuelleborni*, originally described by Hoenpli and Hsü (1929) as *Onchocerca fuelleborni*, whose taxonomic position within the genus *Crassicauda* remains uncertain (Baylis, 1932; Skrjabin, 1969). As indicated above, due to the poor condition of the type material of many *Crassicauda* species, frequently consisting of either caudal or cephalic ends only, the validity of some of them seems questionable. Further work is needed and may result in the establishment of new synonyms.

The males of five *Crassicauda* species namely, *C. boopis* from the urogenital system of roqual whales (Balaenopteridae) and *Megaptera novaeangliae* (Borowski, 1781) (Margolis and Pike, 1955; Skrjabin, 1973; Lambertsen, 1985), *C. tortilis* Skrjabin, 1959 a parasite of *Balaenoptera musculus* (L., 1758) in the same location (Skrjabin, 1959), *C. benneti* Spaul, 1926 from the kidney of *Hyperoodon* sp. (H. planifrons Flower, 1882 according to Gibson and Harris (1979) (Spaul, 1926), *C. grampicola* Johnston et Mawson, 1941 from the cranial sinuses of *Grampus griseus* (Cuvier, 1812) and *Delphinus delphis* L., 1758 (Johnston and Mawson, 1941; Raga, 1987; Gibson, pers. comm.), but also reported in the mammary glands of *Lage-norhynchus acutus* (Gray, 1828) (Geraci et al., 1978) and *C. fuelleborni* (Hoenpli and Hsü, 1929) from the muscles around the vagina of *Neophaeaca phocaenoides* (Cuvier, 1829) (Hoenpli and Hsü, 1929) possess no spicules, and thereby differ from the present material. In contrast, the males of another five species: *C. giliakiana* Skrjabin and Andreeva, 1934 from *Delphinapterus leucas* (Pallas, 1776), *Hyperoodon ampullatus* (Forster, 1770) and *Berardius bairdi* Steiniger, 1883, *C. anthonyi* Chabaud, 1962 (= *C. giliakiana sensu* Lopéz-Neyra (1958)) from various beaked whales (Ziphidae), *C. delamureana* Skrjabin, 1966 from *Balaenoptera borealis* Lesson, 1828, *C. costata* Skrjabin, 1969 from *Eubalaena australis* (Desmoulins, 1822), *C. crassicauda* (Crepilin, 1829) from several roqual whales and *Balaena mysticetus* (L., 1758), all recorded in the urogenital system, and *C. duguyi* Dollfus, 1966 a parasite of subcutaneous neck muscles of *Kogia breviceps* (de Blainville, 1838), possess spicules of different sizes (Skrjabin and Andreeva, 1934; Chabaud, 1962; Dollfus, 1965, 1966; Skrjabin, 1966, 1969, 1973).

The present specimens differ from *C. duguyi* and *C. crassicauda* since the males of these species have unequal spicules in size and greater in length (600/2,000 µm in *C. duguyi* and 720-800/270-320 in *C. crassicauda*) (Dollfus, 1966; Skrjabin, 1973). They also can be distinguished from *C. costata* by the absence of the characteristic cuticular ribs after which the latter species was named (Skrjabin, 1969). The present worms can also be separated from *C. delamureana*, which appears to possess a considerably larger body length: 650-700 mm for the females and 284-310 mm for the males. In addition, *C. delamureana* has a distinctly digitiform cephalic region and the spicules of the males are very small, around 20 µm in length (Skrjabin, 1966). *C. anthonyi* differs from our material in possessing a cuticular collar around the penis of a long-finned pilot whale caught in the region of the Strait of Gibraltar. The absence of male worms...
among his material precluded identification at the specific level and thus the specimens were referred to as *Crassicauda* sp. The morphometry and morphology of these helminths clearly conforms with those of our helminths (Table I). Furthermore, the host species, site of infection and geographical locality of *Crassicauda* sp. are very similar to, if not identical with, those of the present nematodes.

The morphometric study of egg dimensions also supports the synonymy of *Crassicauda* sp. sensu Dollfus (1968) with *C. carbonelli* n. sp. Variation in ranges and averages of maximum and minimum diameter of the eggs of Dollfus’ (1968) specimens did not differ substantially from those of most of our material (Figs 2, 3). Our results indicate that length and width of the eggs can vary significantly between *C. carbonelli* n. sp. females (Fig. 3). This seems to support Dollfus’ (1965, 1968) view, giving poor value to egg size as a criterion for identification of *Crassicauda* sp. Variability of egg size might be related to different stages of maturation of the eggs and other factors, such as host species or host individuals within a given species. However, more *Crassicauda* species should be studied to precisely assess the value of egg size in the taxonomy of this genus.

The pathological changes produced by *C. carbonelli* n. sp. in the host’s tissues are similar to those caused by *C. crassicauda* in the penis of rorqual whales (Baylis, 1922; Dailey, 1985). Worms of the genus *Crassicauda* may exhibit a certain degree of site specificity. Each species appears to occur in a typical location in their definitive hosts. An exception to this seems to be *C. grampicola*, which is usually found in the cranial sinuses of *C. griseus* and *D. delphis* but has been reported parasitising the mammary glands of *L. acutus* (Geraci et al., 1978).

In view of the ecological and trophic differences between odontocetes and mysticetes, it seems unlikely that the same *Crassicauda* species could infect members of both cetacean groups (Dollfus, 1966). So Dollfus (1966) indicated that reports of *Crassicauda* species typical of mysticetes in odontocetes (e. g. Joyceu and Baer (1931), Baylis (1932)) are questionable. According to Arvy (1973-1974), either Skrjabin (1959) or Cockrill (1969) reported *C. tortilis*, a parasite of the blue whale, in *Physeter catodon*. It must be pointed out, however, that neither made no mention of such a record and, thus, Arvy’s (1973-1974) citation is erroneous.

*C. crassicauda* and *C. boopis* are the only *Crassicauda* species so far reported from the penis and clitoris of mysticetes (Gibson, 1973; Skrjabin, 1973). It seems that *C. carbonelli* n. sp. might exploit a similar habitat to that of these two species in odontocetes.

Acknowledgments. — The authors wish to express their appreciation to Dr A. J. Petter (MNHN) for her help in the study of the specimens deposited at the MNHN, and for her advice throughout this study. Thanks are also due to Dr D. I. Gimson (British Museum (Natural History)) for his critical reading of the manuscript. Ms. G. Tapia greatly improved the drawings of the specimens and Dr J. F. Pertusa and Ms. M. Fernández provided invaluable assistance with the morphometric analysis. J. A. Balbuena holds a doctoral scholarship from the Conselleria de Cultura, Educació i Ciència, Generalitat Valenciana. Funds for laboratory work were provided by the DGICVTH of the Spanish Ministry of Science and Education (Project No. PB87-997).

REFERENCES

Baylis H. A.: Note on the habitat and structure of *Crassicauda* (Nematoda). *Parasitology*, 1922, 14, 9-12.

260
NEW SPECIES OF CRASSICAUDA (SPIRUTOIDEA)


