Illuminating the Evolutionary History of a Group of Miniaturized Worms, the Nemertean Genus *Ototyphlonemertes*

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Objectives

To discover evolutionary history of genus *Ototyphlonemertes* and its morphological characters.

Introduction

A few nemertean worms belong to a group of animals called meiofauna, which is loosely defined as organisms able to fit through a 0.5 mm sieve [1]. *Ototyphlonemertes*, currently 26 named valid species, are highly specialized interstitial meiofauna, which means that they move through the aqueous pore space of marine sediments, such as coarse-sand beaches, without burrowing [1]. The species range from 0.1 to 0.35 mm in diameter and from 1 to 40 mm long as adults [1].

Nemertean worms have received little scientific attention, and much of their biology is unknown or speculative [2]. In this study, we wanted to clarify the evolutionary history of the genus *Ototyphlonemertes*; specifically: 1) Is the genus monophyletic? 2) Two broad groups are identifiable, having either a smooth proboscis stylet + oligogranual statoliths (Fig. 1) or a helically sculpted stylet + polygranular statoliths (Fig. 1). Are the two reciprocally monophyletic or is one nested in the other (paraphyletic)? 3) Six general morphotypes have been recognized on the basis of combinations of characters but lack recognized synapomorphies; are these monophyletic?

Materials and Methods

This study used samples collected from beaches in Puerto Rico and French Polynesia. The samples were preserved in ethanol and DNA was extracted at the Smithsonian Institution prior to the beginning of the study.

PCR amplification
Three markers were amplified from the genomic DNA: 1) a xxx-base region of 18S rDNA, with primer pairs Euk and SR7 [3, 4]; a xxx-base region of COI gene with the primer pairs dgLCO and dgHCO [5]; and a xxx-base region of mitochondrial 16S rDNA with primer pairs Ar-L and Br-H [6]. PCR reactions were performed using BIOLase polymerase. After cycling, the samples were purified using EXOSAP and sequenced with BigDye. The products were then cleaned using sephadex and analyzed.

Alignment and phylogenetic analyses
Contigs were made, edited, and aligned using Geneious [7]. Alignments were tested for best-fit model and DNA sequence evolution was evaluated [8]. Trees were constructed using Mr. Bayes and RAxML [9, 10]. For Mr. Bayes, 5 million generations were used with a model for each gene. RAxML used 1000 bootstraps with partition dataset for each gene. The outgroup is monostiliferan hoplonemertean shown to be basal to Ototyphlonemertes in several nemertean molecular phylogenies.
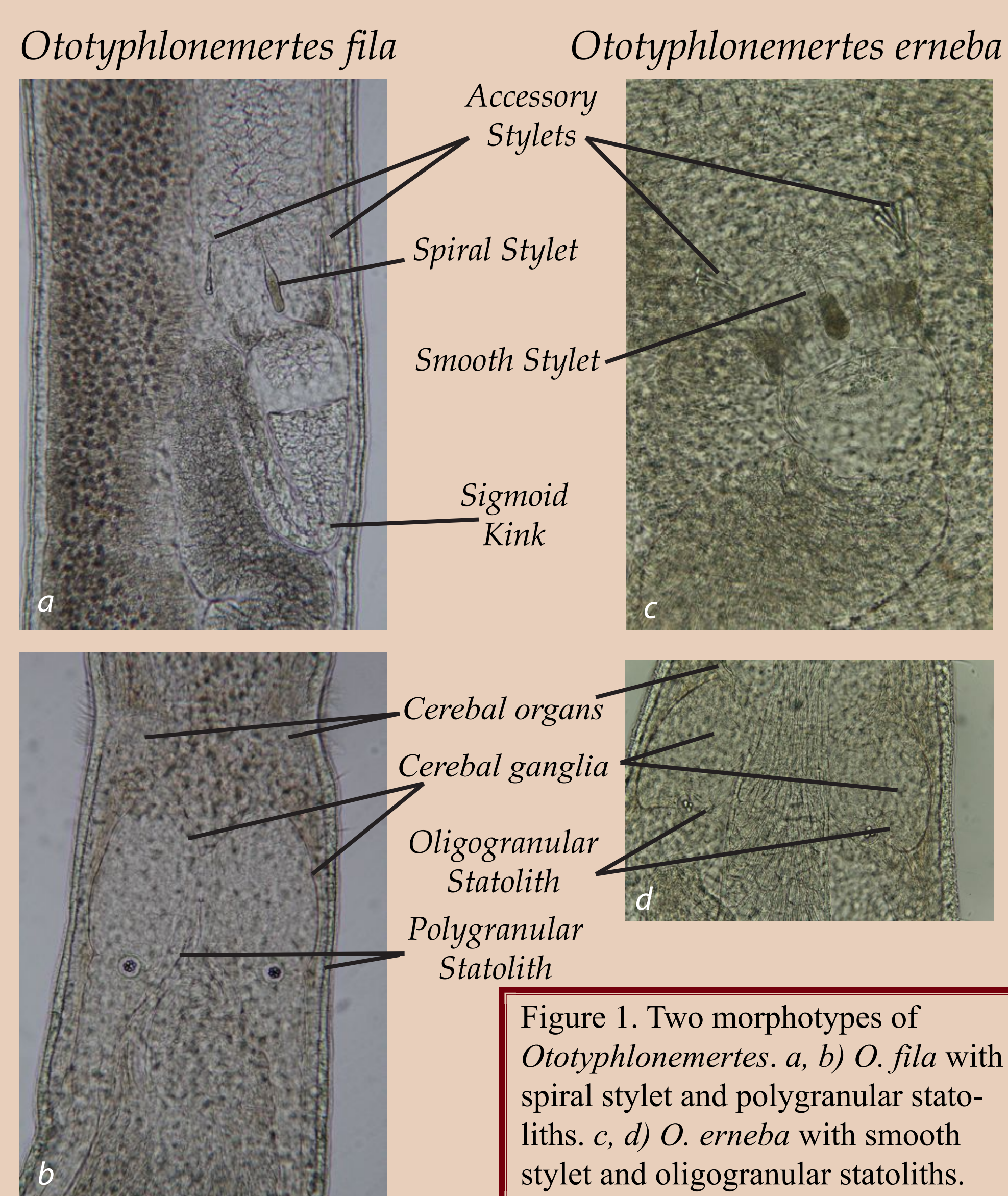
Results

Figure 1. Two morphotypes of *Ototyphlonemertes*. a, b) *O. fila* with spiral stylet and polygranular stato-liths. c, d) *O. erneba* with smooth stylet and oligogranular statoliths.
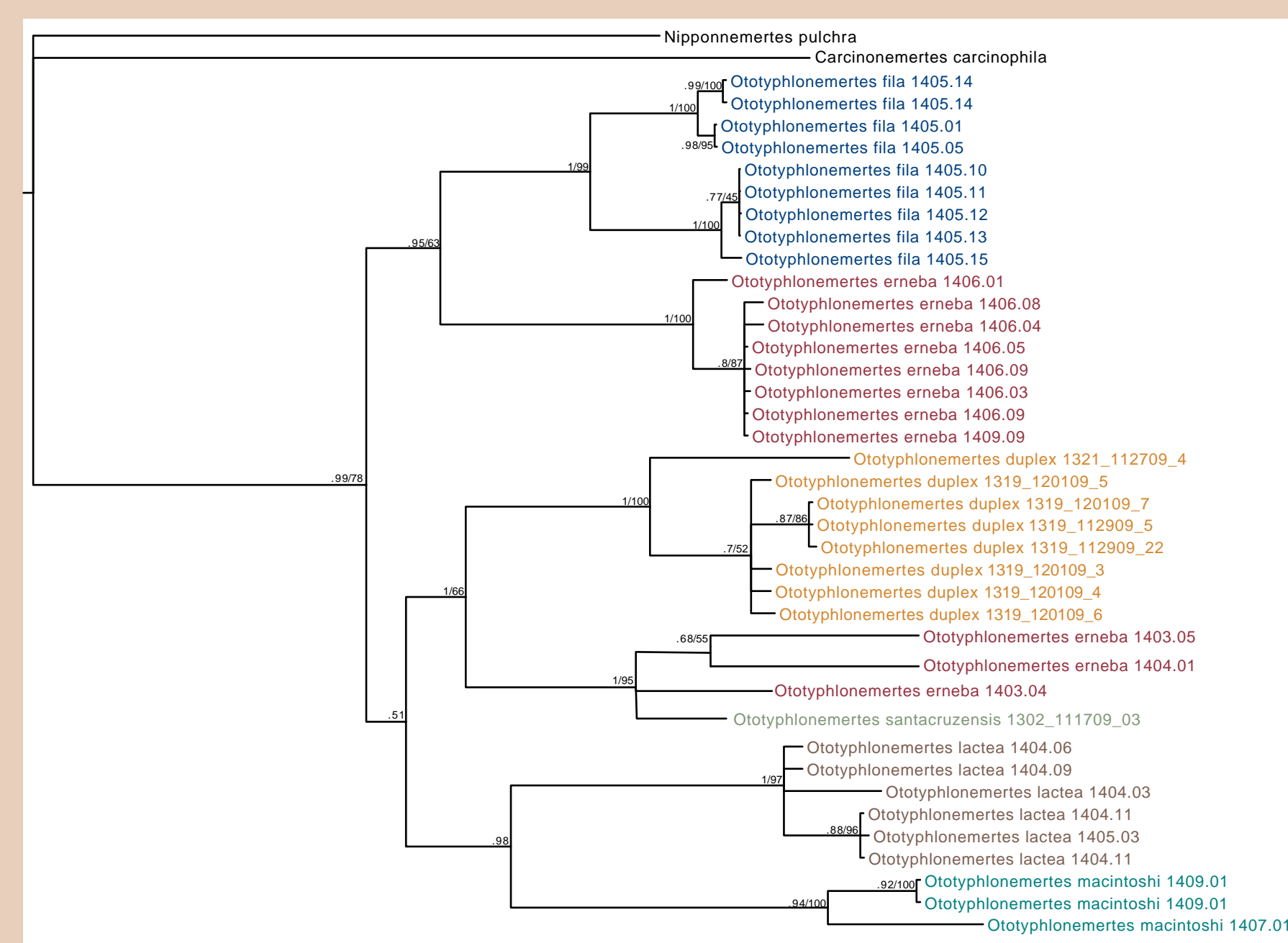

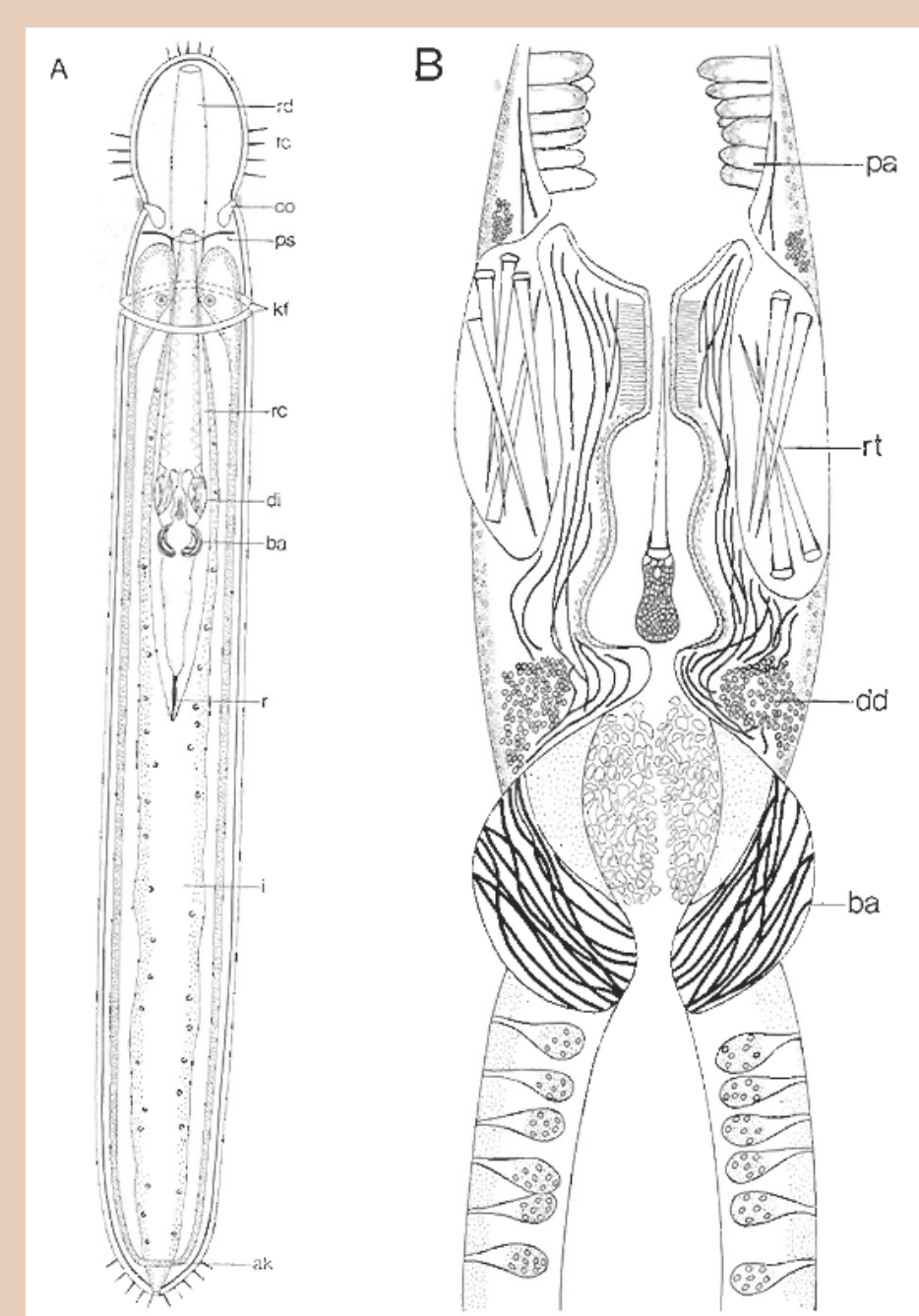Figure 2. Phylogenetic tree showing lineages of 6 morphotypes of *Ototyphlonemertes*.

Figure 3. Illustration of *Ototyphlonemertes erneba*. [11]

Discussion

The genus *Ototyphlonemertes* is monophyletic with our data. The morphotypes characterized as *O. lactea*, *O. fila*, and *O. macintoshi* have helically sculpted stylets and polygranular statoliths. This character association appears to be ancestral. The morphotypes characterized as *O. erneba*, *O. santacruzensis*, and *O. duplex* have smooth stylets and oligogranular statoliths, which appears to be derived. According to our data these paired characteristics have arisen multiple times but there is not strong support for these inferences.

It is apparent that *O. fila* consists of sympatric cryptic species. All samples of *O. fila* were collected from the same beach at similar times. *O. erneba* represents at least two morphologically cryptic species but distinct morphological differences were detected. One group has a rod structure in the proboscis papillae, while the other group does not. *O. duplex* reveals a sub-clade collected at a different station than the rest of the specimens, and there appears to be cryptic speciation within the *O. duplex* morphotype.

O. lactea and *O. macintoshi* are similar morpho-logically, in that both lack the neuroglandular cerebral organs found in all other free-living hoplonemerteans. This loss is revealed by our study to be a synapomorphy. It might be an adaptation to life in a highly dynamic environment. Both species also have a modified mid-proboscis, which is bulb-shaped in all other hoplonemerteans but is a short cylinder in *O. lactea* and a very elongated cylinder in *O. macintoshi*.

Conclusions

Our study demonstrates significant risks in identifying *Ototyphlonemertes* by morphology alone. There is weak support for the deeper nodes of the inferred phylogeny, especially with RAxML. If the pairing of *O. fila* and an *O. erneba* group were true, it would suggest that at minimum the smooth stylet and oligo-granular condition is polyphyletic and that the two main groupings are not reciprocally monophyletic. Our data is insufficiently robust to determine whether the spiral stylet and polygranular statocyst condition is para- or monophyletic. Clearly, we need more data, especially from more variable genetic markers.

References

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