

PRELIMINARY APPROACH TO THE PHYLOGENETIC RELATIONSHIPS OF POLYCLADIDA (PLATYHELMINTHA).



M. TERESA AGUADO², CRISTINA GRANDE³, DANIEL MARQUINA¹, CAROLINA NOREÑA¹

1: Departamento de Biodiversidad y Biología Evolutiva. Museo Nacional de Ciencias Naturales (CSIC), c/ José Gutiérrez Abascal 2, 28006 Madrid, Spain.

2: Universidad Autónoma de Madrid

3: CBM-UAM: Centro de Biología Molecular (CSIC)-Universidad Autónoma de Madrid

Abstract: The Polycladida is a group of marine flatworms with more than 900 species and a world wide distribution. This group was firstly described in 1881, and since then, there have been several conflicting classifications, some of them still coexist nowadays. These previous and current taxonomic disagreements have made difficult the understanding of evolutionary relationships, as well as the identification of geographical distributional patterns of many species. The order Polycladida was firstly divided in 1885 in two suborders Cotylea and Acotylea. Cotylea comprises four families, two of which are well represented, while the other two are monospecific and dubious. Within Acotylea there are three well represented superfamilies, but some genera and families are monospecific and their determination is based only on external characters. This study pursues a deep investigation into the relationships between the different groups. For this purpose we have analyzed morphological (internal and external) information, as well as molecular (genes 16S and COI) data. We provide a phylogenetic framework that facilitates the understanding of the evolution of morphological traits and provides a more stable hypothesis for the systematics.

Materials and methods

Material studied mostly from the Iberian Peninsula, some specimens from Tunes, Napoles and Argentina.

Selection of terminal taxa: Ingroup: specimens representing the two major groups withing Polycladida (Cotylea and Acotylea) as well as the maximum number of families and genera within them. Outgroup: Prior studies suggest Microstomidae and Haplopharyngida as possible sistergroups. *Dugesia* (Tricladida), though initially considered as a possible sistergroup is currently far to be consider as closely related to Polycladida. It was included to root trees and discuss antique evolutionary thypotheses.

Morphological characters: 53 binary (absent/present) and polymorphic and summarized in Table 1. Based on direct observations and literature.

Molecular study: Partial sequences of two mitochondrial genes 16SrRNA and COI were obtained. Outgroups downloaded from GenBank (GB in Figs. 1 & 2). Specific primers designed for polycladids. Alignments accomplished with Mafft. The combined analyses of the two genes was performed through Maximum Parsimony (TNT) and Maximum Likelihood (RAXML). Jackknife and Bootstrap support values for 3000 and 1000 replicates generated in TNT and RAXML, respectively.

Phylogenetic results

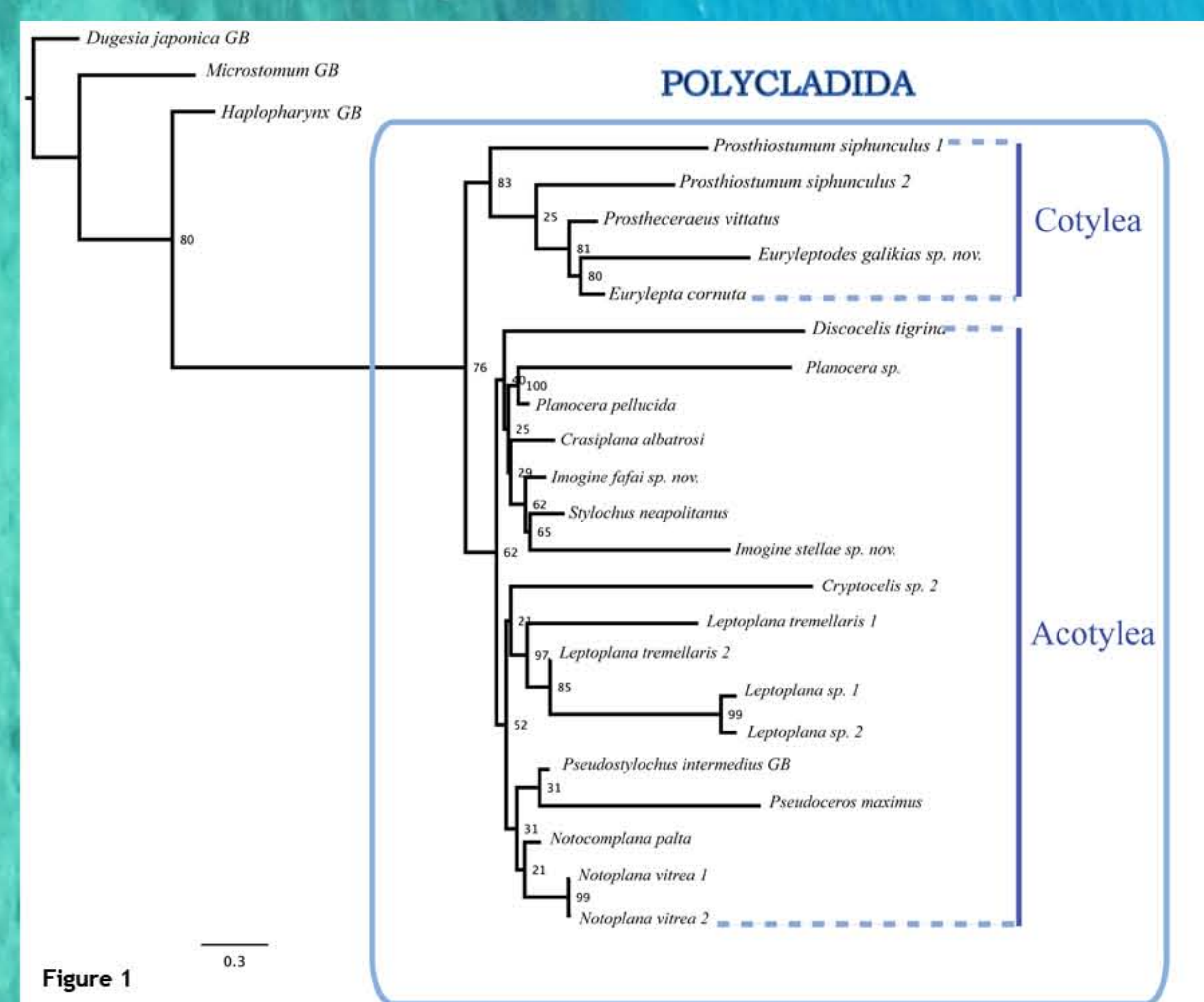


Figure 1

Results of the combined molecular data set (16S + COI) (Fig. 1) and the combined morphology + molecular (16S + COI) (Fig. 2) obtained through MP and ML were congruent in the monophyly of Polycladida, as well as Cotylea and Acotylea. Support values are, in general, not high, excepting Cotylea monophyletic in morpho+molecular analysis (Fig. 2).

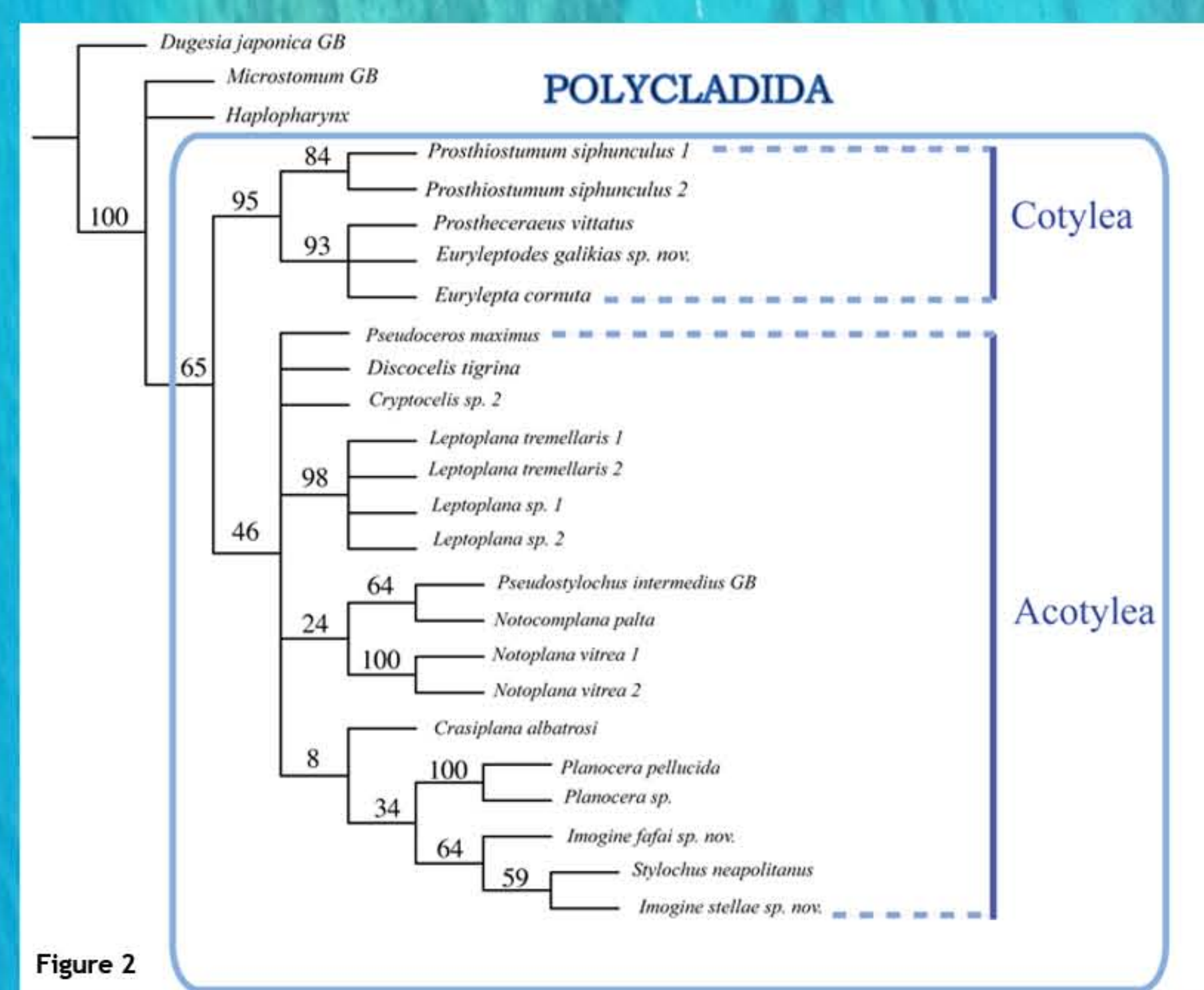


Figure 2

Figure 1. ML tree of the combined molecular analysis (16S + COI). Bootstrap values shown

Figure 2. Strict consensus tree of the MP analysis. Morphology and molecular (16S + COI) combined. Jackknife values above nodes.

Discussion

From both molecular and morphological point of view, our analysis shows a clear separation between the two suborders of Polycladida: Cotylea and Acotylea. Both analysis also show that from the possible taxa which traditionally have been considered as potential sister groups of Polycladida, Tricladida and Macrostomorpha (Macrostomida and Haplopharyngida), the latter share more similarities with the polyclads and is, therefore, the most appropriate sister group of the Order.

After a phylogenetic and kinship analysis between and within the suborders Acotylea and Cotylea, we can conclude that:

☒ Species, genera and families are grouped in molecular and morphologically congruent and well characterized taxa.

In regard to the Superfamilies proposed by Faubel 1984.

☒ By the Acotylea: the Superfamilies Stylochoidea, Ilyplanoidea and Leptoplanoidea, although not well supported, show a clear tendency to form a morphological well characterized clades supported by the following morphological apomorphies. Stylochoidea: with true free prostatic vesicle; Ilyplanoidea: without true prostatic vesicle and Leptoplanoidea: with true interpolated prostatic vesicle.

☒ In Cotylea (and maybe due to the lack of analyzed representatives), from the two known superfamilies, only Euryleptoidea is well defined through the following apomorphies: plicate cylindrical pharynx and well developed tentacles. Whereas Pseudoceroidea, characterised by the apomorphies "plicate ruffled pharynx" and "marginal folds as tentacles (pseudotentacles)", with a single representative (Pseudoceros maximus), needs clearly a further analysis for a justified statement.



Figures 8, 9: *Hoploplana* sp. and *Discocelis trigrina*, from Galicia, Spain. Pictures by Jacinto García.



Figures 3-7, from up to down: *Pseudobiceros flowersi* and different species of the genus *Pseudoceros* that show the enormous variety of colour and colour pattern of the genus, from Lizard Island, Australia. Pictures by Alexander Semenov.