

# Challenges in appendicularian integrative taxonomy and barcoding

E-mail: rade.garic@unidu.hr

Rade Garić<sup>1</sup>, Martin Pfannkuchen<sup>2</sup>,  
Marcell Dénes<sup>3</sup>, Carmela Gissi<sup>4</sup>,  
Per R. Flood<sup>5</sup>, Mirna Batistić<sup>1</sup>



AdMedPlan



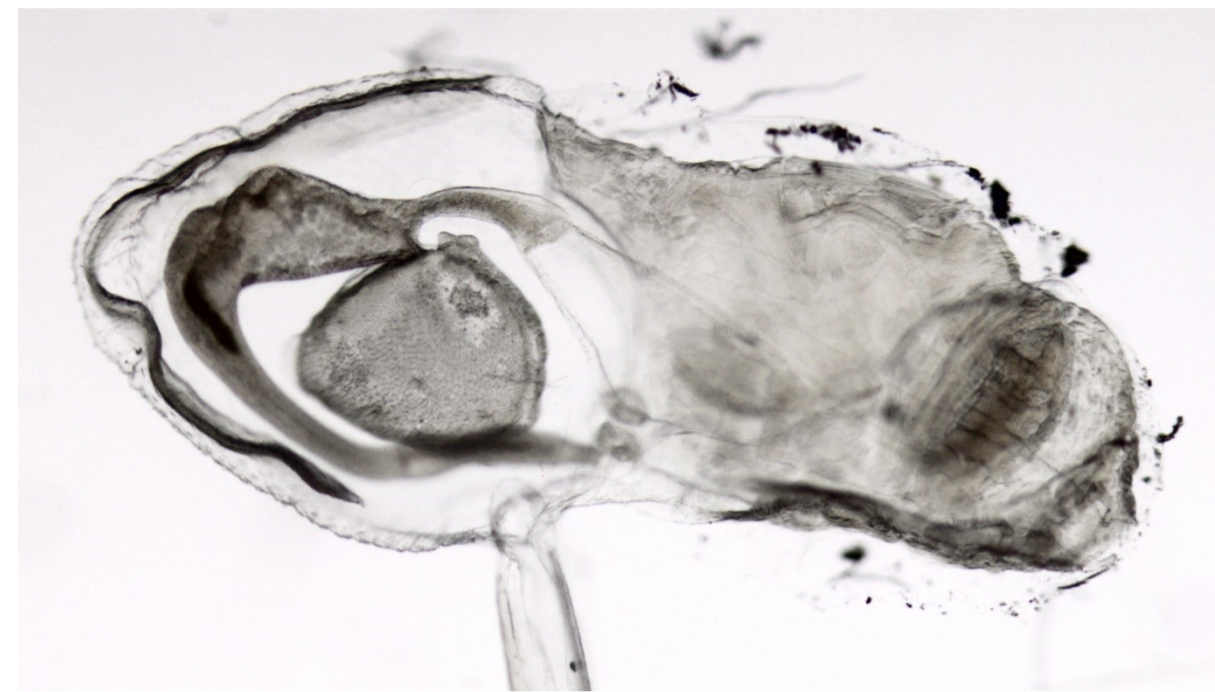
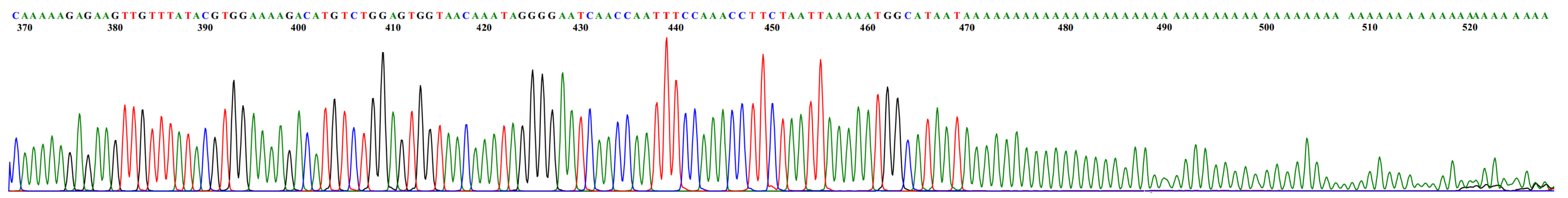
<sup>1</sup> Institute for marine and coastal research (University of Dubrovnik), Dubrovnik, Croatia;

<sup>2</sup> Center for marine research (Ruđer Bošković Institute), Rovinj, Croatia

<sup>3</sup> University of Pécs, Pécs, Hungary

<sup>4</sup> University of Bari, Bari, Italy

<sup>5</sup> Bathybiologica, Bergen, Norway

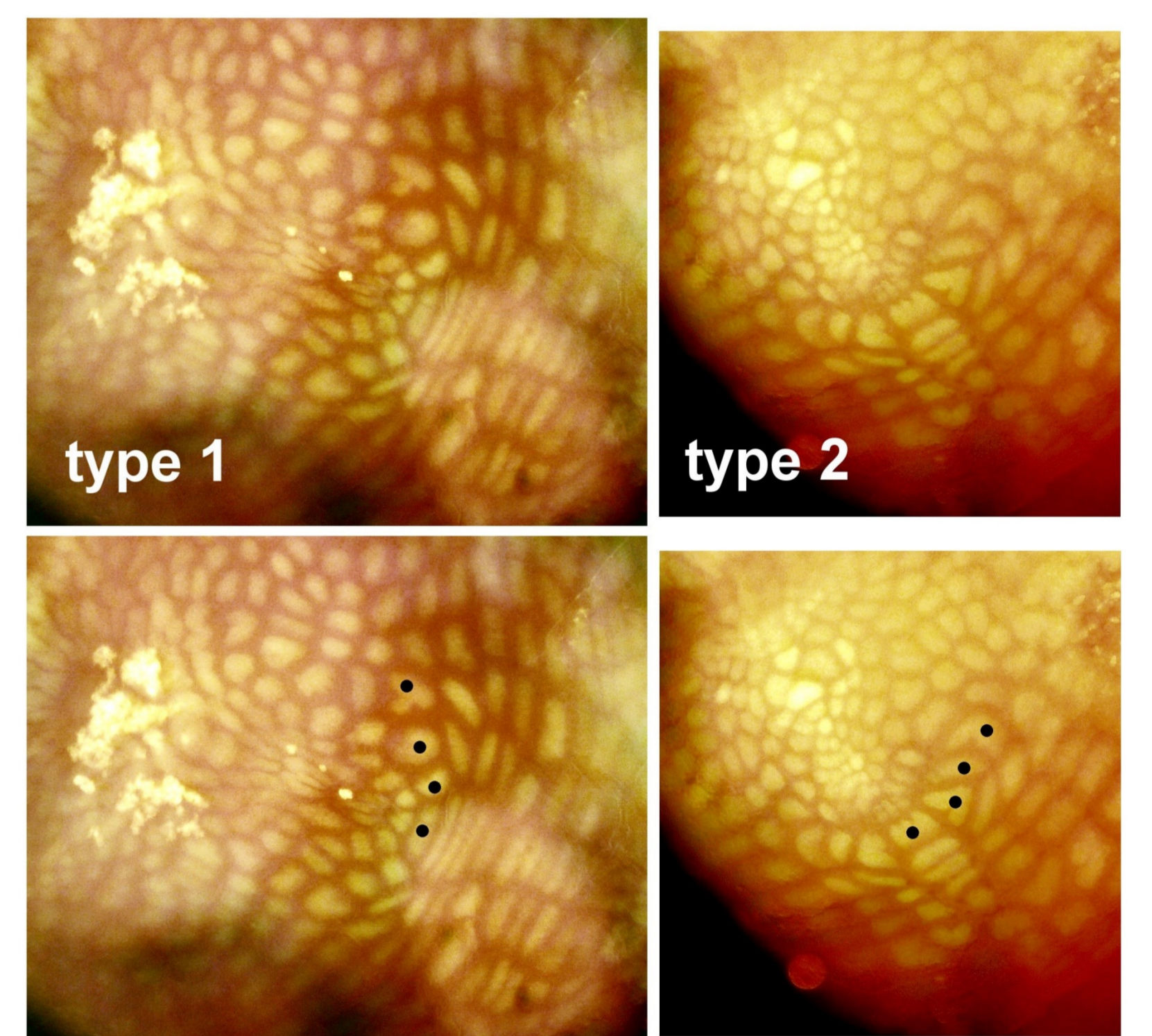
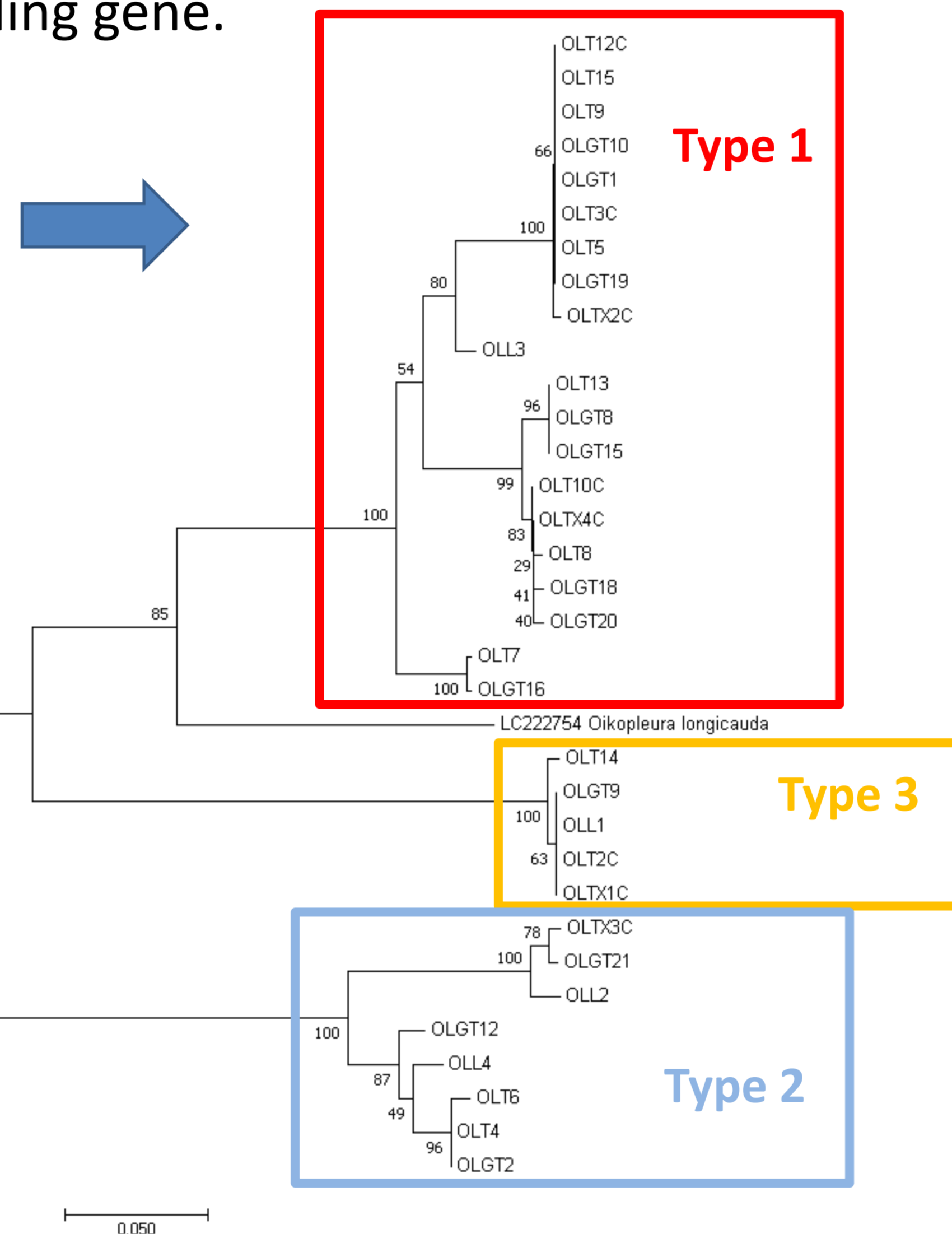


Widely used markers for population genetics such as mt genes are impossible to sequence in *O. dioica* due to presence of long T inserts in + strand (long A inserts in – strand). Such inserts can be in multiple sites per gene and can be more than 50 nucleotides long. Because of this COI is not a possible candidate as appendicularian barcoding gene.

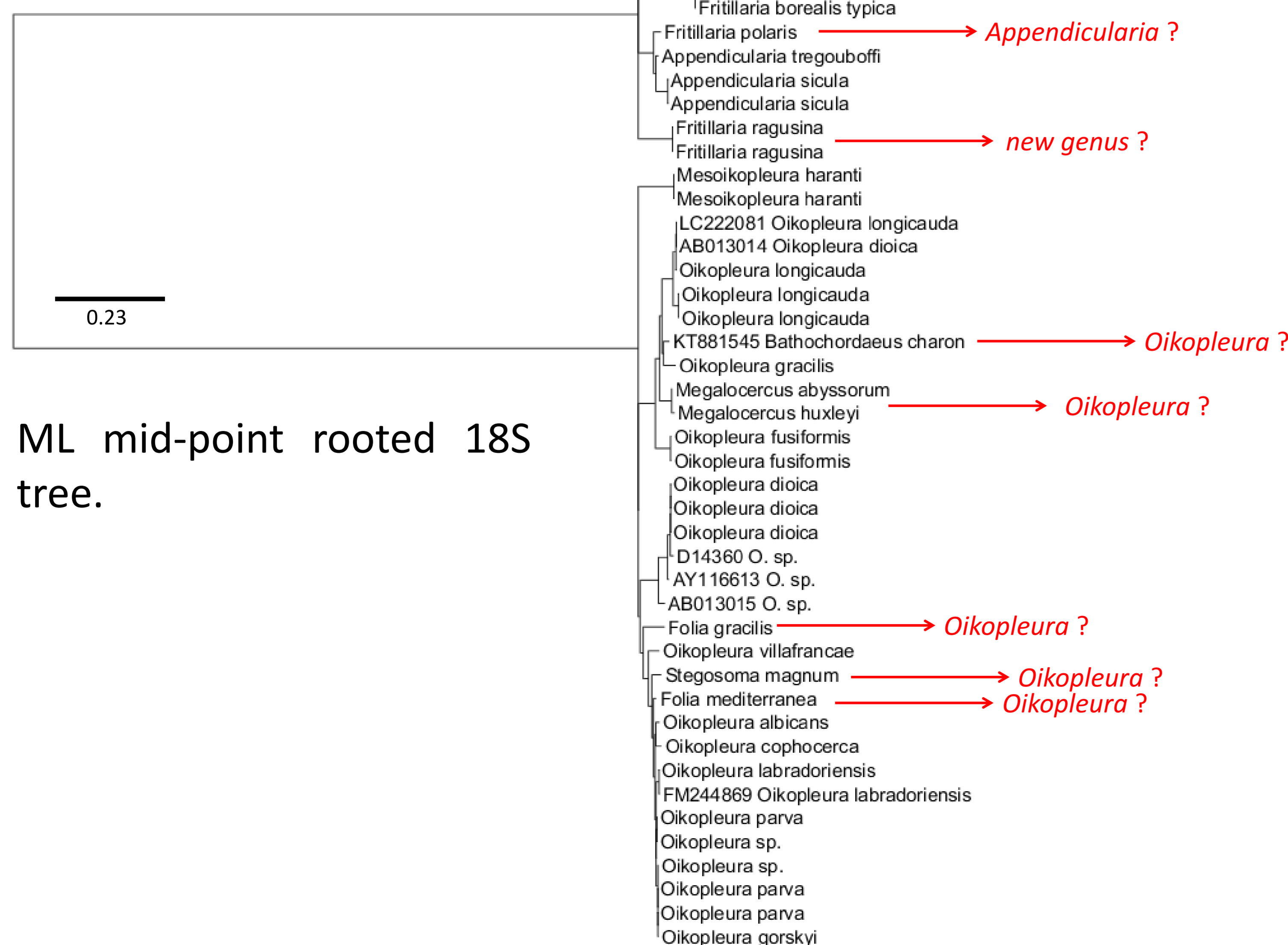
We obtained 307 bp of *O. longicauda* COI. This suggests that not all species have long T inserts, or at least they have long enough stretches without inserts to be usable as markers.

Based on COI, combined with 18S and ITS data, it seems that there are at least 3 cryptic *O. longicauda* species in the Adriatic, which also differ in the pattern of oikoblastic epithelium. The uncorrected p-distances of most distant „type 1” haplotypes is more than 10%. Because of this unusually high species threshold it seems unlikely that designing universal COI primers for appendicularians will be possible.

COI results of *O. longicauda* in the Adriatic suggest that appendicularian cryptic diversity might be substantial. This is further corroborated by 18S and ITS data.



NJ COI *O. longicauda* unrooted tree (left) and oikoblastic cell pattern of 2 cryptic species of *O. longicauda* (above).



ML mid-point rooted 18S tree.

18S data shows long distances between oikopleurid and fritillariid/kowalevskiid branch. For some species, for which more than one sequence is available, 18S sequences are not identical. This further corroborates the hypothesis that there are many cryptic appendicularian species within currently accepted morphological species. 18S has a great potential to be used as appendicularian barcoding gene because of many conserved regions flanking more variable regions.

The drawbacks of 18S is that for some groups of animals it is known to underestimate number of species. Because of appendicularian high evolutionary rate that may not be an issue. 18S might be suitable even for delimitation of closely related appendicularian species. 18S phylogeny of Appendicularia does not correspond completely to current systematics based on morphology. There are multiple genera which are not supported and even validity of Kowalevskiidae as a family is questioned.

ITS region in appendicularians is too variable to be used as a phylogenetic marker but it is useful as a barcoding marker. The ITS region is troublesome to use by PCR because of multiple variants within the same individual, but it could be useful in metabarcoding.

