Challenges in appendicularian integrative taxonomy

and barcoding

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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.

OLGT2

0.050

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.





 Fritillaria haplostoma Fritillaria haplostoma Fritillaria haplostoma Fritillaria formica digitata ^l Fritillaria formica tuberculata Fritillaria urticans Fritillaria helenae - Fritillaria gracilis Fritillaria charybdae Fritillaria fraudax Fritillaria fagei Fritillaria ? Tectillaria fertilis Fritillaria? Kowalevskia tenuis → Fritillaria ? ^l Kowalevskia oceanica -Fritillaria pellucida Fritillaria megachile Fritillaria sp. Fritillaria sp. Fritillaria venusta Fritillaria tenella Fritillaria borealis intermedia Fritillaria sp. I Fritillaria messanensis ¹Fritillaria messanensis Fritillaria borealis sargassi ^IFritillaria borealis sargassi Fritillaria borealis typica ¹Fritillaria borealis typica Appendicularia ? - Fritillaria polaris Appendicularia tregouboffi Appendicularia sicula ¹Appendicularia sicula Fritillaria ragusina → new genus ? Fritillaria ragusina Mesoikopleura haranti Mesoikopleura haranti LC222081 Oikopleura longicauda AB013014 Oikopleura dioica Oikopleura longicauda Oikopleura longicauda ¹Oikopleura longicauda KT881545 Bathochordaeus charon Oikopleura gracilis Megalocercus abyssorum ^LMegalocercus huxleyi Oikopleura fusiformis ¹Oikopleura fusiformis 1Oikopleura dioica Oikopleura dioica Oikopleura dioica ^LD14360 O. sp. ¹AY116613 O. sp. LAB013015 O. sp. → Oikopleura ? - Folia gracilis Oikopleura villafrancae → Oikopleura ? - Stegosoma magnum Folia mediterranea → Oikopleura ? Oikopleura albicans Oikopleura cophocerca Oikopleura labradoriensis FM244869 Oikopleura labradoriensis Oikopleura parva Oikopleura sp. Oikopleura sp. Oikopleura parva Oikopleura parva Oikopleura gorskyi

(above).

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 underrestimate 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 guestioned.

0.23

ML mid-point rooted 18S tree.

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.