

The Bobbit worm dilemma: a case for DNA

(Reply to Salazar-Vallejo *et al.* 2011. Giant Eunicid Polychaetes (Annelida) in shallow tropical and temperate seas. *Rev. Biol. Trop.* 59-4: 1463-1474)

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Received 04-I-2011. Corrected 20-IV-2011. Accepted 31-V-2011.

Whoever came up with the name “Bobbit worm”? It must be a fairly recent idea, given that the “regretful incident” in the Bobbit family only happened in 1993. Unfortunately, it is not clear who coined the name or when it was first used. The name does not do the worms justice and is misleading. For example, one of the explanations for the name that comes up when googling “Bobbit worm” is that the female bites off the male’s penis after copulation (seriously? Since when do they have penises?!). Neither are there any reports of eunicids attacking body parts of unsuspecting humans in their vicinity. The jaws resemble scissors, which is another possible explanation for the name, although actually, the “Bobbit incident” was committed with a carving knife.

Eunice species can indeed be voracious predators with impressive jaws. They are often accidentally introduced into reef tanks, hidden in “live rock”, and wreak havoc with other tank inhabitants. This behavior has given them a bad reputation in the aquarist’s community and beyond. One children’s book even includes them in a list of the most “terrifying and ugly sea creatures” (Christiansen 2008). While ugliness is a matter of taste, I would agree that, in their own little world, they are probably quite terrifying. But that’s still a far stretch from the Bobbits.

Not only is the name misleading with regard to the nature of the worms, it also leads to taxonomic confusion. Some sources specifically apply it to what they believe is *Eunice aphroditois*, others use it for any large eunicid. Given that not even eunicid taxonomists agree on the identity of *E. aphroditois* (well, taxonomists rarely agree with each other, but that’s a different story), it is not too surprising that untrained aquarists or recreational divers lump all the species together under one easily memorable common name.

Does size matter? Salazar-Vallejo *et al.* cite reports of eunicid worms of over 3 m length. The Australian museum even holds a specimen that reportedly was nearly 6 m long when collected (Fauchald 1992 and pers. comm.). (As a side note: six meters is long, but it’s still far from the longest worm ever reported. That honor falls to the nemertean *Lineus longissimus* which can grow to 30 m in length and probably at least twice as long when fully stretched out [McIntosh 1873-1874]). The main problem with using size in taxonomic studies is that even big worms start out as tiny worms and probably take many years to reach their full length and segment number. In most cases we do not know which characters are size-dependent. In traditional, type-based taxonomy, this can pose serious problems.

Color is another issue. One of the reasons Salazar-Vallejo *et al.* were able to shed some light on the taxonomic distinctness of different “Bobbit worms” is that they could identify different color patterns from photographs taken in different geographic regions. Most traditional taxonomists working with museum collections do not have that option, because the color is lost in the preserved specimens. Some early taxonomists such as de Quatrefages had the talent, patience and funds to illustrate their collections in color, based on live specimens, but many others did not. Even today, few taxonomic descriptions of annelids include color illustrations or photographs. Color patterns may be very distinctive in many cases, but color can also be a confounding factor. To what degree, for example, does color vary with diet? How much color variation is based on phenotypic plasticity and how much has a genetic basis? If we find different color morphs in otherwise morphologically similar individuals, how do we decide whether we are dealing with a single, polymorphic species or with separate species? One of the few studies that specifically addresses the latter issue is that by Nygren *et al.* (2011) of *Harmathoe imbricata*. This species has 10 different color morphs along the Scandinavian coasts but there is no genetic evidence suggesting they represent different species.

Salazar-Vallejo *et al.* conducted a lot of detective work to reconstruct the taxonomic history of *Eunice aphroditois* and other large *Eunice* species and make a convincing case for splitting *E. aphroditois* into several species reflecting different morphologies. Their main conclusion is that more type material, including neotypes and topotypes, needs to be examined to tease apart species and delineate their differences and intraspecific variation.

While I believe that the suggested work is crucial for resolving this and similar taxonomic dilemmas, I would also argue that molecular data should be added to this equation. The decreasing costs for DNA sequencing allow more and more researchers access to the technology or opportunities for collaboration.

Molecular data may not be necessary for each and every species description, but for complicated taxonomic problems, such as that of the large eunicids, DNA would add an independent, objective line of evidence.

An impediment for molecular work on annelids is that museum and ecological collections are usually formalin fixed. Formalin is still the best solution for preserving annelid morphology, but it crosslinks with DNA and proteins, making the extraction of quality DNA difficult if not impossible. Protocols for DNA extraction from formalinized tissue or for enzymatically repairing DNA do exist (Schander & Kenneth 2003, Skage & Schander 2007), but they are cumbersome and not reliably successful. New specimens would have to be collected from the type locations and tissue samples preserved in an appropriate manner while ensuring that voucher specimens are available for morphological studies. In many cases, especially for large worms, a small piece of tissue, such as a parapodium, can be preserved for molecular work, whereas the remainder of the specimen is fixed in formalin. That way, the same specimen can be studied morphologically and genetically.

A DNA barcoding approach would be the simplest solution. A few individuals of confirmed *Eunice aphroditois* from the type locality and, if possible, each of the potential other *Eunice* species proposed in Salazar-Vallejo *et al.*'s paper could be sequenced for one or two genes from the mitochondrial genome, such as the common barcoding gene cytochrome *c* oxidase subunit I (COI) or 16S ribosomal RNA, and the sequences of the proposed species could be compared with each other. The approach is not without flaws and often leads to new questions. For example, how different do two sequences, or sets of sequences, have to be before they are considered separate species? The substitution rate in the mitochondrial genome and consequently the amount of variation among species and individuals within species can vary from taxon to taxon (Galtier *et al.* 2009). Usually, if the interspecific variation for COI is greater than the intraspecific variation, a

more convincing case can be made for species boundaries. If not, the researchers should take a second look at the morphology and maybe re-evaluate some of the characters used in their identifications. Sometimes, genetic data show great differences among species that are morphologically identical or almost identical. An example for such a cryptic species complex in eunicids is the genus *Palola* (Schulze 2006). On the other side of the spectrum, the mitochondrial sequence data can help clarify whether the observed variation among samples, such as the color morphs in *Harmathoe imbricata* mentioned above, reflects species differences or intraspecific variation.

A more thorough approach would be to generate a multi-gene phylogeny for the entire genus *Eunice* in which all of the species in question are included. A great start was the paper by Zanol *et al.* (2010) who included 25 species of *Eunice* in their phylogenetic analysis of Eunicidae. Of the larger species mentioned in Salazar-Vallejo *et al.*'s paper only *E. rous-saei* and *E. cf. violaceomaculata* were included and appeared as sister species. Note the "cf." in *E. violaceomaculata*: the fact that not even experienced eunicid taxonomists could confidently identify the species is a further reminder that we need help from sources other than morphology. It would be interesting to know where the other large *Eunice* species fall in the tree. One of the most important finding by Zanol *et al.* (2010) was that *Eunice* is not monophyletic, warranting a revision of the entire family. As such, it is possible that some of the species proposed by Salazar-Vallejo *et al.* belong to different genera.

In conclusion, Salazar Vallejo *et al.*'s approach of examining color images of "Bobbit worms" from around the world highlights some of the taxonomic problems encountered in this group. I fully support the call for examining more topotypes and neotypes of the species in question, but strongly suggest incorporation of molecular data as well. Genetics will never

replace morphology in taxonomic studies, but will add a new dimension and perhaps clarity in delineating species. Rather than using one or the other data source, I believe that "Bobbit worms" are a good case in point for a combination of morphological and molecular data to provide a more complete picture of the evolutionary history of these terrifying and ugly creatures.

ACKNOWLEDGMENTS

Kristian Fauchald and Jon Norenburg helped retrieve some of the information on large eunicids and nemerteans. Elizabeth Borda provided comments that improved the manuscript. The work was supported by NSF AToL grant DEB-1036186.

REFERENCES

- Christiansen, P. 2008. Terrifying and ugly sea creatures. Gareth Stevens, New York, USA.
- Galtier, N., B. Nabholz, S. Glémin & G.D.D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol. Ecol.* 18: 4541-4550.
- Nygren, A., E. Norlinder, M. Panova & F. Pleijel. 2011. Colour polymorphism in the polychaete *Harmathoe imbricata* (Linnaeus, 1767). *Mar. Biol. Res.* 7: 54-62.
- Schander, C. & H.M. Kenneth. 2003. DNA, PCR and formalinized animal tissue - a short review and protocols. *Organisms Diversity & Evolution* 3: 195-205.
- Schulze, A. 2006. Phylogeny and genetic diversity of palolo worms (*Palola*, Eunicidae, Polychaeta) from the tropical North Pacific and the Caribbean. *Biol. Bull.* 210: 25-37.
- Skage, M. & C. Schander. 2007. DNA from formalin-fixed tissue: extraction or repair? That is the question. *Mar. Biol. Res.* 3: 289-295.
- Zanol, J., K.M. Halanych, T.H. Struck & K. Fauchald. 2010. Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. *Mol. Phyl. Evol.* 55: 660-676.