



On the identity and phylogenetic position of *Dero indica* (Clitellata: Naididae)

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Abstract

The identity and validity of the freshwater worm *Dero indica* (Clitellata: Naididae; Naidinae) has been debated, and it has been suggested that it is likely to be identical with *D. digitata*. In this study we combine a newly generated COI sequence of *D. indica* with available sequences from GenBank, to estimate the phylogeny of *Dero* using both Bayesian Inference and Maximum Likelihood. The trees show that *D. indica* is well separated from *D. digitata*, instead it is closest to *D. vaga*, but with low support. Furthermore, the analyses confirm the close relationship between *Dero* and *Branchiodrilus* found in previous studies, and indicates the presence of cryptic species in *D. furcata* and *D. digitata*.

Keywords DNA barcoding · Oligochaeta · India · COI

Introduction

The identity of the naeid worm *Dero indica* Naidu, 1962; pp 110-112 (Clitellata: Naididae: Naidinae) has historically been questioned. In the original description Naidu (1962) described it as being closest to *Dero digitata* and *D. zeylanica*, and in their global compendium on aquatic Oligochaeta, Brinkhurst and Jamieson (1971) regarded *D. indica* as most likely being identical to *D. digitata*. The ambiguity regarding *D. indica* was partly resolved by Naveed (2012) by studying several live and preserved specimens of *D. indica*, and found morphological differences. The two species of *Dero* differ from each other in the number of dorsal chaetae: *D. digitata* is characterized as having one hair and one needle chaeta, *D. indica* as

having two hair and two needle chaetae. However, so far, no genetic data from *D. indica* has been available, and the phylogenetic position is still unknown. *Aulophorus* Schmarda, 1861 is often treated as a junior synonym to *Dero* and are often synonymised, also in this study. Please note that Naidu (1962; pp 137-139) also described *Aulophorus indicus*, which becomes a junior homonym to *D. indica* when the two genera are treated as synonyms.

The aim of this study is to test if *Dero indica* is genetically distinct from *D. digitata* and other congeners, as well as placing it phylogenetically. To obtain this, phylogenies were estimated, with both Bayesian Inference and Maximum Likelihood, on a dataset consisting of newly obtained COI (Cytochrome c oxidase subunit I) sequence of *D. indica* combined with available sequences of *Dero* spp. from GenBank.

Sivabalan Srinivasan and Svante Martinsson contributed equally to this work.

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Material and methods

A specimen of *Dero indica* was collected at Neithavayal Pond (Minjur), Tamil Nadu, India, 13°16'43.1904" N, 80°15'11.7252" E and identified using a combination of the monograph by Brinkhurst and Jamieson (1971), the work of Naidu (2005) on Indian aquatic Oligochaeta and the guide by Timm (2009). The specimen was preserved in ethanol. The DNA extraction and amplification were performed at the Biozone Lab, Chennai, following a phenol chloroform protocol (Pachamuthu et al. 2000). The standard barcoding gene COI

(Cytochrome c oxidase subunit I) was amplified with the primer pair LCO1490 and HCO2198 (Folmer et al. 1994), using the following PCR-program: initial denaturation at 94 °C for 3 min followed with 32 cycles with 94 °C for 1 min, 48 °C for 1 min, and 72 °C for 1 min 20 s, and finishing with the final extension at 72 °C for 7 min. The obtained COI sequence of *D. indica* was deposited in GenBank (accession no. MK302407).

The new COI sequence of *D. indica* was combined with all COI sequences of *Dero* available in GenBank (downloaded 2019-09-02), as well as a set of other representatives of Naidinae as outgroups (see Table 1 for details), in total 27 sequences, the sequences were aligned using MAFFT v7.017 (Kato et al. 2002) as implemented in Geneious 8.1.9 (Biomatters Ltd., Auckland, New Zealand), using default settings.

Phylogenies were estimated using both Bayesian Inference in MrBayes v.3.2.6 (Ronquist et al. 2012) and Maximum likelihood (ML) using PhyML 3.0 (Guindon et al. 2010), as

implemented at the Montpellier Bioinformatics platform (<http://www.atgc-montpellier.fr/>). For the Bayesian analysis the alignment was partitioned according to codon position, partitions were unlinked. Rate variation across sites was set to gamma distribution with a proportion of invariable sites; model jumping was implemented to integrate over substitution model space. The analyses ran for 20 million generations sampling every 10,000 generations, the first 25% were discarded as burn-in, and a majority-rule consensus tree was constructed. For the ML analysis the Smart Model Selection (Lefort et al. 2017) with Bayesian Information criterion was used for automatic model selection; Subtree Pruning and Regrafting were used for tree improvement. Branch support was calculated with the SH-like (Shimodaira-Hasegawa test-like) approximative likelihood ratio test (aLRT) (Anisimova and Gascuel 2006). The trees were rooted according to the result in Erséus et al. (2017). All trees were drawn in FigTree 1.4.2 (Rambaut 2014) and further edited in Adobe Illustrator.

Table 1 Sequences included in the phylogeny, with species, country of collection, GenBank accession numbers, and references

Species	Collection country	GenBank acc. no:	Reference
<i>Dero indica</i> Naidu, 1962	India	MK302407	This study
<i>D. borellii</i> Michaelsen, 1900	United Kingdom	KY633385	Erséus et al. (2017)
<i>D. digitata</i> (Müller, 1774)	USA	AF534835	Bely and Wray (2004)
<i>D. digitata</i> (Müller, 1774)	USA	AF534836	Bely and Wray (2004)
<i>D. digitata</i> (Müller, 1774)	Canada	MF544417	Dewaard, J.R. (unpubl.)
<i>D. digitata</i> (Müller, 1774)	Sweden	KY633397	Erséus et al. (2017)
<i>D. furcata</i> Oken, 1815	USA	KP204260	Zattara and Bely (2015)
<i>D. furcata</i> Oken, 1815	–	HQ691221	Novo et al. (2011)
<i>D. furcata</i> Oken, 1815	USA	AF534837	Erséus et al. (2017)
<i>D. furcata</i> Oken, 1815	Taiwan	KY633388	Erséus et al. (2017)
<i>D. obtusa</i> d'Udekem, 1855	USA	AF534838	Bely and Wray (2004)
<i>D. obtusa</i> d'Udekem, 1855	Canada	MG423030	Dewaard, J.R. (unpubl.)
<i>D. superterrenus</i> (Michaelsen, 1912)	USA	KY633389	Erséus et al. (2017)
<i>D. sp1</i>	USA	GQ355368	Bely and Sikes (2010)
<i>D. sp</i>	USA	AF534840	Bely and Wray (2004)
<i>D. vaga</i> (Leidy, 1880)	USA	AF534839	Bely and Wray (2004)
<i>D. vaga</i> (Leidy, 1880)	USA	KY633412	Erséus et al. (2017)
<i>Allonais gwaliorensis</i> (Stephenson, 1920)	Cambodia	KY633391	Erséus et al. (2017)
<i>Al. inaequalis</i> (Stephenson, 1911)	Peru	KY633390	Erséus et al. (2017)
<i>Amphichaeta raptisae</i> (Chapman, 1981)	USA	GQ355365	Bely and Sikes (2010)
<i>Am. sannio</i> Kallstenius, 1892	Sweden	KY633392	Erséus et al. (2017)
<i>Branchiodrilus</i> spM10	India	MH744942	Martin et al. (2018)
<i>B. cleistocheata</i> Dahl, 1957	Cameroon	MH744913	Martin et al. (2018)
<i>Chaetogaster diaphanous</i> (Gruithuisen, 1828)	USA	AF534831	Bely and Wray (2004)
<i>Nais alpina</i> Sperber, 1948	Sweden	GU902104	Erséus et al. (2010)
<i>N. barbata</i> Müller, 1774	Sweden	JQ519863	Envall et al. (2012)
<i>N. bretscheri</i> Michaelsen, 1899	Switzerland	LN810267	Vivien et al. (2015)

Results

The newly generated sequence of *Dero indica* is 507 base pairs (bp) long, and the COI alignment is 658 bp long, whereof 272 are variable.

The Bayesian phylogenetic estimation resulted in mainly well resolved tree (Fig. 1a). However, *Dero* is not recovered as monophyletic, instead *Dero* and *Branchiodrilus* are found in a well-supported, but unresolved clade. This clade is a polytomy, consisting of a well-supported *Branchiodrilus*, and three groups of *Dero*. One of them consists of *D. indica* and *D. vaga*, but the sister relationship between them are unsupported. The other two groups of *Dero* are well supported. The first group consists of *D. borellii*, *D. furcata*, and *D. superrenus*, *D. furcata* forms two groups, and *D. borellii* is very close to one of them. The second group well-supported group consists of *D. digitata*, *D. obtusa*, and unidentified *D. sp.* the two *D. sp.* are both found together with one *D. digitata*, whereas the other *D. digitata* forms a separate group.

The ML phylogenetic estimation (Fig. 1b) is in most aspect similar to the Bayesian tree, *Dero* and *Branchiodrilus* form a weakly supported clade, and in contrast to the Bayesian analysis, both genera are recovered as monophyletic, the monophyly of *Dero* is weakly supported, whereas *Branchiodrilus* is strongly supported. The same three groups of *Dero* found recovered in

the Bayesian tree are also recovered here, with the same internal relationships as in the Bayesian analysis. The support for the sister-group relationship between *D. indica* and *D. vaga* is weak.

Discussion

Both phylogenetic analyses (Fig. 1) indicated a sister-group relationship between *D. indica* and *D. vaga* but, the support for this relationship is weak in both trees. However, we can conclude that *D. indica* is not closely related to *D. digitata*, as they well-separated in the trees, as well as differing morphologically (Naveed 2012), also summarised in the Introduction. We only have representatives of seven of the about 35–40 described species of *Dero*, and it is likely that *D. indica* is closer to some of the species not included in this study. More species need to be sequenced to find the exact position of *D. indica*.

Of the included species, two have deep divergences, indicating that there could be cryptic species involved. Furthermore, *D. borellii* is very close to one of the lineages of *D. furcata*, indicating that they could be the same species. To properly test this, more genetic markers are needed.

There are very few molecular studies on the clitellate fauna of India (Chakma et al. in press; Martin et al. 2018; see also Lalthanzara et al. 2018) and there are currently only seven

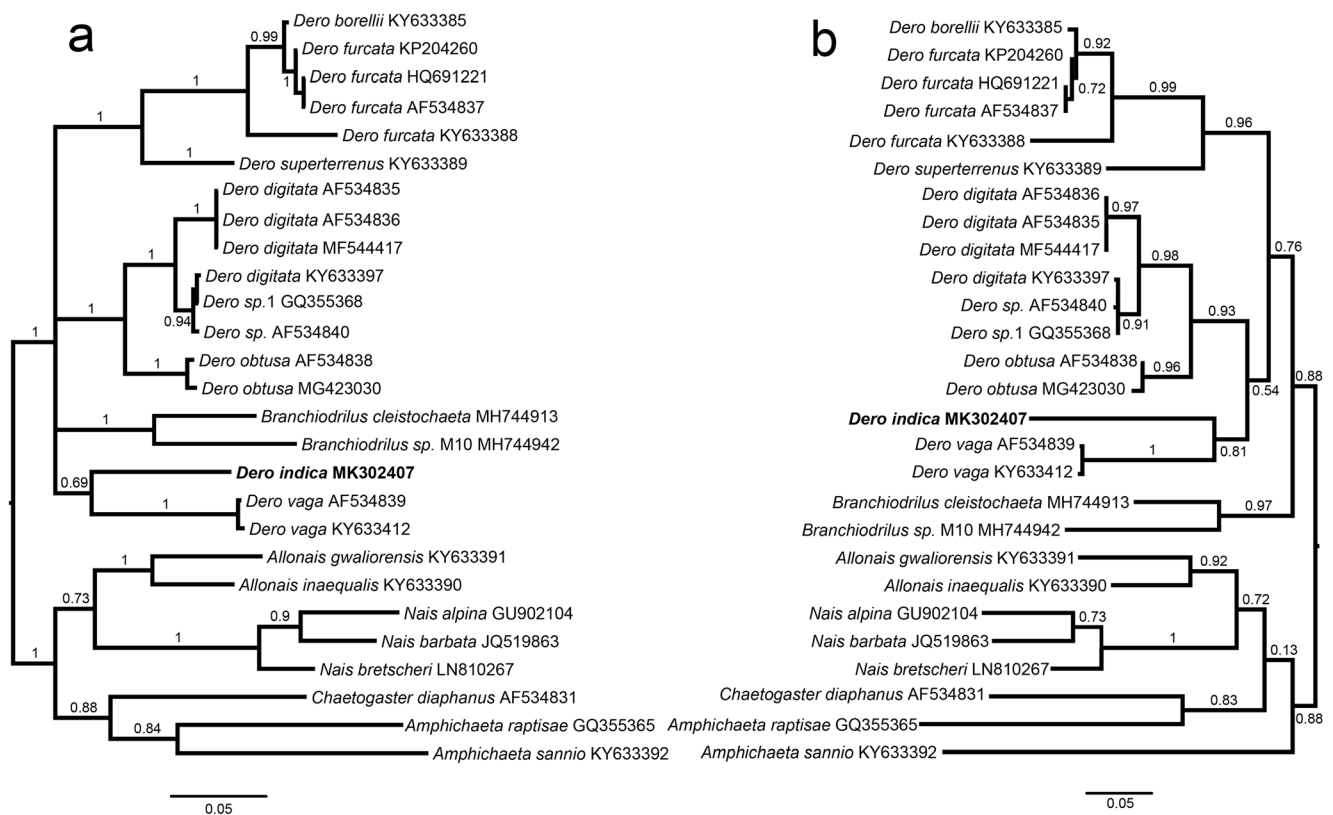


Fig. 1 COI trees of *Dero*. **a** Estimated with Bayesian Inference in MrBayes. Values at branches are posterior probabilities; **b** estimated with Maximum Likelihood in PhyML. Values at branches are SH-like aLRT support values. Scales show expected number of changes per site

records of COI sequences from the family Naididae from India in GenBank (search performed 2019-09-13). There is great potential for the use molecular methods in the exploration of the clitellate fauna of India, and hopefully, there will be more studies in the future, characterising the clitellate fauna of India.

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