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NOTA CIENTÍFICA

# Proposal of a trophic relationship between Chaetognatha and Cnidaria based on Mitochondrial COI sequences

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**ABSTRACT:** This is the first study in Brazil to propose a trophic relationship between Chaetognatha and Cnidaria based on molecular data (COI gene - DNA barcode). Cnidaria COI sequences were detected in tissue samples from two specimens of *Flaccisagitta enflata* collected in the surroundings of Fernando de Noronha archipelago, Tropical Western Atlantic. The methodology used followed all the requirements for a safe molecular manipulation, making the possibility of contamination unlikely. A search carried in BLAST showed that both sequences provided here presented a combination of highest query coverage and match identity with *Nemopilema nomurai* (Cnidaria: Scyphozoa), supporting the hypothesis that they belong to a species of jellyfish. Since gelatinous taxa are known to integrate the broad diet of Chaetognatha, it is possible that F. enflata has ingested this type of item, which was not fully digested until the moment of sampling and was not visualized by the transparency method. Thus, the record of Scyphozoa by the COI marker suggests a trophic relationship between the respective taxon and *F. enflata*. Future studies using the association of markers as well as specific methodologies may constitute a new perspective on how to evaluate the trophic ecology of key zooplankton species, including the abundant *F. enflata* in the Atlantic ocean.
**Keywords:** DNA barcode, food item, oceanic island, tropical Atlantic, Zooplankton, cytochrome oxidase subunit I gene

# Proposta de relação trófica entre Chaetognatha e Cnidaria baseada em sequências COI do DNA Mitocondrial

**RESUMO:** Este é o primeiro estudo no Brasil a propor uma relação trófica entre Chaetognatha e Cnidaria baseada em dados moleculares (gene COI - DNA barcode). Sequências COI de Cnidaria foram detectadas em amostras teciduais de dois espécimes de *Flaccisagitta enflata* coletados nos arredores do arquipélago de Fernando de Noronha, Atlântico Oeste Tropical. A metodologia utilizada seguiu todos os requisitos para uma manipulação molecular segura, tornando improvável a possibilidade de contaminação. Uma busca realizada no BLAST mostrou que ambas as sequências fornecidas aqui apresentaram uma combinação de maior cobertura e identidade com *Nemopilema nomurai* (Cnidaria: Scyphozoa), apoiando a hipótese de que elas pertencem a uma espécie de medusa. Uma vez que táxons gelatinosos podem integrar a vasta dieta de Chaetognatha, é possível que *F. enflata* tenha ingerido este tipo de item, o qual não foi totalmente digerido até o momento da amostragem e não foi visualizado pelo método de transparência. Assim, o registro de Scyphozoa pelo marcador COI sugere uma relação trófica entre o respectivo táxon e *F. enflata.* Estudos futuros compreendendo a associação de marcadores e metodologias específicas podem constituir uma nova perspectiva sobre como avaliar a ecologia trófica de espécies-chave do zooplâncton, incluindo a abundante *F. enflata* no oceano Atlântico.
**Keywords:** DNA barcode, item alimentar, ilha oceânica, Atlântico tropical, zooplâncton, gene citocromo oxidase subunidade I.

## Introduction

Zooplankton is composed of an extensive diversity of taxa, among which Chaetognatha is one of the most representatives in terms of abundance and frequency (CASANOVA, 1999). This phylum is known to gather carnivorous species of voracious predatory character, which makes them important

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builders of the planktonic communities by affecting the distribution, density and occurrence of their prey in environment (CHENEY, 1985). Its ecological relevance also extends to higher trophic levels, since it constitutes an important trophic link between Copepoda and fish of small to large size, including those of commercial interest (REEVE, 1970; ROGER, 1994). Thus, chaetognaths also may act as biological indicators of marine areas with attractive fishing potential.

Among the species widely distributed in the global oceans, *Flaccisagitta enflata* (GRASSI, 1881) is a highly frequent planktonic chaetognath in Brazilian waters (Tropical Western Atlantic). In this region, most studies involving this species focus on its spatial distribution, sometimes discussed with other types of data, such as biomass estimates or analysis of maturity stages (LIANG; VEGA PÉREZ, 1994, 2001, SOUZA; LUZ; MAFALDA JUNIOR, 2014). The trophic ecology, on the other hand, has still been poorly explored for this species (eg. LIANG; VEGA PÉREZ, 1995; MARAZZO; MACHADO; NOGUEIRA, 1997). In a study developed in the Humboldt Current System (Chile), it was demonstrated that *F. enflata* is an important carnivore in the surface layer of the water column, playing an essential role in the zooplankton community structure and in regional trophodynamics (GIESECKE; GONZÁLEZ, 2004). Thus, it is interesting to evaluate aspects related to the feeding of this species, to better understand the role of Chaetognatha as active predators in the marine food web.

The present investigation proposes a trophic relationship between *F. enflata* and Cnidaria (Scyphozoa), based on molecular data obtained with the cytochrome c oxidase subunit I (COI) gene, part of the mitochondrial DNA (DNA barcode). Data of this scope are pioneers in Brazil, and through deeper investigations and refinement of methodologies, may provide a new perspective on how to evaluate the trophic ecology of key zooplankton species.

## Material and Methods

Two specimens of *F. enflata* were collected in the surroundings of Fernando de Noronha, Tropical Western Atlantic (TWA) (03°49.43'S and 32°24.78'W) (Figure 1). Sampling was conducted through of research vessel Transmar I, in the scope of the project "Plankton community in the Saint Peter and Saint Paul's Archipelago and its association with physical mechanisms: vertical distribution of diversity and productivity". The samples were obtained by horizontal tows in the surface layer of the water column, using bongo plankton net of 500 µm mesh size and 0.60 m of mouth diameter. The sampled material was then washed rapidly in 3% sterile saline solution, and immediately fixed and preserved in 100% ethanol. In the laboratory, the chaetognaths were sorted from the sample as soon as possible, and the target species was identified according to specialized literature (CASANOVA, 1999). Each organism was subsequently dissected with pre-sterilized disposable blades. The tissue fragments obtained (~ 25 mm³) were kept in separate Eppendorf tubes, preserved in 100% ethanol and at a temperature of 4°C, until the DNA extraction

The plankton sample addressed in this article is part of a broader study, from which phylogeographic aspects of *F. enflata* were evaluated at neritic and oceanic TWA sites (MELO et al. 2020, in press). All biological material was obtained under the licenses of Ministério do Meio Ambiente (MMA): Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio - Number 17689) and the Sistema de Autorização e Informação de Biodiversidade (SISBio - Number 47270-5).

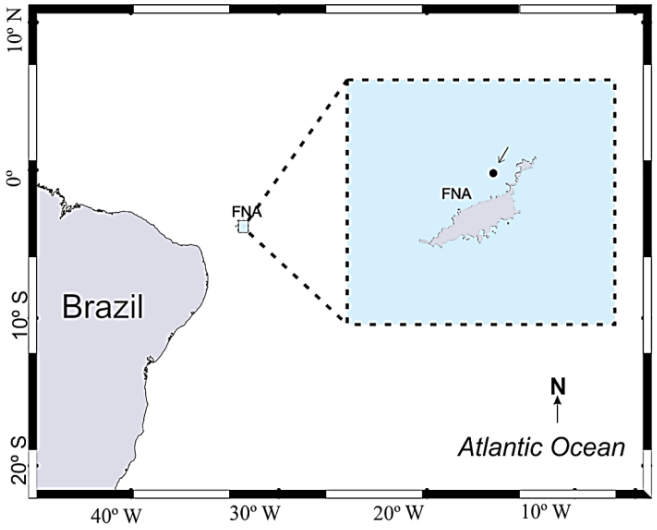


**Figure 1**. Sampling station in Fernando de Noronha archipelago, where the two specimens of *Flaccisagitta enflata* approached in the present study were collected

Total DNA of *F. enflata* was obtained using the Blood and Tissue extraction kit from Qiagen, following the manufacturer's protocol. Posteriorly, a polymerase chain reaction (PCR) was performed using the universal primers proposed by Folmer et al. (1994) for COI gene: LCOI LCO1490 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G - 3') and HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA - 3'). This primer pair amplifies nearly 710 bp. The reactions were established at a final volume of 20 µl, consisting of 10 µl of Master Mix Go Taq G2 C (Promega), 5 pmol of each primer, and 20 to 50 ng of extracted DNA. The reaction protocol involved an initial denaturation step at 95°C for 1 minute; followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 52ºC for 40 seconds and extension at 72ºC for 1 minute; with a final extension step at 72ºC for 5 minutes performed at the end of the last cycle (modified from FOLMER et al. 1994). The ExoSap-IT commercial system (USB Corporation, Cleveland, OH, USA) was used to purify the PCR products. These products were then sequenced and edited to generate the consensus sequences, which were compared with publicly available data in GenBank, using the Nucleotide - Basic Local Alignment Search Tool (BLAST).

The two COI sequences treated in this study were deposited in GenBank with the denomination of Cnidaria sp. (Accession Numbers MK577998 and MK577999).

## Results and Discussion

The present study reports the occurrence of two specimens of *F. enflata* that presented COI sequences incompatible with the genetic identity of this species. This result was obtained through sequencing 118 *F. enflata* organisms, of which 116 were satisfactorily sequenced and evaluated with a phylogeographic approach in the TWA (MELO et al. 2020, in press). It is worth mentioning that all organisms were previously selected, so that only the most conserved after fixation and without apparent stomach contents were sent to the genetic analyzes. The specimens were kept apart of any contaminating source as soon as sorted from the samples, and all steps prior to DNA extraction (sample handling, organisms screening and dissection with disposable slides) were performed under stringent cleaning conditions and using sterile or previously autoclaved materials. DNA extraction, amplification and sequencing also followed the requirements for a safe molecular manipulation and were conducted in proper laboratory environment. Thus, the possibility of biological contamination is considered unlikely.

The two COI sequences obtained had final sizes of 691 and 595 bp (MK57799.8 and MK57799.9, respectively). A search carried in BLAST showed that they presented a combination of highest query coverage and match identity with *Nemopilema nomurai* Kishinouye, 1922 (Cnidaria: Scyphozoa), among all available sequences in GenBank. Both sequences provided here exhibited an identity match higher than 82% with this species. Although we could not identify our sequences at the specific level (since the identity was < 95%), the query coverage and identity values combined corroborate with the hypothesis that they belong to a species of jellyfish. Among the four *N. nomurai* sequences detected by BLAST, three were sampled in Japan (LC055090.1, LC055083.1 and LC055030.1) (GOTOH et al. 2017) and one in China (KU360831.1) (DONG et al. 2016). This fact reflects the general distribution of species, cited as a subtropical pelagic jellyfish (60°N - 25°N, 117°E - 152°E), endemic and highly frequent off the East Asian Marginal Seas (UYE, 2008). No records of its distribution in the Atlantic ocean were found. Therefore, is suggested that the data here presented refers to a species of Scyphozoa of the Atlantic, whose COI sequences are currently not available in GenBank.

It is know that the Chaetognatha's diet is significantly diverse, since they are able to consume a wide size spectrum of classes and stages of zooplankton development (PEARRE, 1980). The species can be considered since opportunistic carnivores, preying those organisms found in greater abundance in the environment (eg. Copepoda - SULLIVAN, 1980; MARAZZO; MACHADO; NOGUEIRA, 1997); to selective carnivores, whose diet would be based mainly on the size, shape and/or aggregation behavior of their prey (FEIGENBAUM, 1991). The food item most consumed and the size of the prey may also be modified according to the stage of maturity of the chaetognaths (PEARRE, 1980; VEGA PÉREZ; LIANG, 1992; LIANG; VEGA PÉREZ, 1995; MARAZZO; MACHADO; NOGUEIRA, 1997). Remarkably, copepods are the main component of the stomach contents of the species, evaluated in the light of several studies (GIBBONS, 1992; VEGA PÉREZ; LIANG, 1992; LIANG; VEGA PÉREZ, 1995; MARAZZO; MACHADO; NOGUEIRA, 1997). However, alternative items can be consumed in smaller proportions, such as fish larvae, other crustaceans (euphausiids, cladocerans) and gelatinous and semi-gelatinous taxa, including other chaetognaths (intra and interspecific cannibalism), appendicularians and jellyfish (eg. ALVARIÑO, 1975; LIANG; VEGA PÉREZ, 1995; GIESECKE; GONZÁLEZ, 2004; VEGA PÉREZ; SCHINKE, 2011). It has been verified, for example, that *F. enflata* is predator of gelatinous items in general (GIESECKE; GONZÁLEZ, 2004), being able to consume hydromedusae in more advanced stages of maturity (MARAZZO; MACHADO; NOGUEIRA, 1997). In this sense, the sequences discussed here may reflect a food item consumed by the species, but which could not be visualized in the intestinal tube from the transparency method. This method may fail

to evidence certain structures consumed that were not fully digested until the moment of sampling, as the example of remaining mandibles of Copepoda (LIANG et al. 2003) and body structures of Cnidaria (LINDSAY et al., 2015). This is likely to be the case of the present investigation, regarding the record of Scyphozoa with the COI marker, suggesting a trophic relationship with *F. enflata*.

It is important to mention that the gene targeted here is satisfactorily applied to the diagnosis of metazoans. This occurs due to its wide distribution in the animal kingdom and to the fact that it is species-specific in many cases, even among phylogenetically close species (HEBERT et al. 2003). Such advantages have allowed its use in several investigations, including studies concerning trophic relationships based on the analysis of stomach contents, from invertebrates to large marine predators (BUCKLIN; STEINKE; BLANCO-BERCIAL, 2011). Although the application of the COI presents limitations for some groups, Lindsay et al. (2015) suggest that this gene will be extremely useful in identifying of Cnidaria, since satisfactory results were obtained with preliminary analyzes using fish and shrimp stomachs, as well as seabird scats. Other authors indicate the use of COI specific primers in analysis of this scope, especially if targeted at predators with diversified diets, such as Chaetognatha (BUCKLIN; STEINKE; BLANCO-BERCIAL, 2011). In addition, the combined use of distinct markers (as COI and 16S, for instance) may help to broaden the list of cataloged prey, as well as investigate other issues more deeply, as different patterns of feeding over an observed period. Future studies on the group should consider such aspects.

This paper is the first proposal turned to the trophic ecology of Chaetognatha based on genetic data in Brazil. Although still costly, molecular analyzes constitute reliable and effective methods at scientific application, and can to establish relevant advance in assessing the complex relationships that make up the marine food chain.

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