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Studying sources of incongruence in arthropod molecular phylogenies: Sea spiders (Pycnogonida) as a case study

Étude des sources de conflit dans les phylogénies moléculaires des arthropodes : l'exemple des pycnogonides

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ABSTRACT

In this report, we analyze the phylogeny of Pycnogonida using the three nuclear and three mitochondrial markers currently sequenced for studying inter- and intrafamilial relationships within Arthropoda: 18S and 28S rRNA genes, Histone H3, cytochrome c oxidase subunit 1 (CO1), 12S and 16S rRNA genes. We identify several problems in previous studies, due to the use of inappropriate sequences (taxonomic misidentification, DNA contamination, sequencing errors, missing data) or taxa (outgroup choice). Our analyses show that most markers are not powerful to study the phylogeny of sea spiders. The results suggest however a recent diversification of the group (Mesozoic rather than Paleozoic) and the early divergence of Austrodecidae, followed by Colossendeidae, Pycnogonidae and Rhynchothoracidae. Except Ammotheidae and Callipallenidae, all other families were recovered as monophyletic. Analyses of synonymous sites in CO1 sequences reveal an extreme heterogeneity of nucleotide composition within sea spiders, as six unrelated species show a reverse strand-specific bias. We therefore suggest that several independent reversals of asymmetric mutational constraints occurred during the evolution of Pycnogonida, as a consequence of genomic inversions involving either the control region or a fragment containing the CO1 gene. These hypotheses are supported by the comparison of two complete mitochondrial genomes of sea spiders (*Achelia bituberculata* and *Nymphon gracile*) with that of *Limulus*.

Mots-clés :
Arthropoda
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Phylogénie
Conflit
Composition nucléotidique
Biais brin-spécifique

RÉSUMÉ

Dans ce travail, nous analysons la phylogénie des pycnogonides (Arthropoda, Pycnogonida) en utilisant les trois gènes nucléaires et les trois gènes mitochondriaux les plus couramment séquencés pour étudier les relations inter- et intrafamiliales au sein des arthropodes : ARNr 18S et 28S, Histone H3, sous-unité 1 de la cytochrome c oxydase (CO1), ARNr 12S et 16S. Nous montrons que les études précédentes ont été affectees par l'utilisation de séquences problématiques (mauvaise identification taxinomique, contam-

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ination d'ADN, erreurs de séquence, données manquantes) et par un choix peu judicieux des groupes externes. Nos analyses indiquent que la plupart des marqueurs ne sont pas suffisamment informatifs pour étudier la phylogénie des pycnogonides. Les résultats favorisent toutefois l'hypothèse d'une diversification au Mésozoïque plutôt qu'au Paléozoïque, et suggèrent la divergence précoce des Austrodecidae, suivie par celle des Colossendeidae, Pycnogonidae et Rhynchothoracidae. À l'exception des Ammotheidae et des Callipallenidae, toutes les familles sont trouvées monophylétiques. L'analyse des sites synonymes du gène CO1 révèle une forte hétérogénéité de la composition nucléotidique, puisque six espèces non apparentées ont subi une inversion du biais brin-spécifique. Ainsi, nous suggérons que l'évolution des pycnogonides a été marquée par plusieurs évènements indépendants d'inversion des contraintes mutationnelles asymétriques, en raison de multiples inversions génomiques impliquant soit la région de contrôle, soit un fragment comprenant le gène CO1. Ces hypothèses sont confirmées par l'analyse comparative des génomes de *Limulus* et de deux espèces de pycnogonides : *Achelia bituberculata* et *Nymphon gracile*.

1. Introduction

Pycnogonids or sea spiders are exclusively marine organisms, found from shallow waters to abyssal depths in all oceans and seas. They comprise 1334 described species arranged in 10 families [1]: Ammotheidae, Austrodecidae, Callipallenidae, Colossendeidae, Endeidae, Nymphonidae, Pallenopsidae, Phoxichilidiidae, Pycnogonidae and Rhynchothoracidae. The group is characterized by several morphological autapomorphies, such as the prominent external proboscis, the ovigers (a specialized pair of appendages on the cephalosoma) and multiple gonopores [2]. Most morphological and molecular systematists consider that they belong to Chelicerata [3–6], and the homology between the chelifores of pycnogonids and chelicerae of euchelicerates (horseshoe crabs and arachnids) has been confirmed using Hox gene expression patterns [7].

Six molecular markers are currently used in most arthropod phylogenies dealing with inter- and intrafamilial relationships: 18S and 28S rRNA genes (18S and 28S), Histone H3 (H3), cytochrome c oxidase subunit 1 (CO1), 12S and 16S rRNA genes (12S and 16S). For instance, they have been used to infer the phylogenetic relationships among centipedes [8], flies [9], decapods [10], and spiders [11]. The phylogeny of Pycnogonida has been also explored recently employing all these six markers [1] or only the 18S gene [12]. These studies have provided conflicting results for interfamilial and intergeneric relationships (Fig. 1). Four families were found monophyletic in both analyses: Colossendeidae, Endeidae, Pallenopsidae and Pycnogonidae. Members of the families Callipallenidae and Nymphonidae were grouped together, but the monophyly of each family was not supported. Concerning the family Ammotheidae, *Ascorhynchus* and *Eurycyde* were found to be distantly related to other ammotheid genera – either as the sister group of Nymphonidae and Callipallenidae [1], or as an early offshoot of sea spiders [12]. Surprisingly, Nakamura et al. [12] included them in their own family Ascorhynchidae, even though their analyses indicated paraphyly of this group. Other genera of Ammotheidae, such as *Ammothea*, *Achelia*, *Sericosura* and *Tanystylum*, were found to belong to a clade including the family Endeidae, as well as *Anoplodactylus*, one of the three genera of the family Phoxichilidiidae. In Arango and Wheeler [1], *Rhynchothorax* (Rhynchothoracidae) was also included in this clade, whereas *Phoxichilidium* was related to Pycnogonidae, suggesting polyphyly of Phoxichilidiidae. On the contrary, Nakamura et al. [12] found *Rhynchothorax* to be the sister-group of Pycnogonidae.

The main aim of the present study is to identify the causes or sources of conflicting results between the molecular studies of Arango and Wheeler [1] and Nakamura et al. [12], in order to provide a better understanding of pycnogonid phylogeny. Several non-exclusive hypotheses can be advanced to explain these conflicts: inadequate taxonomic sampling (including choice of outgroup species), DNA contamination, misidentification of species, missing data, use of different methods of tree reconstruction (direct optimization *versus* multiple alignment, total evidence *versus* separate analyses), over-interpretation of the results (lack of signal), conflicting signals among genes, and significant heterogeneity in nucleotide composition and/or evolutionary rates among taxa.

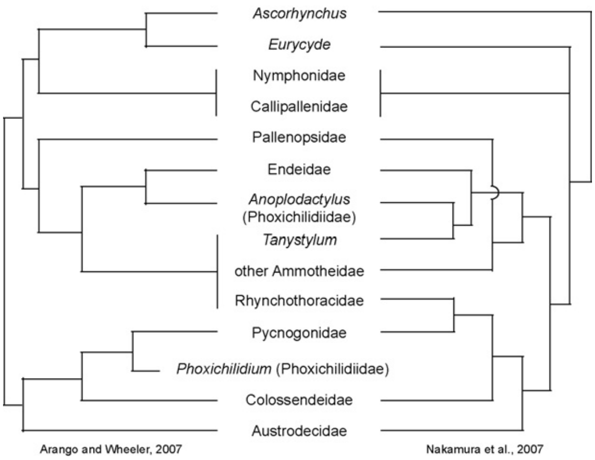

Fig. 1. Conflicts between previous molecular phylogenies of Pycnogonida. The phylogeny of Arango and Wheeler [1] (on the left) is compared to the phylogeny of Nakamura et al. [12] (on the right). Main conflicts lie in the positions of the families Pallenopsidae and Rhynchothoracidae, and those of the genera *Ascorhynchus*, *Eurycyde* and *Tanystylum*.