



Evolution/Évolution

The phylogenetic position of the 'living fossils' *Neoglyphea* and *Laurentaeglyphea* (Decapoda: Glypheidea)*La position phylogénétique des « fossiles vivants » Neoglyphea et Laurentaeglyphea (Decapoda: Glypheidea)*

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ARTICLE INFO

Article history:

Received 18 June 2010

Accepted after revision 27 August 2010

Available online 29 September 2010

Keywords:

Phylogenetics

Reptantia

Achelata

Astacidea

New Caledonia

Mots clés :

Phylogénie

Reptantia

Achelata

Astacidea,

Nouvelle Calédonie

ABSTRACT

The Glypheidea is a group of lobster-like decapods that appeared in the Triassic and that was thought to be extinct until 1975, when a specimen of the species *Neoglyphea inopinata* was caught off the Philippines. More recently, in 2005, a specimen of another glypheid species, *Laurentaeglyphea neocaledonica*, was discovered near New Caledonia. Here, we construct a decapod molecular data set including the two extant glypheid species sequenced from eight nuclear and mitochondrial genes. Our study strongly shows that the two extant genera of glypheids cluster together, and further confirms the status of Glypheidea as a separate infraorder. Moreover the reptantian decapods are divided into two major groups, one including Brachyura, Anomura, and Axiidea, and the other including Astacidea, Polychelida, Achelata, and Glypheidea. Although commonly nicknamed 'Jurassic shrimps' and considered as 'living fossils', glypheids are therefore a derived decapod lineage.

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R É S U M É

Les glyphéides sont des décapodes apparus au Trias que l'on pensait éteints jusqu'en 1975, date à laquelle un spécimen de l'espèce *Neoglyphea inopinata* a été découvert aux Philippines. En 2005, un spécimen d'une autre espèce, *Laurentaeglyphea neocaledonica*, a été collecté près de la Nouvelle Calédonie. Nous présentons ici un jeu de données moléculaires des décapodes, incluant les deux espèces de glyphéides vivantes et basé sur huit gènes nucléaires et mitochondriaux. Notre étude montre que les deux glyphéides actuelles forment un groupe monophylétique et confirme le statut de sous-ordre pour les Glypheidea. Les Reptantia apparaissent divisés en deux groupes principaux : le premier comprend les Brachyura, Anomura et Axiidea et le second inclut les Astacidea, Polychelida, Achelata et Glypheidea. Bien que surnommées « crevettes du Jurassique » et considérées comme des « fossiles vivants », les glyphéides constituent donc une lignée dérivée de décapodes.

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1. Introduction

Crustaceans are the fourth most species-rich group of animals on the planet, following insects, chelicerates, and molluscs. Foremost among the crustaceans in number and morphological diversity are the decapods. With nearly 15,000 extant species, they include the well-known crabs (Brachyura), hermit crabs and their relatives (Anomura), shrimps (Dendrobranchiata, Caridea, and Stenopodidea), lobsters (Astacidea, Gebiidea, and Axiidea), and other lesser known groups: Achelata, Polychelida and Glypheidea [1–3].

The Glypheidae Winckler 1883 are a group of lobster-like decapods that first appeared in the Lower Triassic, flourished during the Jurassic, became less abundant in the Cretaceous, and apparently disappeared before the end of the Eocene [4]. Glypheids were therefore thought to be an extinct group until 1975, when a male specimen of the species *Neoglyphea inopinata*, caught off the Philippines at a depth of 185 meters in 1908 and kept in the collections of the Smithsonian Institution, was described [4]. Subsequent expeditions in 1976, 1980, and 1985, captured additional specimens at the same site, and another specimen was trawled in the Timor Sea. This discovery stimulated new research on the group, and subsequently the glypheid lobsters were recognized as a separate infraorder [5]. More recently, in October 2005, during the EBISCO cruise (MNHN-IRD), a single female specimen of another glypheid species was discovered on a seamount in the Coral Sea, near New Caledonia, at a depth of 400 meters. Nicknamed 'Jurassic shrimp', it received some attention from the media [6]. This specimen has been named *Laurentaeglyphea neocaledonica* in 2006 [7,8].

Although several markers are now available that can resolve deep and shallow relationships within the Decapod Tree of Life [9,10], only four rRNA gene fragments (12S, 16S, 18S and 28S rRNA) have been sequenced from *Neoglyphea inopinata*. Consequently, the exact phylogenetic position of the Glypheidea is still unresolved [9]. Here, we sequenced four additional protein-coding genes, both mitochondrial and nuclear (cytochrome oxidase I, histone H3, phosphoenolpyruvate carboxykinase and sodium-potassium ATPase α -subunit) from the two extant glypheid species in order to investigate their relationships among decapods.

2. Material and methods

We constructed an expanded decapod molecular data set, which included representatives from Dendrobranchiata, Stenopodidea, Caridea, Brachyura, Anomura, Axiidea, Astacidea, Polychelida, Achelata, and the two extant glypheid species *Neoglyphea inopinata* and *Laurentaeglyphea neocaledonica*. The genes sampled are three mitochondrial genes: cytochrome oxidase I (COI), 12S and 16S rRNA, and five nuclear genes: histone H3, 18S and 28S rRNA, phosphoenolpyruvate carboxykinase (PEPCK) and sodium-potassium ATPase α -subunit (NaK).

2.1. Data collection

Table 1 lists the taxa used in the study. All the specimens sequenced for the study are housed in the

crustacean collection at the *Muséum national d'histoire naturelle* in Paris and preserved in 80% ethanol.

DNA extraction was performed using the QIAamp DNA Micro Kit commercialized by Qiagen. Amplification and sequencing were performed using the following sets of primers: L1490 and H2198 for COI [11]; 16Sar and 16Sbr for 16S rRNA [12]; H3F1 and H3R1 or H3F2 and H3R2 for Histone H3 [13]; 12S1 and 12S3 [14] or 12SF and 12SR [15] for 12S rRNA; 1F-5R, 3F-18Sbi and 18Sa2.0-9R [16,17] for 18S rDNA; 28SC1 [18] and 28SC2 or 28SD2 [19] for 28S rDNA; PEPCK for, PEPCK for2, PEPCK rev and PEPCK rev3 [20] for PEPCK; NaK for-b and NaK rev [20] for NaK.

PCR products were sequenced using the BigDye Terminator V3.1 kit (Applied biosystem) and the ABI3730XL sequencer. Both strands of the PCR products were sequenced. The two strands obtained for each sequence were combined using the BioEdit Sequence Alignment Editor program [21].

The 70 sequences generated for this work have been deposited in GenBank under accession numbers HQ241499-HQ241568.

Nucleotide sequences were aligned by eye. Alignment was straightforward for the protein-coding genes, COI, H3, NaK and PEPCK, resulting in datasets of 666, 330, 534, and 570 base pairs (bp), respectively. Variable regions of the 16S, 18S and 28S genes were difficult to align reliably and were removed from the alignments. The final aligned sequences consisted of 438 bp for 16S, 1869 bp for 18S, 358 bp for 28S, and 364 bp for 12S. The few remaining gaps were treated as missing data in all further analyses. Alignments can be obtained from Marie-Catherine Boisselier.

2.2. Phylogenetic analyses

We performed both separate and combined analyses. We built phylogenies using Maximum Likelihood (ML) and Bayesian methods of inference. ML analyses were performed with RAXML 7.0.4 [22,23], and Bayesian analyses were performed with MrBayes 3.1 [24]. We used eight data partitions in the combined analyses, corresponding to the eight genes here sampled.

Bayesian analyses were performed by running 5,000,000 generations in four chains, saving the current tree every 100 generations, with the model inferred by Modeltest using the AIC criterion [25] applied to each partition (GTR). The last 48,000 trees were used to construct a 50% majority rule consensus tree. For the ML analysis, we used the same eight partitions and performed 1000 bootstrap replicates.

3. Results and discussion

As all recent molecular studies found Dendrobranchiata, Stenopodidea and Caridea to be the most basal decapod infraorders, we used them as outgroups [1,9,10,20,26,27]. The remaining decapods (Reptantia) are divided into two major groups supported by ML BP and Bayesian PP values of 100%, one including Brachyura, Anomura, and Axiidea, and the other including Astacidea, Polychelida, Achelata, and Glypheidea (Fig. 1).

Table 1

List of taxa and markers used in this study.

			COI	12S	16S	H3	18S	28S	PEPCK	NaK	
Dendrobranchiata	Penaeoidea	<i>Aristeus</i> ssp.	----	HQ211500	HQ241511	HQ241556	HQ241523	HQ241534	EU427212	EU427143	
		<i>Penaeus</i> ssp.	AY781297	EF584003	AF192088	---	AF186250	AF124597	EU427213	EU427144	
Pleocyemata	Caridea	Crangonoidea	<i>Glyphocrangon</i> ssp.	HQ241546	HQ241502	HQ241513	HQ241558	HQ241525	HQ241537	EU427242	EU427173
		Pandaloidea	<i>Heterocarpus</i> ssp.	HQ241547	HQ241503	HQ241514	HQ241559	HQ241526	HQ241537	EU427243	EU427174
			<i>Plesionika</i> ssp.	HQ241552	HQ241507	HQ241519	HQ241564	HQ241530	HQ241541	EU427249	EU427180
	Stenopodidea		<i>Stenopus</i> ssp.	AF125441	----	DQ079734	DQ079701	DQ079769	DQ079812	EU427247	EU427178
	Axiidea	Callianassoidea	Callianassidae	AF436025	----	DQ079777	DQ079664	DQ079741	DQ079777	EU427236	EU427167
	Achelata	Palinuroidea	<i>Panulirus</i> ssp.	AF339470	----	AF337976	DQ079697	DQ079765	DQ079808	EU427230	EU427161
			<i>Puerulus angulatus</i>	HQ241554	HQ241509	HQ241521	HQ241566	HQ241532	HQ241543	EU427233	EU427164
	Anomura	Galattheoidea	<i>Munida</i> ssp.	HQ241548	HQ241504	HQ241515	HQ241560	HQ241527	HQ241538	EU427188	EU427119
		Lomosoidea	<i>Lomis hirta</i>	AY595672	AY595547	AF436052	DQ079680	AF436013	AY596101	EU427187	EU427118
		Paguroidea	<i>Coenobita</i> ssp.	HQ241545	HQ241501	HQ241512	HQ241557	HQ241524	HQ241535	EU427184	EU427115
	Astacidea	Nephropoidea	<i>Homarus</i> ssp.	AF370853	DQ298427	AF370876	DQ079675	AF235971	AY859581	EU427219	EU427150
			<i>Nephropsis</i> ssp.	HQ241551	HQ241506	HQ241518	HQ241563	HQ241529	HQ241540	EU427221	EU427152
			<i>Thaumastocheles</i> ssp.	HQ241555	HQ241510	HQ241522	HQ241567	HQ241533	HQ241544	EU427223	EU427154
	Brachyura	Cancridea	<i>Cancer</i> ssp.	AF060771	----	DQ079708	DQ079668	DQ079743	DQ079781	EU427196	EU427127
		Portunoidea	Portunidae	AY616443	AY919085	AJ130811	DQ079669	DQ079744	DQ079782	EU427205	EU427136
	Glypheidea		<i>Neoglyphea inopinata</i>	HQ241549	DQ298431	HQ241516	HQ241561	AY583968	AY583986		
			<i>Laurentaeglyphea neocaledonica</i>	HQ241550	HQ241505	HQ241517	HQ241562	HQ241528	HQ241539	HQ241499	HQ241568
	Polychelida	Eryonoidea	<i>Polycheles</i> ssp.	HQ241553	HQ241508	HQ241520	HQ241565	HQ241531	HQ241542	EU427234	EU427165

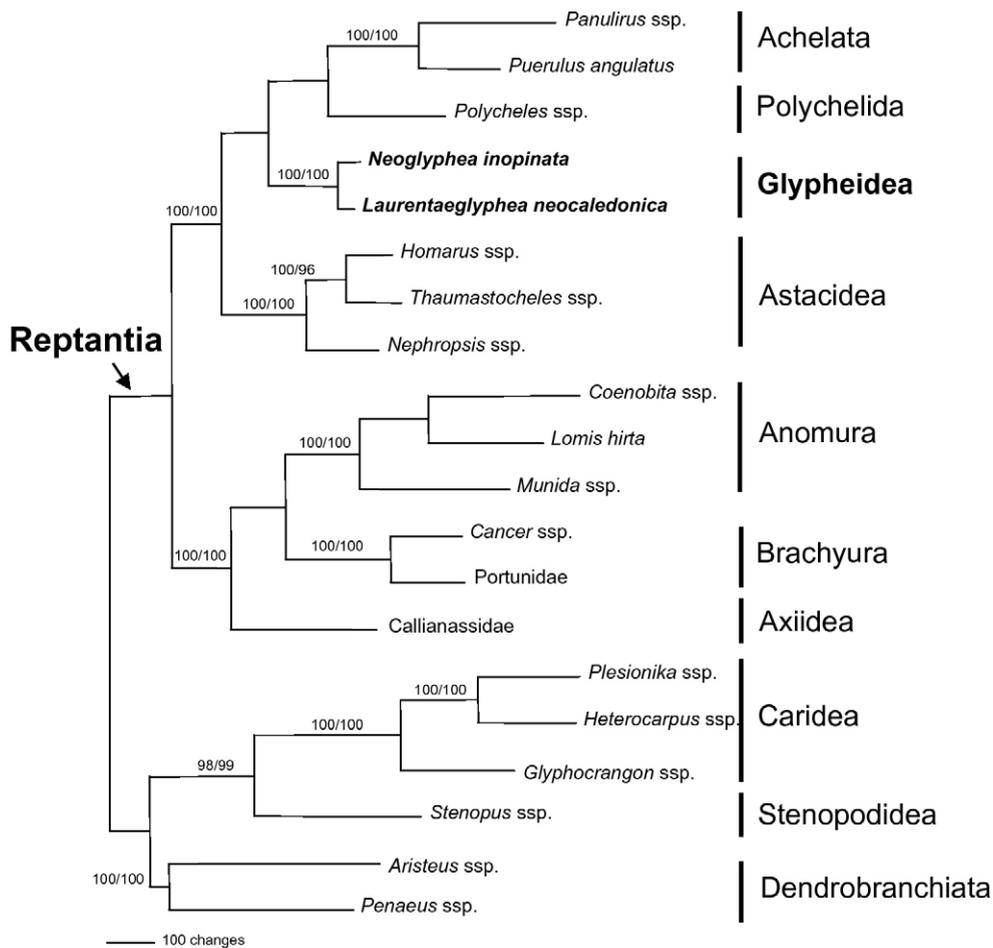


Fig. 1. Bayesian tree obtained from the combined data set (COI, 12S and 16S rRNA, 18S and 28S rRNA, H3, PEPCCK and NaK; 5129 bp). Nodes with values are supported by Bayesian posterior probabilities values and ML bootstrap values above 95%.

The phylogenetic position of the Glypheidea has long been debated [5,28–33] and our study is the first molecular one to strongly support the inclusion of Glypheidea within one of the two major reptantian clades.

Our study also strongly shows that the two extant genera of glypheids cluster together. It further confirms the status of Glypheidea as a separate infraorder [5,34], in disagreement with several authors who considered glypheoids as Astacidea [33,35,36].

Among the major clade identified here that includes Astacidea, Polychelida, Achelata, and Glypheidea, the position of the latter is however unresolved. In the ML analysis, Glypheidea is placed as sister-group to Achelata (BP value < 50%, a result also obtained with weak support by Bracken et al. [9]) while the Bayesian analysis places Glypheidea as the sister-group to Achelata plus Polychelida (both forming the ancient Palinura infraorder) with a PP value of 72%.

Glypheids somewhat resemble crayfish or lobsters and are hypothesized to have an astacidean-like habitus (lifestyle) as well as a growth close to that observed for *Nephrops norvegicus* [34]. They also appear to be close to the Astacidea through their thoracic and abdominal

structures, by their mouthparts and gills, and by the presence of gonopods [7]. The presence of a diaeresis on the uropodal exopod, with a spinose and distinct margin is another common feature and a horizontal position of the first pereopod has been inferred to be a synapomorphy of the Astacura (Glypheidea plus Astacidea) [32]. On the basis of nodal robustness, our results do not exclude a sister relationship of glypheids with astacideans [5].

Conflict of statement

No conflict of interest for any of the authors.

Acknowledgements

We thank A. Crosnier, R. Cleva and J. Brisset for their help in collection management. This work was supported by the “Consortium national de recherche en génomique”, and the “Service de systématique moléculaire” of the Muséum national d’histoire naturelle (CNRS UMS 2700). It is part of the agreement No. 2005/67 between the Genoscope and the Muséum national d’histoire naturelle

on the project “Macrophylogeny of life” directed by Guillaume Lecointre.

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