

BARCODING MARINE CRUSTACEANS: FROM BIODIVERSITY TO ECONOMY



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INTRODUCTION

DNA barcoding based on a fragment of cytochrome c oxidase I (COI) has been proposed as an identification system for the animal kingdom (1,2) and proved to be effective for different groups including crustaceans (3). With 8329 entries for COI in GenBank (May, 2007), there is a first guide for assembling a large database of barcodes for this highly diverse group. A number of marine crustaceans are harvested on large scale as food resources and crustacean fisheries bring an important revenue each year in Atlantic Canada. A potential collapse due to overexploitation caused a search for additional economical species and for accurate identification of larval stages, hence DNA barcoding. Our goal is to provide COI barcodes for all economically important marine crustaceans in NW Atlantic and an additional 150 species of benthic crustaceans (mainly amphipods).

MATERIAL AND METHODS

- Sampling: 127 specimens collected between 2003-2006 in NW Atlantic and the Arctic Ocean (Fig.1).
- DNA extraction: DNeasy Tissue kit (Qiagen) or E.Z.N.A. Tissue DNA Kit (Omega bio-tek).
- PCR: COI fragment of 550-710 base pairs amplified with three alternative sets of primers: LCO/HCO (4), CrustF1/HCO, CrustF2/HCO (3).
- Sequencing: between 1-6 individuals/ species (Fig.2).
- All data (species and collection details, COI sequences, trace files) uploaded on the Barcode of Life Data System (BOLD) (Fig.3).
- COI sequence divergence within species and genus based on the "Distance Summary" tool in BOLD-Management and Analysis System (MAS).
- "Taxon ID Tree" in BOLD used to build a neighbor-joining (NJ) tree with Kimura 2-parameter (K2P) distance model.



Fig.1 Sampling locations throughout the Atlantic and the Arctic Ocean.

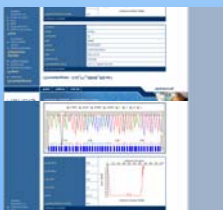


Fig.2 Trace details (quality and viewer) for a specimen as received from the sequencing facility.



Fig.3 Sequence page for a specimen in BOLD (nucleotide sequence, amino acid translation and barcode).



RESULTS

- Sequenced: 127 specimens from 66 species, 53 genera, 32 families, six orders and two classes of crustaceans.
- Economical importance (in the present or the near future): 19 species.
- Mean genetic distances: 0.648% within-species, 12.25% within-genus (Table 1).

Table 1. Pairwise COI divergences at different taxonomic levels ("Distance summary" in BOLD).

	n	Taxa	Comparisons	Min	Mean	Max	SE
Within Species	127	66	80	0	0.048	8.031	0.12
Within Genus	127	53	65	0	12.25	24.512	0.038
Within Family	127	32	292	3.029	24.485	41.964	0.36
Within Order	127	6	2517	20.622	30.762	49.968	0.114
Within Class	127	2	3075	20.085	34.467	53.409	0.08

- Very high intraspecific divergences for two amphipod species, *Tmetonyx cicada* (8.03%) and *Onisimus litoralis* (5.33%). In both cases only two specimens/species were available (Fig. 4 and 5).



Fig.4 *Tmetonyx cicada*.



Fig.6 *Sclerocrangon aff. ferox*.

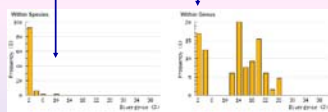


Fig.5 Frequency distribution of intraspecific and congeneric COI distances for 127 specimens.

- Very low congeneric divergences for two shrimp genera, *Sclerocrangon* (0-1.79%) and *Crangon* (1.02-1.78%) (Fig.5 and 6).

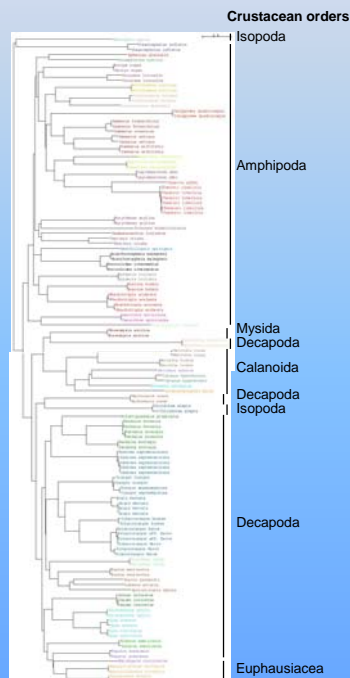


Fig.7 NJ tree with K2P distance model and crustacean families identified by the different colours.

DISCUSSION

The high intraspecific divergence in the case of above-mentioned amphipods strongly suggests the presence of cryptic species, unidentified by traditional taxonomy, and sequencing additional specimens will help clarify this point.

The low congeneric divergence in relation to very low intraspecific divergence indicates the existence of 'recently evolved' species (*Crangon*). Moreover, questionable morphological identification of samples was clarified by DNA barcoding (*Sclerocrangon aff. ferox*).

The fact that all specimens grouped with their families in the NJ tree (except for *Bythocaris irene*) (Fig.7) suggests that barcoding can be an effective tool at this level.

CONCLUSIONS

DNA barcoding proved to be very useful in our case by pointing out the existence of potential cryptic species, of 'recently evolved' species and by providing an accurate identification tool.

In addition, this system will be very effective when using crustacean larval stages for stock predictions (e.g. shrimps, crabs) or the identification of marine invaders (e.g., green crab in the Gulf of St. Lawrence).

FUTURE WORK

- Sampling within the Gulf of St Lawrence and the Bay of Fundy (2007); other samples will be obtained from different trawl surveys of Fisheries and Oceans Canada off the Atlantic coast.

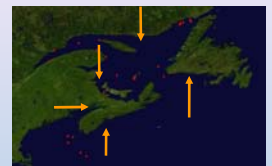


Fig.8 Sampling locations for 2007.

- Additional sequencing to a final five specimens (different geographic localities) for each species for a better understanding of intraspecific versus interspecific genetic differentiation.

- Providing DNA barcodes for all economically important crustacean species together with another 150 benthic species (especially amphipods) from NW Atlantic.

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