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DNA barcoding reveal patterns of species diversity among northwestern Pacific molluscs

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Abstract

The marine molluscs present a significant challenge for morphological approaches to specimen identification because they exhibit differences in life stage, frequently have morphologically cryptic taxa, and substantial phenotypic plasticity, which hampered the conservation and management of the richest diversity of this taxa. In this sense, reliable specimen identification and biodiversity monitoring of organism in the field is quite necessary.

In total, 2801 DNA barcodes belonging to 569 species from China, Japan and Korea were analyzed. An overlap between intra- and interspecific genetic distances was present in 71 species. We tested the efficacy of this library by simulating a sequence-based specimen identification scenario using Best Match (BM), Best Close Match (BCM) and All Species Barcode (ASB) criteria with three threshold values. BM approach returned 89.15% true identifications (95.27% when excluding singletons). The highest success rate of congruent identifications was obtained with BCM at 0.053 threshold. The analysis of our barcode library together with public data resulted in 582 Barcode Index Numbers (BINs), 72.2% of which was found to be concordantly with morphology-based identifications. In Neighbour-Joining phenogram, 2,320 (83.0%) queries formed 355 (62.4%) species-specific barcode clusters allowing their successful identification. 33 species showed paraphyletic and haplotype sharing. 62 cases are represented by deeply diverged lineages.

This study represents the first comprehensive molecular assessment of northwestern Pacific molluscs, and suggest an increased species diversity in this region, highlighting taxonomic revision and conservation strategy for the cryptic complexes.

Result and Discussion

Distance summary

Table 1 COI genetic divergences according to different taxonomic levels.

Comparison	Min Dist(%)	Mean Dist(%)	Max Dist(%)	SE Dist(%)
Within species	0.00	0.97	26.16	0.002
Within genus, between species	0.04	18.67	36.98	0.011
Within family, between genera	6.52	22.47	40.28	0.019
Within order, between families	17.20	25.30	45.32	0.022
Within class, between order	19.33	30.60	50.59	0.026

A hierarchical increase in mean divergence was found in different taxonomic levels (Table 1). The average intraspecific divergence of the northwestern Pacific molluscs was about two times higher than that of any other marine groups, including Australian marine fishes (0.39%)[1], Australian decapods (0.46%)[2], Australian echinoderms (0.62%)[1] and Canadian polychaetes (0.38%)[3]. Such a high level of intraspecific divergence may be explained by the limited dispersal capabilities of molluscs, which promote lineage divergence and enhanced speciation rates[4].

Barcode gap analysis

The distance-based approach assumes that a species can be correctly identified when the mean distance to the most closely related species (NN, nearest neighbor) is higher than the maximum intraspecific distance[5].No barcode sharing was detected among individuals of different species and a barcode gap was present for all but 71 cases.

Success of sequence-based specimen identification

Table 2 Identification success based on Best Match (BM), Best Close Match (BCM) and All Species Barcodes (ASB).

	BM	BCM (%)			ASB (%)		
		0.01	0.021	0.053	0.01	0.021	0.053
TRUE	89.15 (95.34)	68.62 (73.27)	74.94 (79.99)	76.29 (81.41)	68.62 (73.27)	72.47 (77.36)	72.69 (77.70)
FALSE	10.92 (4.73)	1.14 (0.88)	1.75 (1.07)	2.46 (1.11)	1.14 (0.88)	1.43 (0.73)	2.03 (0.65)
Ambiguous	-	14.28 (15.27)	15.42 (16.49)	15.49 (16.57)	14.28 (15.27)	18.21 (19.47)	19.53 (20.73)
No id	-	15.96 (10.58)	7.89 (2.44)	5.75 (0.92)	15.96 (10.58)	7.89 (2.44)	5.75 (0.92)

BM approach returned 89.15% true identifications (95.27% when excluding singletons).The highest success rate of congruent identifications was obtained with BCM at 0.053 threshold (Table 2). The ASB criterion is more restrictive than BCM. BCM criterion looks only at the closest match below a defined threshold, while ASB assigns a match according to all sequences under that threshold. Thus, when sequences from different species have distances values falling below the threshold, ASB criterion returned a misidentification[6].

BIN discordance report

The analysis of our barcode library together with public data resulted in 582 Barcode Index Numbers (BINs).The majority of BINs (66.5%) was found to be taxonomically concordant with other barcode data on BOLD. 33.4% of the BINs were found to be discordant. Most of the discordances between our data and that already incorporated in the BIN pipeline were caused by the use of synonymies, inadequate taxonomy and misidentifications.

The taxonomic reliability of DNA barcodes usually be evaluated by analysing new data together with already published sequences. Thus, our result highlights the need for an accurate taxonomic review of already published DNA barcode data, which will be one of the most relevant issues to increase the reliability of international barcode reference libraries like BOLD[7].

Neighbour-joining analysis

In Neighbour-Joining phenogram, 2,320 (83.0%) queries formed 355 (62.4%) species-specific barcode clusters allowing their successful identification. 33 species showed paraphyletic and haplotype sharing (e.g., *Patelloida* spp and *Meretrix* spp). 62 cases are represented by deeply diverged lineages (e.g., *Echinolittorina vidua* and *Serratina capsoides*, Fig. 2A-D).

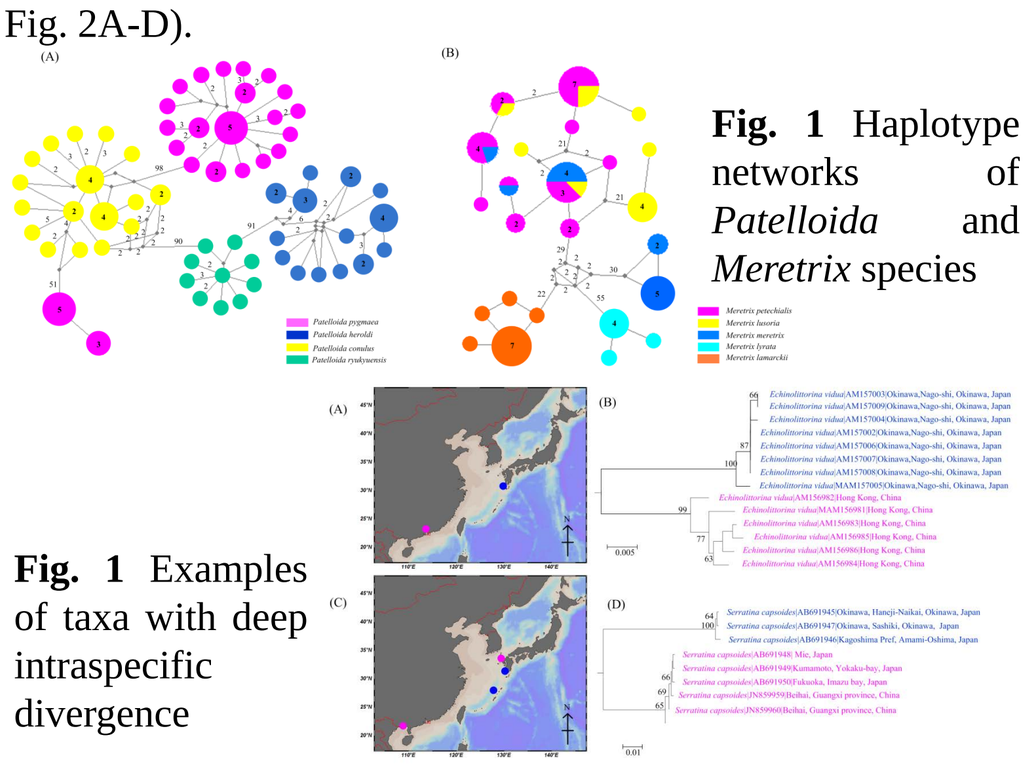

Fig. 1 Haplotype networks of *Patelloida* and *Meretrix* species

Fig. 1 Examples of taxa with deep intraspecific divergence

In general, interspecific haplotype sharing has four possible explanations: hybridization, incomplete lineage sorting, inadequate taxonomy or misidentification[8-9]. High genetic variability within a species can result from phylogeographic processes or geographically incomplete sampling[6,10]. The species with deeply diverged lineages revealed the previously unrecognized cryptic diversity, which need to be taken into account in protection strategies.

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