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PCR survey of 50 introns in animals: Cross-amplification of homologous EPIC loci in eight non-bilaterian, protostome and deuterostome phyla

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Abstract:

Exon Primed Intron Crossing (EPIC) markers provide molecular tools that are susceptible to be variable within species while remaining amplifiable by PCR using potentially universal primers. In this study we tested the possibility of obtaining PCR products from 50 EPIC markers on 23 species belonging to seven different phyla (Porifera, Cnidaria, Arthropoda, Nematoda, Mollusca, Annelida, Echinodermata) using 70 new primer pairs. A previous study had identified and tested those loci in a dozen species, including another phylum, Urochordata (Chenuil et al., 2010). Results were contrasted among species. The best results were achieved with the oyster (Mollusca) where 28 loci provided amplicons susceptible to contain an intron according to their size. This was however not the case with the other mollusk *Crepidula fornicata*, which seems to have undergone a reduction in intron number or intron size. In the Porifera, 13 loci appeared susceptible to contain an intron, a surprisingly high

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number for this phylum considering its phylogenetic distance with genomic data used to design the primers. For two cnidarian species, numerous loci (24) were obtained. Ecdysozoan phyla (arthropods and nematodes) proved less successful than others as expected considering reports of their rapid rate of genome evolution and the worst results were obtained for several arthropods. Some general patterns among phyla arose, and we discuss how the results of this EPIC survey may give new insights into genome evolution of the study species. This work confirms that this set of EPIC loci provides an easy-to-use toolbox to identify genetic markers potentially useful for population genetics, phylogeography or phylogenetic studies for a large panel of metazoan species. We then argue that obtaining diploid sequence genotypes for these loci became simple and affordable owing to Next-Generation Sequencing development. Species surveyed in this study belong to several genera (*Acanthaster, Alvinocaris, Aplysina, Aurelia, Crepidula, Eunicella, Hediste, Hemimysis, Litoditis, Lophelia, Mesopodopsis, Mya, Ophiocten, Ophioderma, Ostrea, Pelagia, Platynereis, Rhizostoma, Rimicaris*), two of them, belonging to the family Vesicomydae and Eunicidae, could not be determined at the genus level.

Keywords: Universal primers ; Alternative barcoding ; Non-model species ; Genetic marker ; Intron

1. Introduction

Population genetics and genomics of non-model species (including ecologically relevant model species) are hampered by the lack of knowledge of their genome and the absence of universal primers (e.g. Chenuil 2006). This is a particular problem for phyla of marine invertebrates which encompass a much wider phylogenetic range than terrestrial metazoans. Next-Generation Sequencing (and, to a lesser extent, Next-Generation Genotyping) methods underwent a significant diversification and decrease in cost. With respect to population genetics, the starting material may be mRNA, good quality genomic DNA for Rad-Seq (Narum et al 2013), or PCR products (amplicons). Amplicons remain the most convenient solution relative to field sampling constraints; they also still correspond to the cheapest approaches when hundreds of markers are not requested. In particular, with the development of biodiversity studies using barcoding and metabarcoding and the need of multilocus data, the need of universal primers for rarely studied phyla is growing. Introns are non-coding genomic regions susceptible to provide highly variable molecular markers. Primer pairs were recently designed to amplify introns in a very wide phylogenetic spectrum of species; the design was based on the choice of intron positions that are well conserved across metazoan phyla and which were embedded within highly conserved exon sequences which do not appear duplicated in annotated genomes (Chenuil et al., 2010). About 50 introns, framed by one or several alternative primer pairs in exons, were tested for PCR amplification and an average of 24 introns per species appeared promising in Bilaterian species. Among those promising introns, five were amplified successfully in all 10 species including cnidarians. Some of these loci were sequenced in numerous individuals and proved useful for population genetic and phylogeographic studies (Penant et al., 2013; Pivotto et al. in prep.). By providing nuclear markers in non-model species, these loci allowed for example disentangling intricate phylogeographic situations within species complexes like the sea urchin *Echinocardium* sp. (Egea, 2011; Egea et al., unpublished), the gastropod *Hexaplex trunculus* (Marzouk et al., unpublished) and the cockle *Cerastoderma glaucum* (Chenuil & Tarnowska, unpublished). They also provided codominant nuclear markers such as microsatellites useful for populations genetic studies in different species, *e.g.* the sea urchin *Abatus cordatus* (Ledoux et al., 2012), or the brittlestar *Ophioderma longicauda* (Weber et al., submitted). In the present study, we aimed to extent this EPIC survey to additional phyla. We designed more than 70 additional alternative primers for the same set of loci and we investigated their amplification patterns in 23 species, not tested previously, from seven different phyla. The phyla were chosen to encompass a very wide phylogenetic spectrum. They included the two main non-bilaterian phyla, Porifera and

Cnidaria, and the most diverse bilaterian phyla. We surveyed four Protostomian phyla (i.e. two Ecdysozoan phyla, Arthropoda and Nematoda, and two Lophotrochozoan phyla, Mollusca and Annelida) and a Deuterostomian phylum (Echinodermata). Another Deuterostomian phylum, Urochordata, had been investigated in a previous study (Chenuil et al 2010), and for Vertebrata, numerous markers including EPICs (Atarhouch et al 2003) are already available.

2. Materials and methods

The method for primer design and the sequences of previously designed primers were given in Chenuil et al. (2010). New primers were specifically designed in this study in order to improve complementarity with ecdysozoan (*i.e.* arthropods and nematodes) and cnidarian genomes (but not for poriferans (*i. e.* sponges). For those phyla, new expressed sequenced tags (EST) sequences were aligned with genome sequences of the gene families previously selected for EPIC design by Chenuil et al. (2010). The set of new primer sequences (several combinations were tested) is given in Table 1. The alternative primers we designed (for a given locus and a given amplification direction) most often corresponded to different levels of ambiguity for the same positions, and in some cases to a slight positional shift (Table 1). The PCR reaction contained the following quantities: 2. 4 µl sterile distilled water, 2.5 µl of MgCl2 at 25mM, 2.5 µl of 5X green buffer (flexi-go taq Promega), 2 µl of a mixture of dNTP (0.2 mM each), 0.25 µl of a solution of 50 µM for each primers, 0.06 µl of flexi GoTaq ® polymerase (concentrated at 5u.µL-1) and 1 µl of DNA extract at 5ng.μl^{-1}. The PCR program was: 2 min at 94°C; 14 cycles of 1 min at 94°C, 1 min at hybridization temperature from 58°C for the first cycle to 45°C for the 14^{th} cycle, 1 min at 73°C; 25 cycles of 40 sec at 94°C, 40 sec at 58°C, 1 min at 72°C, and finally 3 min at 73°C. For each sample, 5 µl of PCR products were checked on large 1.5% agarose gel electrophoresis as in Chenuil *et al.* (2010). For small sized species, i.e. *Hemimysis margalefi* and *Litoditis marina,* DNA extracts from distinct specimens had to be used for different sets of EPIC loci. DNA extraction methods varied according to organisms: DNeasy tissue kits (Qiagen) were used for all cnidarian and ophiuroids species, QiaAMp DNA minikit (Qiagen) for *Acanthaster, Aplysina, Hemimysis* and *Platynereis*, Nucleospin® Multi-96 Tissue Kit (MACHEREY-NAGEL) were used for *Crepidula fornicata,* CTAB protocols with proteinase-K incubation at 55°C were used for the Vesicomydae sp., the Eunicidae sp., *Rimicaris* and *Alvinocaris* spp. (Doyle & Doyle 1990, Teixera et al, 2013), a customized CTAB protocol (Remerie et al 2006) for *Mesopodopsis*, a protocol explained in Derycke et al (2005) for *Litoditis* (which was named *Pellioditis)* and the innuprep DNA minikit (Analytik Jena) for *Hediste*. After excluding individuals that were not amplified for any intron, the result for each primer pair in each species was

classified into one of three categories: (1) P (promising) which corresponds to amplification in all individuals of the species, without multiple bands, and of sufficient size to potentially contain an intron of at least 70 bp (the expected size of a putative intron after removal of the exonic fragment are reported for each primer pair in Chenuil *et al.* (2010)); (2) I (intron) corresponds to less intense amplifications or cases with multiple bands; (3) A (amplification) correspond to other cases resulting in amplification products, yet particularly amplicons which are too small to contain an intron, and excluding those producing only primer dimers or small size artefactual amplification products. However, we cannot exclude that occasionally some particularly large artefactual amplification products were erroneously classified as "A" results, since we did not sequence the amplicons. A precise estimation of the frequency of such mis-classifications is not available, but amplicons from about two dozens of different loci or species (including two 'A' loci) were sequenced by some of us and other colleagues and the results always provided sequences embedded within the expected exonic sequence (unpublished or cited in introduction). In one or two cases, we also observed, among the sequenced clones, an artefactual sequence not embedded in the expected exonic regions (unpublished data) which was smaller. DNA extracts from different species were distributed among three different 96-well plates, for which we did not test exactly the same combination of primer pairs for each locus. Each combination of forward and reverse primer was given a name reported in Table 2. Primer pairs tested for each plate appear in Table S1 (Supplementary material). The plate "ECDY-Platy" was mainly composed of samples from ecdysozoans, and for this plate we preferentially tested the new primers specially designed for ecdysozoans (a total of 69 primer pairs was tested). For logistic reasons (*i.e.* filling of 96-well plates, to allow the use of multichannel pipets and to limit the number of agarose gels), we also used two non-ecdysozoan DNA samples in this plate, corresponding to *Platynereis dumerilii* (Polychaeta) which were thus tested using the same primer pairs, *a priori* non-optimal for this taxon. The plate "CNI-POR-Hedi" contained a majority of cnidarians but also two non-cnidarian species, *Hediste diversicolor* (Polychaeta) and *Aplysina cavernicola* (Porifera). Some primers designed for cnidarians were preferentially used for this plate, which was tested with 68 primer pairs. The third plate contained exclusively lophotrochozoans (mollusks and polychaetes) and echinoderms (named "LOPHO-ECHI") and was used for 75 primer pairs. The number of samples for each species is given in parenthesis after the species name. In the plate « ECDY-Platy », we tested the nematode *Litoditis marina* (2), the arthropods *Rimicaris exoculata* (4), *Alvinocaris muricola* (3) and *Alvinocaris markensis* (3) which afterwards appeared to belong to the same species (Teixeira et al., in press), *Hemimysis margalefi* (3), *Mesopodopsis slabberi* (4), and the polychaete *Platynereis dumerilii* (2). In the plate "CNI-POR-Hedi", we tested the cnidarians *Eunicella cavolinii* (3), *Eunicella verrucosa* (2), *Lophelia pertusa* (4), *Pelagia noctiluca* (2), *Rhizostoma pulmo* (2), *Aurelia*

aurita (2), but also *Aplysina cavernicola* (Porifera) (1) and *Hediste diversicolor* (Polychaeta) (4). In the "LOPHO-ECHI" plate, we tested the echinoderms *Acanthaster planci* (2), *Ophiocten sericeum* (3), *Ophioderma longicauda* (3), the mollusks *Crepidula fornicata* (4), Vesicomyidae sp. (1), *Ostrea edulis* (3), and the polychaetes Eunicidae spp. (3), and *Platynereis dumerilii* (2) for which some samples were also tested in the plate "ECDY-Platy", *i.e.* with slightly different primer pairs for some loci. After these tests, a new plate (named "IV (i21-i51)") has been composed of a variety of samples from the former plates for which we increased or decreased the DNA amount (3-fold increase for *Hemimisys* and *Mesopodopsis*, 3-fold dilution for *Crepidula*), and from an additional mollusk species, *Mya arenaria* (4 specimens), and to be tested exclusively with two loci (i21 and i50) that appeared particularly successful in (Chenuil et al., 2010) with the original set of primers, excluding newly designed primers (supposedly adapted to ecdysozoan or cnidarian). The other ecdysozoan species were also tested in this plate, without changing their DNA concentrations (*Litoditis*, *Rimicaris* and the two *Alvinocaris* species).

3. Results

The results for each species across the set of loci vary considerably among species (see Table 3 for detailed results and Table 4 for a summary per species). The best results were obtained for the oyster with 28 loci providing amplicons of sufficient size to contain an intron. The sponge *A. cavernicola* successfully amplified 13 distinct EPIC loci with amplicon sizes suggesting the presence of an intron (P+I results). This is noteworthy as no sponge genome sequence data were used when we designed primer sequences (Chenuil et al., 2010). The Porifera phylum branches before all the other phyla surveyed in the tree of life and phylogenetic divergence is a major parameter influencing primer design efficiency. In the two cnidarians of the genus *Eunicella*, we obtained 24 loci with intron size amplicons, despite their phylogenetic distance with genomes that most influenced primer design.

Three Ecdysozoans globally did not provide good and regular amplification, in particular *Hemisysis* and *Mesopodopsis*, yet the two deep sea shrimps *Alvinocaris* and *Rimicaris* obtained good results. In plate "i21-i51" for which we used the initial set of primers from (Chenuil et al., 2010) instead of the newly designed primers based on ecdysozoan ESTs, we obtained better results in some cases (i.e. in *Litoditis* and *Rimicaris*, for which DNA concentration were unchanged) but not always.

The gastropod *Crepidula fornicata* displayed a significantly higher proportion of amplicons too short to contain an intron as compared to the average computed across the other species (exact test, $p<0.001$) (Table 4). This proportion is even more extreme in the arthropod *Hemimysis* and is also high in the arthropod *Mesopodopsis* and the nematode *Litoditis* but since few primer pairs amplified in this species (4 to 8), the estimated proportion of short amplicons is not precise at all.

4. Discussion

The good results obtained for cnidarians are not due to the design of special primers using cnidarian EST information, because, contrary to the ecdysozoans for which most primers were newly designed, few newly-designed primers were used for cnidarians (Table 1 -Table S1). This, together with the good results obtained for the sponge, confirms that our approach enables finding candidate loci across the genome, for species for which only very few polymorphic markers are available, across a very wide phylogenetic range.

Attempts to reconstruct phylogenetic trees (not shown) based on the amplification patterns ("P", "I" and "A" contingency tables) obtained for each locus evidenced a strong influence of the DNA plate (thus of the primer pair combinations), and of the proportion of successful loci per species, species with good results being grouped together (and the reverse). Within genera (*i.e.* the two *Eunicella* species, and the *Alvinocaris* species) the results were highly similar though not strictly identical (Table 2). At a higher taxonomic level however, we found no influence of taxonomical relatedness. As a consequence, to identify, for a new species, potentially useful EPIC loci from our set of markers, it is recommended to first test the primer pairs that globally appeared as the best one on the whole range of phyla tested, rather than to choose those that worked in the most closely related taxa (except if congeneric species or close genera were surveyed). Those "first choice" loci appear on Table 3 (e.g. locus i50) and generally correspond to the best ones identified in Chenuil et al. (2010).

The contrasted patterns observed across the study taxa may be explained by several possible causes (Table 5). Firstly, DNA damage is expected to decrease the number of successfully amplified loci (leading to low values of the triplet (A+P+I)), and increase the proportion of short amplicons (A) among successful amplifications, because short fragments are more likely to remain intact in target DNA. Secondly, high evolutionary rates are expected to increase mispriming of the PCR primers, decreasing the number of successfully amplified loci. This process would equally affect the loci containing an intron or not and consequently the proportion of short amplicons should not be

influenced. Finally, natural selection for a reduction of intron length in a genome should turn patterns 'P' and 'I' into 'A', but should not decrease the amount of successful loci. Those three hypotheses lead to different patterns and can theoretically be distinguished (Table 5). Natural selection favouring large introns, contrary to selection for small introns, seems unlikely to affect the genome globally and is a less relevant hypothesis to explain the proportion of P, I and A results of a taxon; however in case it occurs, this would significantly decrease the amount of amplifying loci, since we rarely obtained amplicons exceeding 1000 bp with our experimental conditions (this corresponds to intron sizes between 720 and 930 bp, most often of 850 bp after removing the exonic fragment length). Comparing the results obtained for the different taxa (Table 4) with the three scenarios above (Table 5), we suggest that *Crepidula* introns may have been affected by natural selection for length reduction. One hypothesis that has been proposed to explain introns evolution is linked to life cycle parameters such as generation duration (Jeffares *et al.*, 2006). *Crepidula fornicata* is a perennial species –living 8-12 years- but some authors have hypothesized that it may be better described as a species with an r-strategy life cycle (Richard *et al.*, 2006). In such species intron loss may allow replication time reduction (Jeffares et al., 2006). Although large-scale ESTs libraries were built-up (Henry et al. 2010) for this species, there is no genome data available for this species to confirm this scenario. The patterns displayed by two arthropods (*Hemimysis* and *Mesopodopsis*) and the nematode *Litoditis* at first sight are best explained by damaged DNA, and these species are the smallest of the survey. Note however that in the case of *Litoditis* the second scenario cannot fully be rejected as this species is a very strong colonizer (r-strategy) and this may contribute to its high proportion of short amplifications due to reduction in intron size. In the "i21-i51" plate, DNA concentration was doubled for these two arthropods resulting in a gain of amplification, for locus 21, for one of those two species, whereas i50 remained unamplified; therefore the influence of DNA quantity for these samples is not clearly established. However, an influence of DNA quality on our results is strongly supported by the profiles of DNA extracts on agarose gels: the oyster samples displayed, by far, the best profiles (a very neat band of high molecular weight and no degradation smear), and *Hemimysis* and *Mesopodopsis* displayed degraded migration profiles (though comparable to those from other species that performed better on PCR tests). Nematodes and Arthropods generally display less and smaller introns and appear to have lost them (Cho et al., 2004; Hawkins, 1988; Rogozin et al., 2003). By contrast with ecdysozoans, *Platynereis* (Raible et al., 2005) and the cnidarians (Zimek and Weber, 2008) were reported to have highly conserved genome sequences and intron-exon structures (our own experience based on their nucleotide alignments supports this view). However, three of the arthropods we surveyed displayed numerous successfully amplified loci and a low proportion of amplicons too short to contain an intron, as the majority of the species. While we designed new

primers, we observed a lot of variation among arthropods, more than within other phyla and we actually expected that those new primers may not improve PCR efficiency. The annelid *Platynereis* which was tested both in the ECDY-Platy plate (with numerous ecdysozoan primers) and in the LOPHO-ECHI plate (mostly with the initial set of primers), obtained slightly more amplification results (P, I or A) in the ECDY-Platy plate (22 *versus* 19), suggesting that the bad results obtained for some ecdysozoan species are not directly explained by the design of the new PCR primers dedicated to ecdysozoans, but rather by their high evolutionary rate or a global intron reduction (Raible et al 2005, Zimek and Weber 2008).

This second survey of the EPIC loci isolated in Chenuil et al (2010) confirms that those EPIC primers may potentially amplify any metazoan species. Combining the present and the former study, some loci appear more likely to successfully amplify an intron : i1, i2, i5, i8, i9, i11, i21, i34, i36, i50 for Mollusks (five species, four genera tested), i5, i12, i15, i22, i29, i30, i53 for Cnidarians (eight species, seven genera), i1, i2, i5, i9, i21, i22, i29, i36, i50, i51 in echinoderms (eight genera), and i26, i29, i35, i50, i51 for Arthropods (four genera). We emphasize however that amplification results are very poorly correlated to phylogeny and it is strongly recommend to test all the EPIC loci (if possible, combining several species for the same session).

Recently, Li et al. (2013) developed a hybridization capture method which allows finding hundreds of coding sequences in highly divergent vertebrate species. This promising method however does not target highly variable genomic regions. Furthermore, it is more complex and expensive than an EPIC PCR survey, even when PCRs are followed by a Next-Generation Sequencing run. For example, amplicons from all intronic loci can be pooled in a MISEQ run using up to 184 tags to label the different individuals. For about 3000 € one can obtain more than ten millions of paired-end reads (250 bp x 2 each) for 96 tagged individuals, resulting in more than 1000 paired-end reads per locus for each individual in average. With such a sequencing depth, diploid sequence genotypes can be safely inferred as explained in Chenuil (2012): in particular, the analysis of the distribution of read numbers within individuals allows detecting whether a marker corresponds to a single and diploid locus or whether there is polyploidy or paralogy, and allows determining the level of multiplication; loci prone to (and alleles generated by) PCR or sequencing errors also are identifiable using such distributions.

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Raible, F., Tessmar-Raible, K., Osoegawa, K., Wincker, P., Jubin, C., Balavoine, G., Ferrier, D., Benes, V., de Jong, P., Weissenbach, J., Bork, P., Arendt, D., 2005: Vertebrate-type intron rich genes in the marine annelid *Platynereis dumerilii*. Science 310,1325-1326.

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Mini-CVs of authors

- KG is an evolutionary biologist interested in biogeographic processes that drive Southern-Hemisphere marine diversity especially in Antarctic waters.
- EG is a research assistant on eel conservation. She is interested in evolution and conservation, particularly in catadromous fish.
- SAH is an evolutionary ecologist with a strong interest in the understanding of factors involved in the divergence of populations, particularly in the marine environment.
- DA is an evolutionary biologist interest in population genetics and adaptive processes
- RB is a senior scientist at the university of Rostock, working on phylogeny and phylogeography, biological invasions, ecophysiology.
- PC is a Senior CNRS researcher working primarily on the Mediterranean marine biodiversity, with an emphasis on underwater cave biota and their relation with deep-sea communities. Among model organisms studied are sponges, cnidarians, echinoderms and mysid crustaceans.
- Sofie Derycke is a postdoctoral fellow. Her research focus is on population genetics, phylogeography, cryptic speciation and taxonomy of marine invertebrates. She also has a keen interest in assessing taxon and functional diversity of marine sediments using DNA barcoding, metagenetics and metagenomics and in linking this diversity to ecosystem stability and resilience.
- RH is head of the German Federal Research Institute of Fisheries Ecology in Hamburg. He is a marine biologist interested in causes and pathways of adaptation and speciation in the sea.

- SL is geneticist and is developing research in population genetics/genomics and selective breeding of marine molluscs.

- CL is a molecular ecologist studying the effects of large scale disturbances (mainly climate change and biological invasions) on biodiversity and the adaptive responses of organisms.

- SM is a population geneticist. He is interested in population genomics, forces shaping genetic variation in populations and species and in methods related to the analysis of sequence polymorphism.

- AR Andreja Ramšak is a molecular biologist and her research interest is focused on phylogeography and population genetics of scyphozoans.

- TR finished his PhD in Marine Biology at Ghent University, and participated in several research projects on marine genetic biodiversity (phylogeography, population genetics, phylogeny of marine invertebrates). In 2009 he started as a lecturer at the Artevelde University College, where he is involved in natural sciences education, as part of the teacher training program.

- F.V. is a senior CNRS researcher. Her interests include molecular ecology and evolutionary biology to examine dispersal and adaptation processes of marine coastal species, in particular invasive species.

- J-P.F is a senior CNRS researcher. He aims to understand the origin, the maintenance and the erosion of biodiversity taking into account the mode of development of marine benthic invertebrates and environmental factors in continuous and insular systems.

- AC is an evolutionary biologist working on population genetics, population genomics and phylogeography of marine organisms.

Table 1: New primers (altogether 104) designed for this study. Nucleotides at ambiguous sites are marked using the IUPAC ambiguity code.

i1F2	GAATCAGGCCTGTCCATGGTNAVBTGG
i1R3	TGGCCATATTCCATTGACCAAATGMAYTTRAAYTC
i3F2	TTGATTTGGCGTATGCTATCGAACARATGTGGSA
i3R2	CAACTGTCAGCAATTACTAACAKYTCRTKRTA
i4F2	ATCTAGAGCTCATCATAGATTTACAGGRSCNCARAT
i4R2	GTTTTCGGTCTTAATATTCATAARRTTCATNCC
i5F2	TGTTCCCAGCAGAATATCCNATGMARCC
i5R3	CATATTTTCTTGTTTAATTCAAAACGHCCATTHGG
i5R4	TCCATGATGGTTGCCATGTTTCYGGRTGRTR
i5R5	TCCATGATGGTTGCCATGTYTCYGGRTGRT
i8F2	TTCCAGTGGTCATGTGGCATGGMATGGGYGA
i8R2	CTATTTTTCCCAAACTTAATGGRTTRCARCA
i9F3	TGCCTCTCCATTTCCGGCTATCAYCCRGARAC
i9R2	TATAGCGCCCTCTCCTTTGGTAGGCAKRAANSCAAT
i11F2	TATGTTTTTGGTTGGAATGCAGRAYAARAARAT
i11R2	ACTGCCTGCAAGTGACGATCRTAYTCYTG
i12F2	GATGATAAAAGTGTCAGARTNTGGGARTGGGA
i12R2	TGAAGTCCAACATTTTGAATAAGTTTYRTRTCNAC
i13F2	TGGGTGCTCATTGGACACGARTWYATGGA
i13R2	ATAATATCATACATTTGTCCAARNCCRTACCA
i17F2	ATTGGTGTATATATTATAGAYMGDTAYAC
i17R2	ATGTTGGAAGATTCGCGAAGATCCRAARAARTC
i19F2	GAAACCGATTGATGTGAAAACAAARTTYTAYARYGC
i19F3	GAAACCGATTGATGTGAAAACAAAGTTYTAYARYGC
i19R3	TGTATTGTTCCGAACTTTCAAGTTCSACCTTYTCSAG
i21F3	AAAACCAATTTACAATCCTGCTGGAAARTAYAYGWT
i21R3	GATCCAGGAAAGTCATATCCTCCCATAASYTTCATRTA
i21R4	GATCCAGGAAAGTCATATCCTCCCATNSTTCATRTA
i22F3	GCTGCTGGAGAAGGCCTACATKAARGTSAT
i22F2	TACATGAAGGTTATGGGAGGVTAYGAYTT
i22R2	GTATCGTTCAATTCAATTCTTTCHGGWATCCA
i22R3	CAATTCAATTCTTTCAGGAATCCADCCBGTYA
i24F2	AAGAGTTTATCACTCTTATTGTGTAYRAVAVY
i24R3	GAATAGTTGATTGTATTGGTTTTYTCRTAYTG
i25F3	AGCGTGGATGGACACCTGAARTTYTGGAARAA
i25R3	TCCAGCTTTATCATGTTGATCATRTCRAARTT
i26F3	TGATGAATGTCCGAAAACCGTKGARAAYTTCTG
i26R3	AAATTCATCTTCAAAATCTCYDCCCCADAT

Table 1 (continued)

i29F2	ATCGGTGATCGATTTCGATGAGATSGCGGHGG
i29F3	GATTTCGATGAGATGGCCGGAGGTBYVAACAA
i29R2	GTTTTACCGGATCCCTGCAAACCNACRAACATKA
i29F4	ATGGCAGCTGGTCTCAACAAACGCARRATGATHCAR
i30F2	TTCCGTGCTGGTGCTTTCGATCAAATMAARCARAAYGC
i30F3	TTCCGTGCTGGTGCTTTCGATCAAATAAARCARAAYGC
i30R2	GTATCCACAATGATGATTTCRAANCCYTC
30R3	CCGCTAGTATCCACAATGATGAHYTCRAARTT
i34F2	GACATGTATGAGCAGTTCCAGAACATYATGAARATGGG
i34R3	TTCCTTATCACTCATAGTGTCCATSAYNGTCAT
34R4	TTCTTCATCCTTCATACTGTCCATBATNRTCAT
i35F3	CAATACAAGAAATTCTCTGCTGTGGTAAAGAARATKGG
i35F4	CAATACAAGAAATTCTCTGCTGTGGTHAAGAARATKGG
i35R3	GGATCCATCATTTTTGCCATYTGHTGRTT
i35R4	GGATCCATCATTTTTGCYATYTGHTGRTT
i36F2	TTCAAGGGAACCATCATGGAAGARTGGTWYTTY
i36R3	ACGTTTCCGCTGAGTACATTTGCTGGSAWCATYTG
i37F3	TGTCGAACATTCTTCTCCACNCAYTAYCA
i37F2	TGTCGAACATTCTTCTCCACNCAYTATCA
i37R2	GGATCCTCATTGTTCTCCTTATCCACCATGCANKY
i38F2	AACGCGAGAGTTCGTGTTCTCACVTACACYGAYGA
i38R2	GATCCGGATGGTTATTGAACCAYACKCCRTACATR
i41F2	AACCCAATGGAGGCCTATTACTTCACDGTRGC
i41F3	CGTGGAACCCAATGGAGGCCTATTACTTCACDGTR
i41R3	ACTGGATGCTCCATATAACGCATGTCRWABGTRTA
i41R4	GACGGAGGCCATTCGTTTTGTGTAGTADAYDTCYC
i42F2	GGAAAACGATTGGTAATGTTYGGMAARTR
i42R3	GCCAATCCCATGTGAAGGAAYGGKGTRTKRTG
i42R4	TTAGCTGCACGACTGCTCTTGTARTTRTGNG
i43R3	CAATATGGGTTTCGACCGTGATGKACMCKRTGATG
i44F2	AGAATAAAATTTATAGATCTTATATYGGAATGGGW
i44F3	AGAATAAAATTTATAGATCTTAYATYGGAATGGGW
i44R2	CCCTGTGAGATTTCTGCTTGGTATGGDTRTACTG
i44R3	CCCTGTGAGATTTCTGCTTGGTAHGGDGTRTACTG
44F4	AGAATAAAATTTATAGATCTTATATYGGMATGGGNTA
i45F2	CAAGTTTATTTGGATGGAGCCAAYATGAATGCYC
i45F3	CAAGTTTATTTGGATGGAGCYAAYATGAATGCYC
i45R2	GGACCACCTCCTCCGTGTGGAATRCARAAKGT
i45R3	GGACCACCTCCTCCGTGTGGWATRCARAAKGT
i46F2	CGAAGTACACAAATTCCGTTGGGARACNTGYTG
i46R2	GCCGCATTATTCTTCATTTCCATRAAYTCRTG
i47F2	GACAGTGAGCATGCGATCAAGTTCTTYCARMGVGC
i47R2	ATGATATCATACATTTGGCCGARNCCRTACCA

Table 1 (end)

48F4	GGAGATTATGAAAATGCTGAGAAGMWHTGYATGCW
i48F2	CAATCAGGAAATTATGTGGAAGCAGAAARRYWTTG
i48F3	CAATCAGGAAATTATGTGGAAGCAGARARRYWTTG
i48R2	GCTGCGGCTAAATTGATGTAACCATCAATRAAWTC
i49F3	GGAAAACTAAACGACGCCATACTCCAYTAYAARGA
i49R3	ATTCGAATGAGCATCGGCAAATGCTGGRTTRATYT
i49R4	ATTCGAATGAGCATCGGCAAATGCTGGRTTDATYTK
i50F2	GATGGAATCCACATTCTCATTAAYATGAAYGG
i50F3	GATGGAATCCACATTCTCATNAAYATGAAYGG
i50R3	GATGTGACAGCATCCGTGATGAWRTAATCCATRAA
i50R4	GGTGATGTGACAGCATCCGTGATGATATAATCCAT
i51F3	GATGACGCTATTGTGTTTTGCAATTTYAAYCAGCT
i51F4	GATGACGCTATTGTGTTTTGCAAYTTYAAYCAGCT
i51R3	ATCAGCCAGTTGTCCTCGACGAACRTGYTCYTCYT
i51R4	ATCAGCCAGTTGTCCTCGACGAACATGYTCYTCYT
i52F2	GTAACTCATGCTCTCAGAACCACTGARTAYCAYGA
i52R2	GCAACAATAAATTGCTTCAATCCHTCVACHGTCA
i53F2	ACTGTTCGAGGAGTTATGAGAAGAGGMWTGACDRT
i53R3	TTCTTGTTGAACGCCCAAATYTTRTCCCAYTCCAT
i56F2	CATCATCTCGGTCAAAACTTCTCCAAVATGTTCRA
i56R3	GGCACTCCCTTCAGCTCCCAGTGRTTRWAYTTCCA
i57F2	ACAACGTCACCACCACCGAGGATCCVRTNAT
i57R2	CTCCGATTTTGTAGGCAACAATATCCCANGARTA

Table 2: Combinations of EPIC primer pairs used for PCR amplification. The sequences of the primers designed for this study are given in Table 1, the others are provided in Chenuil et al. (2010).

Primer pair name	1a	1b	1c	2a	3a	3b	3c	4a	5a	5b	5c	5d	5e
Forward primer	i1-F	i1-F	i1-F2	i2-F	i3-F	i3-F2	i3-F3	i4-F2	i5-F	i5-F	i5-F2	i5-F2	i5-F2
Reverse primer	i1-R	i1-R2	i1-R3	i2-R	i3-R	i3-R2	i3-R2	i4-R2	i5-R	i5-R2	i5-R3	i5-R4	i5-R5

Primer pair name	8a	8b	9a	9b	9c	11a	11b	12a	12b	13a	15a	15b	17a
Forward primer	i8-F	i8-F2	i9-F	i9-F2	i9-F3	i11-F	i11-F2	i12-F	i12-F2	i13-F2	i15-F	i15-F2	i17-F2
Reverse primer	i8-R	i8-R2	i9-R	i9-R	i9-R2	i11-R	i11-R2	i12-R	i12-R2	i13-R2	i15-R	i15-R2	i17-R2

Primer pair name	19a	19b	19c	19d	21a	21b	21c	21d	21e	21f	22a	22b	22c
Forward primer	i19-F2	i19-F3	i19-F	i19-F	i21-F	i21-F	i21-F3	i21-F3	i21-F3	i21-F3	i22-F	i22-F2	i22-F2
Reverse primer	i19-R3	i19-R3	i19-R	i19-R2	i21-R	i21-R2	i21-R	i21-R2	i21-R3	i21-R4	i22-R3	i22-R2	i22-R3

Primer pair name	22d	22e	22f	24a	24b	25a	25b	25c	26a	29a	29b	29c	29d
Forward primer	i22-F3	i22-F3	i22-F	i24-F2	i24-F2	i25-F	i25-F2	i25-F3	i26-F3	i29-F	i29-F	i29-F2	i29-F3
Reverse primer	i22-R	i22-R3	i22-R	i24-R2	i24-R3	i25-R2	i25-R2	i25-R3	i26-R3	i29-R	i29-R2	i29-R2	i29-R2

Primer pair name	29e	29f	30a	30b	30c	30d	34a	34b	34c	34d	34e	35a	35b
Forward primer	i29-F4	i29-F4	i30-F	i30-F	i30-F2	i30-F3	i34-F	i34-F	i34-F2	i34-F2	i34-F2	i35-F	i35-F3
Reverse primer	i29-R	i29-R2	i30-R	i30-R3	i30-R2	i30-R2	i34-R	i34-R4	i34-R	i34-R3	i34-R4	i35-R3	i35-R3

Primer pair name	35c	35d	35e	36a	36b	36c	37a	37b	38a	38b	39a	39b	40a
Forward primer	i35-F3	i35-F4	i35-F4	i36-F	i36-F	i36-F2	i37-F2	i37-F3	i38-F	i38-F2	i39-F	i39-F2	i40-F
Reverse primer	i35-R4	i35-R3	i35-R4	i36-R	i36-R3	i36-R3	i37-R2	i37-R	i38-R3	i38-R2	i39-R	i39-R2	i40-R2

Primer pair name	40b	40c	40d	40e	40f	41a	41b	41c	41d	41e	42a	42b	42c
Forward primer	i40-F2	i40-F2	i40-F2	i40-F3	i40-F3	i41-F	i41-F2	i41-F2	i41-F3	i41-F3	i42-F2	i42-F2	i42-F
Reverse primer	i40-R2	i40-R2	i40-R3	i40-R2	i40-R3	i41-R2	i41-R3	i41-R4	i41-R3	i41-R4	i42-R3	i42-R4	i42-R

Primer pair name	42d	43a	43b	43c	43d	44a	44b	44c	44d	44e	45a	45b	45c
Forward primer	i42-F	i43-F	i43-F	i43-F3	i43-F3	i44-F2	i44-F2	i44-F3	i44-F3	i44-F4	i45-F2	i45-F2	i45-F3
Reverse primer	i42-R4	i43-R	i43-R3	i43-R3	i43-R4	i44-R2	i44-R3	i44-R2	i44-R3	i44-R2	i45-R2	i45-R3	i45-R2

Primer pair name	45d	46a	46b	47a	47b	48a	48b	48c	48d	48e	48f	49a	49b
Forward primer	i45-F3	i46-F	i46-F2	i47-F2	i47-F2	i48-F2	i48-F3	i48-F4	i48-F4	i48-F	i48-F	i49-F	i49-F3
Reverse primer	i45-R3	i46-R	i46-R2	i47-R	i47-R2	i48-R2	i48-R2	i48-R	i48-R2	i48-R	i48-R2	i49-R4	i49-R3

Primer pair name	50a	50b	50b'	50c	50d	50e	50f	50g	51a	51a	51b	51b	51c
Forward primer	i50-F	i50-F	i50-F	i50-F2	i50-F2	i50-F2	i50-F3	i50-F3	i51-F	i51-F3	i51-F	i51-F3	i51-F2
Reverse primer	i50-R	i50-R2	i50-R2	i50-R	i50-R3	i50-R4	i50-R3	i50-R4	i51-R	i51-R2	i51-R2	i51-R3	i51-R

Primer pair name	51c	51d	51d	52a	52b	53a	53b	53c	54a	54b	54c	54d	55a
Forward primer	i51-F4	i51-F2	i51-F4	i52-F	i52-F2	i53-F	i53-F	i53-F2	i54-F	i54-F2	i54-F	i54-F2	i55-F
Reverse primer	i51-R2	i51-R2	i51-R3	i52-R	i52-R2	i53-R	i53-R2	i53-R3	i54-R	i54-R	i54-R2	i54-R2	i55-R

Primer pair name	56a	56b	57a	57b	57c	58a
Forward primer	i56-F	i56-F2	i57-F	i57-F	i57-F2	i58-F
Reverse primer	i56-R	i56-R3	i57-R	i57-R2	i57-R2	i58-R

Table 3 (to be continued): Results per locus for each species.

Letters in the table refer to the primer pairs (see Table 2) for which results were obtained for a given locus and species. Loci which were not tested for a species are in grey cells with the label ‘NT’. For other cells, different primer pairs were tested among species according to main plates (*cf* materials and methods). The format of the font refers to the amplification pattern obtained: Bold for “Promising”, normal for “Introns”, italics for “Amplification” (see text for detailed explanations). Background colours indicate the best primer pair result: white for “promising”, yellow for “intron”, blue for “amplification”, black for loci which did not provide any amplicon except, occasionally, primer dimers. When results were obtained from the additional fourth plate, the primer pair letter is underlined. Loci which amplified in none of the species were not reported here (*e.g.* i24). *: The name of the “main plate” refers to all results in the corresponding rows, except the underlined results, which correspond to plate IV with increased or decreased DNA concentration.

Table 3 (continued)

Main Plate*	**Taxon**	**1**	**2**	**3**	**4**	**5**	**8**	**9**	**11**	**12**	**15**	**19**	**21**	**22**	**25**
CNI-POR-Hedi	*Aplysina (1)*	**b**			NT	**b***a*	a	a		**a**	**a**	NT	**ab**		
CNI-POR-Hedi	*Aurelia (2)*	**b**	**a**		NT	**b**	a	b		**a**	**a**	NT		**de**	
CNI-POR-Hedi	*Eunicella cav. (3)*	**ab**	**a**		NT	**b***a*	a	b		**a**	**a**	NT	**b**a	**ae**	
CNI-POR-Hedi	*Eunicella ver.(2)*	**a**b	**a**		NT	**b***a*	a	ab		**a**	**a**	NT	**b**a	**a**e	
CNI-POR-Hedi	*Hediste (4)*	ab	**a**		NT	ba	a	b		a	**a**	NT	**b**a	**e**ad	
CNI-POR-Hedi	*Lophelia (4)*	**ab**			NT	b		**b**		a	a	NT		**d**	
CNI-POR-Hedi	*Pelagia (2)*				NT	b				a	**a**	NT		**a**	
CNI-POR-Hedi	*Rhizostoma (2)*	*b*	a		NT					**a**	a	NT			
ECDY-Platy	*Alvinocaris mar.* (3)*		NT		**a**	c		c	b	**b**					
ECDY-Platy	*Alvinocaris mur.* (3)*		NT		**a**	c	**b**	*c*	b	*b*					
ECDY-Platy	*Hemimysis (3)*		NT										**b**		
ECDY-Platy	*Mesopodopsis (4)*		NT						**b**						
ECDY-Platy	*Litoditis (2)*		NT							*b*					
ECDY-Platy	*Platynereis (2)*		NT	**c**	*a*	**c**	**b**	*c*	b	**b**					
ECDY-Platy	*Rimicaris (4)*		NT		**a**			*c*		**b**					
LOPHO-ECHI	*Acanthaster (2)*	**b**a	*a*	a	NT	**a**b	a	**b***a*	**a**			*c*		**a**fe	
LOPHO-ECHI	*Crepidula (4)*	a			NT	**b**	a	b							
LOPHO-ECHI	*Eunicidae (3)*	**ab**	**a**		NT	**b**		b	**a**			*c*	**ab**	**a**fe	**b**
LOPHO-ECHI	*Ophiocten (3)*	**ab**			NT	**b**	a	b				**c**		a	
LOPHO-ECHI	*Ophioderma (3)*	ab	a		NT	**a**b	a	b	a				ab	**a**fe	
LOPHO-ECHI	*Ostrea (3)*	**a**b	a	**a**	NT	**a**b	**a**	**b**	a				**b**a	**a**ef	**b**
LOPHO-ECHI	*Platynereis (2)*		**a**		NT	**ab**	a	b					**ab**	**a**fe	**b**
IV (i21-i51)	*Mya (4)*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	**b**	NT	NT

Table 3 (continued)

Main Plate*	**Taxon**	**26**	**29**	**30**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**
CNI-POR-Hedi	*Aplysina (1)*	NT			**a**					a	**b**				*e*
CNI-POR-Hedi	*Aurelia (2)*	NT	**b**	**a**b				**b**		a				a	e
CNI-POR-Hedi	*Eunicella cav. (3)*	NT	**abef**	**b**a	**abc**	**a**		b		*a*	b		d	**a***b*	e
CNI-POR-Hedi	*Eunicella ver.(2)*	NT	**abef**	**b**a	**abc**	**a**		b		*a*	b			**a**	e
CNI-POR-Hedi	*Hediste (4)*	NT	**abef**	**b**	**abc**	**a**				**a**			**d**	**a**	**e**
CNI-POR-Hedi	*Lophelia (4)*	NT	b	b	**a**						b				**e**
CNI-POR-Hedi	*Pelagia (2)*	NT		**b**				b		a					
CNI-POR-Hedi	*Rhizostoma (2)*	NT	**a**	**ab**	**a**	*a*				**a**					
ECDY-Platy	*Alvinocaris mar. (3)*	a	**c**d			**e**d	c	a	**b**	b	**dc***e*	**b**c			a
ECDY-Platy	*Alvinocaris mur. (3)*	a	**c**d			**e**cd	c	a	**b**	b	**df**c*e*	**b**			a
ECDY-Platy	*Hemimysis (3)*		c								*c*				
ECDY-Platy	*Mesopodopsis (4)*	**a**				d									*a*
ECDY-Platy	*Litoditis (2)*		c			**de**c	*c*						**a**		**d**
ECDY-Platy	*Platynereis (2)*	*a*	**cd**			**de**	*c*	a	**b**	b	f*cde*	**e**bc	**a**		a*d*
ECDY-Platy	*Rimicaris (4)*	**a**	**c**d			**c**	**c**	**a**	b	*b*	*cd*	**b**c	**a**		
LOPHO-ECHI	*Acanthaster (2)*	NT	**b***ae*	a	**a**	**a**	**a**b	b		**a**	**b**		NT	**a**	**e**
LOPHO-ECHI	*Crepidula (4)*	NT	f*e*	*b*		*a*	b	*b*					NT		
LOPHO-ECHI	*Eunicidae (3)*	NT	be	**ab**		**a**	**ab**	**b**			**b**		NT		
LOPHO-ECHI	*Ophiocten (3)*	NT					b			**a**	*b*		NT	ab	*e*
LOPHO-ECHI	*Ophioderma (3)*	NT	be*a*	b		a	**ab**	b		**a**	**b**		NT	**a**	*e*
LOPHO-ECHI	*Ostrea (3)*	NT	**af**be	**ab**	**abce**	**a**	**a**b	b	**a**	*a*	**b**		NT	**a**b	*e*
LOPHO-ECHI	*Platynereis (2)*	NT	*ab*				**b**	**b**		**a**			NT		*e*
IV (i21-i51)	*Mya (4)*	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Table 3 (end)

Main Plate*	**Taxon**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**
CNI-POR-Hedi	*Aplysina (1)*	NT	**a**				ab	*b*		*a*	NT		**a**		
CNI-POR-Hedi	*Aurelia (2)*	NT	**a**				**ac**	bd*c*		a	NT			**a**	
CNI-POR-Hedi	*Eunicella cav. (3)*	NT	**a**		**cd**		c**a**	*abcd*		**ab**	NT		**a**		**a**
CNI-POR-Hedi	*Eunicella ver.(2)*	NT	**a**		**d**		c**a**	*abcd*		**ab**	NT		**a**	**b**	**a**
CNI-POR-Hedi	*Hediste (4)*	NT	a		**c**		**abc**	b		**ab**	NT		**a**		**a**
CNI-POR-Hedi	*Lophelia (4)*	NT						*b*		**b**	NT				
CNI-POR-Hedi	*Pelagia (2)*	NT						*abc*		a	NT				
CNI-POR-Hedi	*Rhizostoma (2)*	NT						*b*			NT				**a**
ECDY-Platy	*Alvinocaris mar. (3)*	*abcd*					**c**ef	**f**e			NT	NT			NT
ECDY-Platy	*Alvinocaris mur. (3)*	*abcd*					**e**f	**f**e			NT	NT			NT
ECDY-Platy	*Hemimysis(3)*	*abcd*									NT	NT			NT
ECDY-Platy	*Mesopodopsis (4)*	*a*									NT	NT			NT
ECDY-Platy	*Litoditis (2)*						**c**ed	*g*			NT	NT			NT
ECDY-Platy	*Platynereis (2)*	*abcd*				*9b*	**ef**	**eg**f			NT	NT			NT
ECDY-Platy	*Rimicaris (4)*	*abcd*			**b**		**c**d	**f**e			NT	NT			NT
LOPHO-ECHI	*Acanthaster (2)*	NT		**a**	**ec***d*		**ab**	**a***cd*	*a*	*ab*	**ac**			**ab**	
LOPHO-ECHI	*Crepidula (4)*	NT					b*a*	*bcd*		*b*					*a*
LOPHO-ECHI	*Eunicidae (3)*	NT	a	*b*	f	9a	a	*bcd*		b*a*	bc*a*			**ab**	
LOPHO-ECHI	*Ophiocten (3)*	NT	a				**abc**	**abcd**		*b*					
LOPHO-ECHI	*Ophioderma (3)*	NT	**a**	b				**acd**b	*a*	b	**abc**				
LOPHO-ECHI	*Ostrea (3)*	NT	a	**b**	**def**	9a	**bb'c**		*a*	*ab*	**acd**b	a		**ab**	a
LOPHO-ECHI	*Platynereis (2)*	NT	**a**		**e**		**ab'c**	**abc**d		*b*	**dc**	*a*			
IV (i21-i51)	*Mya (4)*	NT	NT	NT	NT	NT	**ab**	NT	NT	NT	NT	NT	NT	NT	NT

*: These *Alvinocaris* species were later shown to belong to a single genetic entity (with the mitochondrial COI and 18S rDNA genes), the few differences being due to individual variation (Teixeira et al, in press).

Table 4: Summary of results per species ranked according to the plate (note that *Platynerei*s was studied on two distinct plates): numbers correspond to all loci obtaining P, I or A results, respectively. When several primer pairs were tested for a locus we only considered the best of the results (P>I>A) to characterize the locus. The proportion of amplicons which are too short to contain an intron is the number of "A" divided by "A+I+P". Extreme values commented in the discussion are highlighted in bold except for *Hemimysis*. The 50% value for *Hemimysis* is not considered as reliable since its estimation is probably uncertain due to overall low positive results (see text).

Taxa (number of individuals)	P	I	A	(A+I+P)	Amplicons too short to contain an intron
CNI-POR-Hedi plate					
Aplysina (1)	10	3	3	16	19 %
Aurelia (2)	12	7	0	19	**0 %**
Eunicella cav. (3)	18	6	2	26	8 %
Eunicella ver.(2)	19	5	2	26	8 %
Hediste (4)	17	7	0	24	**0 %**
Lophelia (4)	6	6	1	13	8 %
Pelagia (2)	3	5	1	9	11 %
Rhizostoma (2)	6	2	3	11	27 %
ECDY-Platy plate					
Alvinocaris mar. (3)	9	8	3	20	15 %
Alvinocaris mur. (3)	9	7	3	19	16 %
Hemimysis(3)	1	1	2	**4**	50 %
Mesopodopsis (4)	2	1	2	**5**	40 %
Litoditis (2)	4	1	3	8	38 %
Platynereis (2)	12	4	6	22	27 %
Rimicaris (4)	12	1	4	17	24 %
LOPHO-ECHI plate					
Acanthaster (2)	19	4	4	27	15 %
Crepidula (4)	**1**	6	6	13	**46 %**
Eunicidae *(3)*	13	7	3	23	13 %
Ophiocten (3)	6	6	3	15	20 %
Ophioderma (3)	10	11	2	23	9 %
Ostrea (3)	**21**	7	2	**30**	7 %
Platynereis (2)	13	2	4	19	21 %

Table 5: Expected consequences of some molecular and evolutionary processes on the patterns observed.

	Total amplification success A+P+I	Proportion of amplicons too short to contain an intron: A/(A+P+I)	Taxon
Damaged DNA	↘	↗ (small target DNA less damaged)	*Hemimysis Mesopodopsis (Litoditis)*
r-strategy : selection for rapid replication rate, thus for smaller introns	No effect (P or I loci are turned into A)	↗	*Crepidula*
High genome evolutionary rate	↘ (more mispriming)	No effect	*Hemimysis* Mesopodopsis* (Litoditis*)*

*: These ecdysozoan species display an increased proportion of short amplicons, but the estimation of this proportion is affected by a high variance, due to their low number of successful amplifications (A+P+I); thus we do not rule out the possibility of a role of high evolutionary rate (having in theory no effect on this proportion.