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Abstract

The subfamily GH13_1 of alpha-amylases is typical of Fungi, but it is also found in some unicellular eukaryotes (e.g. Amoebozoa, choanoflagellates) and non-bilaterian Metazoa. Since a previous study in 2007, GH13_1 amylases were considered ancestral to the Unikonts, including animals, except Bilateria, such that it was thought to have been lost in the ancestor of this clade. The only alpha-amylases known to be present in Bilateria so far belong to the GH13_15 and 24 subfamilies (commonly called bilaterian alpha-amylases) and were likely acquired by horizontal transfer from a proteobacterium. The taxonomic scope of Eukaryota genomes in databases has been greatly increased ever since 2007. We have surveyed GH13_1 sequences in recent data from ca. 1600 bilaterian species, 60 non-bilaterian animals and also in

unicellular eukaryotes. As expected, we found a number of those sequences in non-bilaterians: Anthozoa (Cnidaria) and in sponges, confirming the previous observations, but none in jellyfishes and in Ctenophora. Our main and unexpected finding is that such fungal (also called Dictyo-type) amylases were also consistently retrieved in several bilaterian phyla: hemichordates (deuterostomes), brachiopods and related phyla, some molluscs and some annelids (protostomes). We discuss evolutionary hypotheses possibly explaining the scattered distribution of GH13_1 across bilaterians, namely, the retention of the ancestral gene in those phyla only and/or horizontal transfers from non-bilaterian donors.

Key words: alpha-amylase, gene loss, horizontal gene transfer, hemichordates, brachiopods, phoronids, bryozoans, molluscs, annelids, Bilateria, glycosyl hydrolase, introns

Introduction

Alpha-amylases are enzymes that are almost ubiquitous in the living world, where they perform the hydrolysis of starch and related polysaccharides into smaller molecules, to supply energy to the organism through digestion. They belong to glycosyl hydrolases, a very large group of enzymes which have been classified in a number of families according to their structures, sequences, catalytic activities and catalytic mechanisms (Henrissat and Davies 1997). Most alpha-amylases are members of the glycoside hydrolase family 13 (GH13), which includes enzymes that can either break down or synthetize α -1,4-, α -1,6- and, less commonly, α -1,2- and α -1,3-glycosidic linkages. Sucrose and trehalose are also substrates for enzymes of this family (MacGregor *et al.* 2001). The numerous family GH13 is divided into 42 subfamilies, of which only three occur in Metazoans: GH13_1, GH13_15 and GH13_24 (Stam *et al.* 2006; Da Lage *et al.* 2007; Lombard *et al.* 2014). The latter two include the common animal alpha-amylases, while the former was first described in Fungi for which it represents the canonical alpha-amylase (Stam *et al.* 2006). Da Lage *et al.* (2007) described the subfamilies GH13_15/24 as private to Bilateria among metazoans. In the same article, they retrieved sequences belonging to the subfamily GH13_1 from the sponge *Amphimedon queenslandica* (named *Reniera sp.* in their paper) and the sea anemone *Nematostella vectensis*, besides the unikont choanoflagellates and amoebozoans, and also excavates and ciliates. They dubbed “Dictyo-type” this alpha-amylase, referring to the slime mold *Dictyostelium discoideum* (Amoebozoa Mycetozoa). The authors proposed that this amylase, ancestral to the Unikont clade, is shared among non-bilaterian metazoans (e.g. sponges, sea anemones and corals, and Placozoa), but was replaced in Bilateria by an alpha-amylase of bacterial origin, whose sequence is close to the typical animal amylases.

Given that a wealth of new genomes have been sequenced in the twelve years after that publication, we decided to explore again the diversification of this enzyme subfamily among the Eukaryota. We will focus mainly on Metazoa, in which we show unexpected situations of co-occurrence of both subfamilies GH13_1 and GH13_15/24 in the same genomes. We will discuss two mutually exclusive explanations that may be proposed: either the retention of the ancestral GH13_1 gene along with the typical bilaterian GH13_15/24 in some phyla, or horizontal transfer(s) from non-bilaterian animal donor(s) which would have to be identified.

Materials and methods

In order to further characterize the distribution of GH13_1 genes in Metazoa, we used the sequence of the sponge *Amphimedon queenslandica* GH13_1 (GenBank XP_019851448) as a query to perform BLASTP and TBLASTN searches on various online databases available in Genbank (nr, proteins, genomes, assembly, SRA, TSA, WGS), and also in the more specialized databases compagen.org, marinegenomics.oist.jp, reefgenomics.org, marimba.obs-vlfr.fr, vectorbase.org, PdumBase (pdumbase.gdcb.iastate.edu), AmpuBase (https://www.comp.hkbu.edu.hk/~db/AmpuBase/index.php) (Ip *et al.* 2018), between October 2018 and August 2019. Fungi were not searched further in this study because they are known to have a GH13_1 member as the usual alpha-amylase. To increase the chances to retrieve potential cnidarian or ctenophoran sequences, the starlet sea anemone *Nematostella vectensis* amylase (XP_001629956) was also used to query those databases. After the discovery of GH13_1-like sequences in Bilateria, the sequence XP_013396432 of the brachiopod *Lingula anatina* was also used for specific search in Bilateria. Non-animal eukaryote species were investigated using the *Dictyostelium discoideum* sequence XP_640516 as query. We chose a stringent BLAST threshold because glycosyl hydrolases from other GH13 subfamilies might be retrieved otherwise, owing to the presence of stretches of amino acids that are conserved across the subfamilies despite other enzymatic specificities (Janeček 1994; Janeček *et al.* 2014; MacGregor *et al.* 2001; van der Kaaij *et al.* 2007). Therefore, the BLAST hits (or High-scoring segment pairs HSPs) were considered further when expectation values (e-values) were better (lower) than 1e-100 for BLASTP or 1e-75 for TBLASTN in annotated or assembled genomes or transcriptomes which returned full-size or near full-size GH13_1 sequences. When only partial sequences could be retrieved using BLAST we collected a large genome region encompassing and flanking the BLAST hit and tried to reconstitute the full-size sequence. The stringent threshold was obviously not applied to constitutively small HSPs such as SRA (sequence read archives). These were only considered when several highly significant hits (typically 1e-10) covered a large part of the query sequence. Since SRA HSPs generally did not overlap, we could not assemble longer sequences and thus we did not use such sequences in alignments or phylogenies. SRA and transcriptome databases are prone to contamination, thus we checked by reciprocal BLAST that the retrieved sequences were not contaminations. SRA databases were used firstly when no or few assembled genomes or transcriptomes were available (e.g. Nemertea, Bryozoa). If a GH13_1 sequence was found in an annotated or assembled genome, SRA HSPs were also used in order to increase the sampling of related taxa and then added some support to the presence of GH13_1 in the lineage considered. On the other hand, we considered that within a given lineage, the absence of GH13_1 sequence in a reliable annotated or assembled genome,

combined to the detection of GH13_1 in SRA databases from related species would suggest that the GH13_1 gene was lost within this lineage in some taxa but not all. Conversely, if no GH13_1 sequence at all was found in any annotated genome and in any other database, we considered that the gene was lost in an ancestor of this lineage. When working with "assembled genomes" (non-annotated), we reconstituted exon-intron gene structure as well as the the protein sequence from the TBLASTN results. Finally, for phylogenetic analyses we kept only sequences which lay inside long contigs, or full-size or near full-size transcripts. We also checked once again the absence of animal-type alpha-amylase (GH13_15 or 24) outside the Bilateria using the sequence of the bivalve *Corbicula fluminea* (AAO17927) as a BLASTP query. The CAZy database (cazy.org (Lombard *et al.* 2014)), which is devoted to glycosyl hydrolases and related enzymes was used to check assignment of some of the sequences we found to the GH13_1 subfamily.

Intron-exon gene structures were recovered either from alignments between genomic sequences and their mRNA counterparts, or using annotated graphic views when available in the databases. In cases of likely erroneous annotations we reanalyzed the gene region by eye, correcting dubious frameshifts if necessary (see. Fig. S1 as an example). In some cases, for unannotated genes, the N-terminal and/or the C-terminal parts of the retrieved genomic sequences were uncertain, and were not retained in the analyses.

Alignments were performed using MUSCLE (Edgar 2004), as implemented in Geneious (Biomatters Ltd.). A maximum likelihood (ML) tree was built using PhyML's (Guindon and Gascuel 2003) current implementation at the phylogeny.fr portal (Dereeper *et al.* 2008). To this end, we first trimmed the N-terminal protein sequences up to the first well conserved motif LLTDR. C-terminal parts were also truncated at the last well aligned stretch. Gaps were removed from the alignments and data were analyzed under WAG (Whelan and Goldman 2001) with among-site rate variation modeled by four discretized rate categories sampled from a gamma distribution. Both the alpha parameter and the proportion of invariable sites were estimated from the data. The robustness of the nodes was estimated using an approximate likelihood ratio test (aLRT) (Anisimova and Gascuel 2006). The tree was drawn at the iTOL website (Letunic and Bork 2016). Metazoans and choanoflagellates were clustered as the ingroup.

The protein sequences, Fasta alignment and Newick-formatted tree are available at FigShare: https://figshare.com/articles/GH13_1_metazoa/9959369. Supplementary Figures and Tables are available at FigShare: https://figshare.com/articles/Suppl_data_GH13_1/9975956

Results

The sequences retrieved from the databases are listed in Table 1. The metazoans investigated are listed in Tables S1 (non-bilaterians) and S2 (bilaterians) with indication of the current state of genome/transcriptome sequencing, the database, the presence or absence of GH13_1 sequences, and the number of gene copies, where possible. A general protein alignment of the sequences found in this study along with already known GH13_1 sequences is shown in Fig. S2.

GH13_1 sequences retrieved from unicellular taxa

We confirmed the presence of GH13_1 in dictyostelids, in ciliates and also in oomycetes, some representatives of which (but not all) are indicated in Table 1. In two oomycetes, *Saprolegnia diclina* and *Achlya hypogyna*, the GH13_1-like sequences were the C-terminal half of longer sequences, the N-terminal half of which was similar to unclassified GH13 sequences found in e.g. *Acanthamoeba histolytica* (GenBank accession BAN39582), according to the CAZy database. In our general phylogenetic tree (Fig. 1), these sequences were used as outgroups. In choanoflagellates, where *Monosiga brevicollis* was already known to harbor a GH13_1 sequence (Da Lage *et al.* 2007), we found a GH13_1 sequence in the genome of *Salpingoeca rosetta*. A partial sequence was also returned from incomplete genome data from *Monosiga ovata* (at Compagen, not shown).

GH13_1 sequences retrieved from non-bilaterian animals

In Cnidaria, a number of GH13_1 sequences were recovered from many Anthozoa species (sea anemones, corals and allies), from genome as well as transcriptome data, at the Reefgenomics database (Table S1). Interestingly, we found no alpha-amylase sequences at all in Medusozoa (jellyfishes, hydras) nor in Endocnidozoa (parasitic cnidarians). In the general tree (Fig. 1), cnidarian sequences form a clear cluster with two main branches, grouping Actiniaria (sea anemones) and Pennatulacea (soft corals) on one branch, and Scleractinia (hard corals) and Corallimorpharia (mushroom anemones) on the other branch.

In sponges (Porifera), data were less abundant. No alpha-amylase sequence was found in *Sycon ciliatum* (Calcarea) and *Oscarella carmela* (Homoscleromorpha). All the sequences we retrieved belonged to Demospongiae. Similarly, we found no amylase sequence at all in the phylum Ctenophora (*Mnemiopsis leidyi*, *Pleurobrachia bachei*), the phylogenetic position of which is controversial: it has been recovered as the most basal metazoan (Whelan *et al.* 2017), as

Cnidaria's sister group e.g. Simion *et al.* 2017, Philippe *et al.* 2009), re-establishing Coelenterata, and also as the earliest branch in the Eumetazoa (animals with a digestive cavity and/or extra cellular digestion) e.g. Pisani *et al.* 2015.

GH13_1 sequences retrieved from bilaterian animals

The surprising finding of this study, on which we will focus our attention, is the consistent, albeit sparse, occurrence of GH13_1 alpha-amylase sequences in several bilaterian phyla: hemichordates, which are deuterostomes, brachiopods, phoronids (Brachiozoa) and Bryozoa, and in some molluscs and annelids (Eutrochozoa), which are all protostomes. In the well annotated genomes of the brachiopod *Lingula anatina* and the phoronid *Phoronis australis*, two paralogs were found (Table 1). In both species, the two copies are located on different contigs. The paralog sequences are rather divergent, given their positions in the tree (Fig 1) and each paralog groups the two species together. This indicates that not only duplication, but also the divergence between paralogs is ancestral to these species, dating back at least to basal Cambrian, according to the TimeTree database (Kumar *et al.* 2017). GH13_1 sequences were found in other brachiopods and phoronids as sequence reads (SRA) from transcriptome data only, with no available genomic support (listed in Table 1 and S2). We must be cautious when only transcriptome data are available, as transcripts from contaminating symbionts or parasites may generate false positives (Borner and Burmester 2017) and/or the lack of expression of the targeted sequence in the investigated tissues may lead to false negatives. However, seven different brachiopod species returned positive hits, giving some robustness to our finding. Two phyla are related to Brachiozoa : Bryozoa and Nemertea (Kocot 2015; Luo *et al.* 2018, but see Marlétaz *et al.* 2019). We found clues for the presence of GH13_1 in four Bryozoa species, but only transcriptome reads were available. In contrast, in Nemertea, none of the 14 species investigated returned any GH13_1 sequence, including the annotated genome of *Notosperma geniculatus*.

Similarly, we found three gene copies in the genomes of the hemichordates *Saccoglossus kowalevskii* and *Ptychodera flava*. In both species, two copies are close to each other (XP_006816581 and XP_006816582 in *S. kowalevskii*, and their counterparts in *P. flava*) as shown by the topology of the gene tree (Fig. 1). This could suggest independent gene duplication in each species. However, we observed that the two duplicates were arranged in tandem in both species, which would rather suggest concerted evolution of two shared copies. In

P. flava, this genome region is erroneously annotated as a single gene at the OIST Marine Genomics database. The third paralog is very divergent from the two other copies, so its divergence from the ancestral copy probably occurred before the species split, as well. The three copies were therefore probably already present before the split of the two lineages, some 435 mya (Kumar *et al.* 2017). Three other hemichordate species, *Schizocardium californicum*, *Torquaratoridae antarctica* and *Rhabdopleura sp*. harbor a GH13_1 gene, as shown by SRA search in GenBank (Table 1). A positive result was also retrieved from the genome of *Glandiceps talaboti* (Héctor Escrivà, Oceanology Observatory at Banyuls-sur-mer, personal communication).

In molluscs, we found BLAST hits with significant e-values in gastropod species from two clades only, the Vetigastropoda (e.g. the abalone *Haliotis* sp.) and the Caenogastropoda (e.g. Ampullariidae such as *Pomacea canaliculata*). We consistently found one copy in eight species belonging to the family Ampullariidae. In *P. canaliculata*, the genome of which has been well annotated, the GH13_1 sequence (XP_025109323) lies well inside a 26 Mbp long scaffold (linkage group 10, NC_037599) and is surrounded by *bona fide* molluscan genes (Table S3). GH13_1 sequences were found in other Caenogastropoda from SRA or transcriptome databases (Table 1 and S2). We also found GH13_1 sequences in several bivalve clades: Mytiloida (e.g. the mussel *Mytilus galloprovincialis*), Pterioida (e.g. the pearl oyster *Pinctada imbricata*), Arcoida (e.g. *Scapharca broughtoni*) and in the Unionoida *Cristaria plicata*. For sequences retrieved from the TSA or SRA databases (see Table 1), whose issues were mentioned above, we performed reciprocal BLAST in GenBank nr. Almost always *Lingula anatina* was recovered as the best hit. However, as an example of the necessary careful examination of results, we found a significant HSP in a transcriptome database of the sea hare *Aplysia californica* (TSA GBDA01069500). This sequence was not found in the well annotated *A. californica* genome, and turned out to be related to ciliates. We found no occurrence of GH13_1 in Veneroida, Pectinoida and Ostreoida, for which annotated and/or assembled genomes exist, nor in cephalopods.

In annelids, we found occurrences of GH13_1 genes in a few species, the genomes of which are still not fully assembled, namely the “polychaetes” *Hydroides elegans*, *Pygospio elegans* and *Spirobranchus lamarcki* but not in the well-annotated genome of *Capitella teleta*. We also recovered HSPs from the clitellate *Glossoscolex paulistus* but not from *Amynthas corticis* or *Eisenia fetida*. We found no GH13_1 sequences in Hirudinea (leeches). To summarize, in molluscs as well as in annelids, the presence of GH13_1 genes is scattered and patchy across and

within lineages. Interestingly, we found that some of the mollusc GH13_1-like sequences, especially in bivalves, were much shorter, either truncated at the C-terminal, or this region was so divergent from the query sequence (*L. anatina)* that it was impossible to identify, assemble and align it with our data set (Fig. S2). In addition, we found that the annelid *Hydroides elegans* had an internal deletion, which precluded its inclusion in the phylogenetic analysis. This suggests that those sequences may not have alpha-amylase activity.

Gene tree analysis: position of bilaterian sequences

The goal of the gene tree analysis is to examine whether the occurrence of GH13_1 genes in bilaterian animals may be due to independent horizontal gene transfers (HGT) or if they descend from a GH13_1 alpha-amylase copy ancestral to Unikonts. In the first case, the bilaterians GH13_1 sequences are unlikely to cluster together and the gene tree topology will likely display one or more nodes that are inconsistent with the bilaterian phylogeny. In the second case, the bilaterian sequences are expected to recover a bilaterian clade and to have a cnidarian clade as its sister group (Laumer *et al.* 2018). The actual tree topology (Fig. 1) is not that straightforward when it comes to the bilaterian relationships, although we may rule out any proximity of bilaterians GH13_1 sequences with unicellular or fungal sequences, regardless of tree rooting.

All Cnidarian orthologs form a well-supported cluster. The sister relationship between Corallimorpharia and Scleractinia reflects what was recovered in species trees using different markers (e.g. Rodríguez *et al.* 2014), although the Scleractinia topology disagrees with previous phylogenetic analyses of the order (e.g. Barbeitos *et al.* 2010). The other cluster within Cnidaria is mainly composed of actiniarian (sea anemones) sequences, but it also includes, with strong support, the sequence queried from the sea pen *Renilla reniformis* (order Pennatulacea). This order belongs to the sub-class Octocorallia and not to Hexacorallia, the monophyletic sub-class in which scleractinians, corallimorpharians and sea anemones are found (e.g. Rodríguez *et al.* 2014). We used RAXML-NG v0.80 (Kozlov *et al.* 2019) to conduct a constrained search under WAG for a ML tree in which Hexacorallia was monophyletic and *R. reniformis* was placed as its sister group (e.g. Chang *et al.* 2015; Zapata *et al.* 2015)) and employed a simple LR test to statistically evaluate the difference betweeen the observed and expected (phylogenetic) placement of the *R. reniformis* sequence (Kozlov *et al.* 2019). The log-likelihood difference between the unconstrained (lnLh = -29,155.37) and constrained (lnLh = -29,208.38) ML tree

scores was 53.01. According to Kass and Raftery (1995), there is very strong support for the highest likelihood hypothesis (in our case, the ML tree in Fig. 1) when the double of this difference (i.e. 2 x 53.01 = 106.02) exceeds 10 log-likelihood units. Thus, there is significant inconsistency between the position of *R. reniformis*' GH13_1 copy and the phylogenetic placement of this species. This may be due to a horizontal transfer event that would have occurred within Cnidaria, but additional data from well-sequenced Pennatulacea would be welcome to check this possibility. Nevertheless, it is noteworthy that the genome of *Dendronephthya gigantea* (Octocorallia, order Alcyonacea) returned no result. Most bilaterian sequences are clustered with Cnidaria, as phylogenetically expected in the case of a shared ancestral gene, as a robust cluster grouping one Brachiozoa (brachiopod/phoronid) copy, the molluscs and the annelids, which is consistent with the phylogeny. However, the tandem hemichordate duplicates and the other Brachiozoa genes are not included in the bilaterian clade, but remain ingroup relative to the sponge sequences.

Interestingly, the two remaining hemichordate sequences are the earliest diverging lineage of the Metazoa + Choanoflagellata cluster, since they are branched with the placozoan *Trichoplax adhaerens* sequence, this relationship being strongly supported whatever the tree reconstruction method employed (Fig. 1, and data not shown). In order to check for the possibility of a long branch attraction (LBA), which would artificially cluster hemichordate and placozoan sequences, we performed Tajima's relative rate tests (Tajima 1993) using MEGA7 (Kumar *et al.* 2016). The sequence of *S. kowalevskii* XP_006819810, suspected to evolve fast, was compared with its paralog XP_006816581, using five different outgroups, i.e. the three sponges and the two choanoflagellates. Unexpectedly, the χ^2 tests returned non-significant values in two tests and significant values in three tests (Table S4). Therefore, with our data, LBA cannot be entirely ruled out in this particular case.

Analysis of intron positions

Intron positions may be valuable markers when reconstituting gene histories. We identified 56 intron positions from the subset of species of the general tree for which we could find data (Fig. 2). Only one intron position is widely shared among these GH13_1 gene sequences. It is the first position reported in the alignment, and it lies just upstream to the first conserved part of the alignment. The main observation is the numerous conserved positions across bilaterian sequences (10 positions), and between bilaterian sequences and the sponge and

the Placozoa (7 positions). In addition, three positions are common to bilaterians and the choanoflagellate *Monosiga brevicollis*. In contrast, the Cnidaria have few introns, with positions different from the sponge and the bilaterians, except for position 1. The other species under examination, i.e. protists and fungi, have essentially specific intron positions. Therefore, the overall conservation of intron positions across bilaterians + sponges is a further argument to state that an explanation of the occurrence of GH13_1 alpha-amylases in some bilaterians does not involve non-animal species.

Discussion

The evolutionary scenario proposed by Da Lage *et al.* 2007, suggested that the GH13_1 alpha-amylase gene ancestral to Unikonts (Amoebozoa and Opisthokonts, i.e. Fungi and Metazoa/Choanoflagellata) was totally absent from Bilateria, due to its complete replacement by a new alpha-amylase, originating from a bacterium through HGT. Here, we have shown that a limited number of bilaterian lineages, all aquatic species, namely hemichordates, brachiozoans, bryozoans, and some sparse molluscs and annelids, actually do harbor GH13_1 alpha-amylase genes. Note that all those species also have at least one classical animal alpha-amylase of the GH13_15/24 subfamilies. Several species with whole genome well sequenced and annotated were found to harbor such genes in each phylum Hemichordata, Brachiozoa and molluscs. They were investigated in more details, especially regarding the genomic environment of their GH13_1 genes. We are quite confident that the GH13_1 sequences we found are not due to contaminating DNA. First, the bilaterian sequences retrieved from annotated genomes were inside long contigs, and mostly surrounded by genes showing bilaterian best BLAST hits (Table S3). However, the *S. kowalevskii* XP_006819810 gene could appear somewhat dubious, since it is placed at the distal end of a contig, with only two other genes on the contig (Table S3), one of which has a placozoan best hit. But its *P. flava* counterpart is well inside a gene-rich contig. Therefore, these seemingly non-bilaterian genes are well in bilaterian genomic contexts. Second, a lot of additional sequences from other species belonging to these phyla were gathered from more sketchy data, i.e. lower-quality assembled genomes, transcriptomes or sequence read archive databases, which added some support to the presence of these amylase genes. Although transcriptome and rough genomic data should be handled with care, this lends support to our observations. Moreover, reciprocal BLAST from the transcriptome hits always returned a

bilaterian (*L. anatina* or *S. kowalevskii*) best hit, not fungal, protist or other non-bilaterian GH13_1 sequence.

The new data unveils an evolutionary story more complicated than previously supposed. There are two alternative explanations. The first explanation is that several HGTs occurred from non-bilaterian to both hemichordate and Lophotrochozoa ancestors. The second explanation is that the ancestral GH13_1 gene was not lost in all bilaterian lineages, but remained (given the current data) in hemichordates, Brachiozoa, Bryozoa, and in scattered lineages across Mollusca and Annelida.

The hypothesis of HGT requires several such events between metazoans. It implies that HGTs obviously happened after the split of the two main branches of bilaterians, protostomes and deuterostomes, otherwise the transferred copies should have been lost in most phyla, like in the alternative hypothesis. More precisely, in the case of Lophotrochozoa, this would have occurred before the diversification of this clade and after its divergence from the Platyzoa, some 700 mya (Kumar *et al.* 2017); in the case of hemichordates, after diverging from their common ancestor with the echinoderms, and before the divergence between *S. kowalevskii* and *Ptychodera flava*, i.e. between 657 and ca. 435 mya (Kumar *et al.* 2017). Therefore, we may infer *at least* two HGTs, each early in the evolution of the phyla, with a number of subsequent losses in Lophotrochozoa (Fig. 3A). The donor species, given the sequence clustering in the trees, could be related to cnidarians. However, we have underlined that the intron-exon structures of the bilaterian sequences were most similar to the one of the sponge, and that the cnidarian GH13_1 amylases had very different structures. This may be possible if the donors were related to cnidarians, perhaps an extinct phylum or an ancestor of extant Cnidaria, but had conserved the ancestral structures exemplified by the sponge and the placozoan. Indeed, if the structure shared by the sponge, the placozoan and the bilaterians reflects the ancestral state, cnidarians must have undergone a drastic rearrangement of the intron-exon structure of this gene. This would be in line with the long internal branch leading to this clade in the trees (Fig. 1), which suggests accelerated evolution.

The alternative hypothesis of massive GH13_1 gene loss in most phyla except the ones where we found such sequences seems no more parsimonious. It requires many losses, the number of which depends on the phylogeny used. For instance, considering the phylogeny shown in Fig. 3B, regarding deuterostomes, one loss occurred in echinoderms and another one in chordates. In protostomes, one GH13_1 loss in ecdysozoans, and independent losses in Platyzoa and in several lophotrochozoan lineages would be required to produce the observed pattern.

However, although not parsimonious in terms of number of events, we would rather favor the gene loss hypothesis, because this is a common phenomenon, especially given how ubiquitous co-option is (Flores and Livingstone 2017; Hejnol and Martindale 2008). In this respect, the GH13_15/24 gene that was acquired from a bacterium is a type of horizontal transfer akin to what Husnik and McCutcheon called a "maintenance transfer" since it allowed the original function to be maintained while the primitive GH13_1 gene became free to evolve or even to be lost (Husnik and McCutcheon 2018) (see also Da Lage *et al.* 2013). In contrast, while numerous cases of HGT from bacteria to metazoans, or from fungi to metazoans have been reported (e.g. Wybouv *et al.* 2016; Dunning Hotopp 2011, 2018; Haegeman *et al.* 2011; Crisp *et al.* 2015; Cordaux and Gilbert 2017), very few HGT events have been inferred that involve a metazoan donor and a metazoan receiver (Rödelsperger and Sommer 2011; Graham *et al.* 2012; Gasmi *et al.* 2015). Thus, our current knowledge on HGT suggests that this type of transfer might be very rare between metazoans, and that two or more such events would be quite unlikely to explain the current taxonomic distribution of metazoan GH13_1 genes. In addition, it has been shown that a seemingly patchy gene distribution suggestive of HGT may, after more comprehensive taxon sampling, turn out to be rather due to recurrent gene losses, as discussed in Husnik and McCutcheon (2018). The conservation of the intron-exon structure across phyla, probably ancestral to the metazoans, would not be surprising (Sullivan *et al.* 2006 ; Srivastava *et al.* 2010 ; Srivastava *et al.* 2008). For instance, 82% of human introns have orthologous introns in *T. adhaerens* (Srivastava *et al.* 2008).

In the present study we used the results of BLAST searches (BLASTP and TBLASTN) as raw material using the GH13_1-like alpha-amylases found in non-bilaterian animals (Da Lage *et al.* 2007) as query sequences. The stringent threshold we have set avoids retrieving irrelevant sequences belonging to other GH13 subfamilies or even other GH families. For instance, HMM search, such as in PFAM (pfam.xfam.org), shows that the domain composition of e.g. the *Lingula anatina* sequence XP_013396432 consists in an alpha-amylase domain linked to a DUFF1966 domain (DUFF1266 is also present in several fungal proteins, including obviously the GH13_1 amylase). The alpha-amylase domain is actually present in many glycosyl hydrolase families. Interestingly, the sequences found in some molluscs do not have a complete alpha-amylase domain, because they are shorter than usual (see Results). We assumed nonetheless that all the sequences we recovered belong to the GH13_1 subfamily, due to sequence similarities, as shown by the easy sequence alignment. Further, some of them have been assigned to this

subfamily in the reference database CAZy.org (see Table 1). In addition, if we add sequences from the closest subfamilies, namely GH13_2 or GH13_19 (Stam *et al.* 2006) in the alignment and in the phylogenetic tree, the putative GH13_1 and the ascertained GH13_1 remain well clustered together (not shown). It is possible that modifications of a few amino acid positions could bring a change in the substrate or catalytic activity. For instance, concerning the substrate affinity, when the genome of *L. anatina* was released, the authors hypothesized a biomineralization pathway that involves acid proteins, as found in scleractinians and molluscs (Marin *et al.* 2007; Ramos-Silva *et al.* 2013). Given the calcium binding activity of alpha-amylases (Boel *et al.* 1990; Grossman and James 1993; Svensson 1994; Pujadas and Palau 2001), the presence of both GH13_1 and GH13_15/24 subfamilies in *L. anatina* opens the possibility for the neofunctionalization of one of them in the biomineralization process. In the analyses performed by those authors, no amylase was found in the shell matrix, but this does not exclude the possibility of its presence in the pathway. Moreover, the fact that in some molluscs, the sequences are incomplete compared to the brachiopod query or to the sponge and cnidarian GH13_1 amylases, and therefore probably devoid of an amylolytic function, would add credence to another function, especially considering that they are transcribed. This conjecture requires further investigation. On the other hand, the full-size GH13_1 sequences only present in a few bilaterians could have remained true alpha-amylases with the classical function, but this would make even more enigmatic why they have been conserved, either by descent or by horizontal transfer.

Acknowledgments: We want to thank Pedro E. Vieira for introducing AD to molecular analyses. We are grateful to Didier Casane and Emmanuelle Renard for fruitful advise and discussion and two anonymous reviewers for critical reading of the manuscript. We thank Héctor Escrivà for sharing sequence data. This work was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process no. 141565/2017-9) to AD and regular funding of the CNRS to JLDL and CG. CG was also supported by a grant from Agence Nationale de la Recherche (ANR-15-CE32-0011-01 TransVir).

References :

Anisimova, M., and O. Gascuel, 2006 Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Systematic Biology 55 (4):539-552.

Barbeitos, M.S., S.L. Romano, and H.R. Lasker, 2010 Repeated loss of coloniality and symbiosis in scleractinian corals. Proc Natl Acad Sci U S A 107 (26):11877-11882.

Boel, E., L. Brady, A.M. Brzozoxski, Z. Derewenda, G.G. Dodson *et al.*, 1990 Calcium-binding in α-amylases: an X-ray diffraction study at 2.1 Å resolution of two enzymes from *Aspergillus*. Biochemistry 29:6244-6249.

Borner, J., and T. Burmester, 2017 Parasite infection of public databases: a data mining approach to identify apicomplexan contaminations in animal genome and transcriptome assemblies. BMC Genomics 18 (1):100.

Chang, E.S., M. Neuhof, N.D. Rubinstein, A. Diamant, H. Philippe *et al.*, 2015 Genomic Insights into the Evolutionary Origin of Myxozoa within Cnidaria. Proceedings of the National Academy of Sciences of the U.S.A. 112 (48):14912–14917.

Cordaux, R., and C. Gilbert, 2017 Evolutionary significance of *Wolbachia*-to-animal horizontal gene transfer: Female sex determination and the f element in the isopod *Armadillidium vulgare*. Genes 8 (7): doi: 10.3390/genes8070186.

Crisp, A., C. Boschetti, M. Perry, A. Tunnacliffe, and G. Micklem, 2015 Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. Genome Biology 16:50

Da Lage, J.-L., M. Binder, A. Hua-Van, S. Janecek, and D. Casane, 2013 Gene make-up: rapid and massive intron gains after horizontal transfer of a bacterial alpha-amylase gene to Basidiomycetes. BMC Evolutionary Biology 13:40.

Da Lage, J.-L., E.G.J. Danchin, and D. Casane, 2007 Where do animal α-amylases come from? An interkingdom trip. FEBS Letters 581:3927-3935.

Dereeper, A., V. Guignon, G. Blanc, S. Audic, S. Buffet *et al.*, 2008 Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Research 36 (Web Server Issue):W465-469.

Dunning Hotopp, J.C., 2011 Horizontal gene transfer between bacteria and animals. Trends in Genetics 27 (4): 157-163.

Dunning Hotopp, J.C., 2018 Grafting or pruning in the animal tree: lateral gene transfer and gene loss? BMC Genomics 19:470.

Edgar, R.C., 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32 (5):1792-1797.

461 Flores, R.L., and B.T. Livingstone, 2017 The skeletal proteome of the sea star *Patiria miniata*
462 and evolution of biomineralization in echinoderms. BMC Evolutionary Biology 17
463 (125):1-14.
464 Gasmi, L., H. Boulain, A. Hua-Van, K. Musset, A.K. Jakubowska *et al.*, 2015 Recurrent
465 domestication by Lepidoptera of genes from their parasites mediated by Bracoviruses.
466 PLoS Genetics 11 (9):e1005470.
467 Graham, L.A., J. Li, W.S. Davidson, and P.L. Davies, 2012 Smelt was the likely beneficiary of
468 an antifreeze gene laterally transferred between fishes. BMC Evolutionary Biology 12
469 (190): doi: 10.1186/1471-2148-12-190.
470 Grossman, G.L., and A.A. James, 1993 The salivary glands of the vector mosquito *Aedes*
471 *aegypti*, express a novel member of the amylase gene family. Insect Molecular Biology
472 1 (4):223-232.
473 Guindon, S., and O. Gascuel, 2003 A simple, fast, and accurate algorithm to estimate large
474 phylogenies by maximum likelihood. . Systematic Biology 52:696-704.
475 Haegeman, A., J.T. Jones, and E.G. Danchin, 2011 Horizontal gene transfer in nematodes: a
476 catalyst for plant parasitism? Molecular Plant and Microbe Interactions 24 (8):879-887.
477 Hejnol, A., and M.Q. Martindale, 2008 Acoel development indicates the independent evolution
478 of the bilaterian mouth and anus. Nature 456:382-386.
479 Henrissat, B., and G. Davies, 1997 Structural and sequence-based classification of glycoside
480 hydrolases. Current Opinion in Structural Biology 7 (5):637-644.
481 Husnik, P., and J.P. McCutcheon, 2018 Functional horizontal gene transfer from bacteria to
482 eukaryotes. Nature Reviews Microbiology 16:67-79.
483 Ip, J.C.H., H. Mu, Q. Chen, J. Sun, S. Ituarte *et al.*, 2018 AmpuBase: a transcritome database
484 for eight species of apple snails (Gastropoda: Ampullariidae). BMC Genomics 19:179.
485 Janeček, Š., 1994 Sequence similarities and evolutionary relationships of microbial, plant and
486 animal alpha-amylases. European Journal of Biochemistry 224:519-524.
487 Janeček, Š., B. Svensson, and E.A. MacGregor, 2014 α-amylase: an enzyme specificity found
488 in various families of glycoside hydrolases. Cellular and Molecular Life Science
489 71:1149-1170.
490 Kass, R.E., and A.E. Raftery, 1995 Bayes Factors. Journal of the American Statistical
491 Association 90 (430):773–795.
492 Kocot, K.M., 2015 On 20 years of Lophotrochozoa. Organisms Diversity and Evolution
493 16:329-343.

494 Kocot, K.M., T.H. Struck, J. Merkel, D.S. Waits, C. Todt *et al.*, 2017 Phylogenomics of
495 Lophotrochozoa with considerations of systematic error. Systematic Biology 66
496 (2):256-282.
497 Kozlov, A.M., D. Darriba, T. Flouri, B. Morel, and A. Stamatakis, 2019 RAxML-NG: A Fast,
498 Scalable and User-Friendly Tool for Maximum Likelihood Phylogenetic Inference.
499 Bioinformatics doi.org/10.1093/bioinformatics/btz305.
500 Kumar, S., G. Stecher, M. Suleski, and S.B. Hedges, 2017 TimeTree: a resource for timelines,
501 timetrees, and divergence times. Molecular Biology and Evolution 34 (7):1812-1819.
502 Kumar, S., G. Stecher, and K. Tamura, 2016 MEGA7: Molecular Evolutionary Genetics
503 Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-
504 1874.
505 Laumer, C.E., H. Gruber-Vodicka, M.G. Hadfield, V.B. Pearse, A. Riesgo *et al.*, 2018 Support
506 for a clade of Placozoa and Cnidaria in genes with minimal compositional bias. eLife
507 7:e36278.
508 Letunic, I., and P. Bork, 2016 Interactive tree of life(iTOL) v3: an online tool for the display
509 and annotation of phylogenetic and other trees. Nucleic Acids Research 44 (W1):W242-
510 245.
511 Lombard, V., H. HGolaconda Ramulu, E. Drula, P.M. Coutinho, and B. Henrissat, 2014 The
512 carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research
513 42:490-495.
514 Luo, Y.-J., M. Kanda, R. Koyanagi, K. Hisata, T. Akiyama *et al.*, 2018 Nemertean and
515 phoronid genomes reveal lophotrochozoan evolution and the origin of bilaterian heads.
516 Nature Ecology and Evolution 2:141-151.
517 Luo, Y.-J., T. Takeuchi, R. Koyanagi, L. Yamada, M. Kanda *et al.*, 2015 The *Lingula* genome
518 provides insights into brachiopod evolution and the origin of phosphate
519 biomineralization. Nature communications 6:8301.
520 MacGregor, E.A., S. Janecek, and B. Svenssson, 2001 Relationship of sequence and structure
521 to specificity in the α-amylase family of enzymes. Biochimica et Biophysica Acta
522 1546:1-20.
523 Marin, F., G. Luquet, B. Marie, and D. Medakovic, 2007 Molluscan shell proteins: primary
524 structure, origin, and evolution. Current Topics in Developmental Biology 80:209-276.
525 Marlétaz, F., K.T.C.A. Peljnenburg, T. Goto, N. Satoh, and D.S. Rokhsar, 2019 A New
526 spiralian phylogeny places the enigmatic arrow worms among Gnathiferans. Current
527 Biology 29 (2):312-318.

528 Philippe, H., R. Derelle, P. Lopez, K. Pick, C. Borchiellini *et al.*, 2009 Phylogenomics revives
529 traditional views on deep animal relationships. Current Biology 19 (8):706-712.
530 Pisani, D., W. Pett, M. Dohrmann, R. Feuda, O. Rota-Stabelli *et al.*, 2015 Genomic data do not
531 support comb jellies as the sister group to all other animals. Proc Natl Acad Sci U S A
532 112:15402-15407.
533 Plazzi, F., A. Ceregato, M. Taviani, and M. Passamonti, 2011 A molecular phylogeny of
534 bivalve mollusks: Ancient radiations and divergences as revealed by mitochondrial
535 genes. PLoS one 6 (11):e27147
536 Pujadas, G., and J. Palau, 2001 Evolution of α-amylase: architectural features and key residues
537 in the stabilization of the $(\beta/\alpha)_8$ scaffold. Molecular Biology and Evolution 18:38-54.
538 Ramos-Silva, P., J. Kaandorp, L. Huisman, B. Marie, I. Zanella-Cléon *et al.*, 2013 The skeletal
539 proteome of the coral *Acropora millepora*: the evolution of calcification by co-option
540 and domain shuffling. Molecular Biology and Evolution 30:2099-2112.
541 Rödelsperger, C., and R.J. Sommer, 2011 Computational archaeology of the *Pristionchus*
542 *pacificus* genome reveals evidence of horizontal gene transfers from insects. BMC
543 Evolutionary Biology 11:239.
544 Rodríguez, E., M.S. Barbeitos, M.R. Brugler, L.M. Crowley, A. Grajales *et al.*, 2014 Hidden
545 among Sea Anemones: The First Comprehensive Phylogenetic Reconstruction of the
546 Order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) Reveals a Novel Group of
547 Hexacorals. PLoS one 9 (5):e96998.
548 Simion, P., H. Philippe, D. Baurain, M. Jager, D.J. Richter *et al.*, 2017 A large and consistent
549 phylogeny dataset supports sponges as the sister group to all other animals. Current
550 Biology 27:958-967.
551 Srivastava, M., E. Begovic, J. Chapman, N.H. Putnam, U. Hellsten *et al.*, 2008 The *Trichoplax*
552 genome and the nature of placozoans. Nature 454:955-960.
553 Srivastava, M., O. Simakov, J. Chapman, B. Fahey, M.E.A. Gauthier *et al.*, 2010 The
554 *Amphimedon queenslandica* genome and the evolution of animal complexity. Nature
555 466:720-726.
556 Stam, M.R., E.G.J. Danchin, C. Rancurel, P.M. Coutinho, and B. Henrissat, 2006 Dividing the
557 large glycoside hydrolase family 13 into subfamilies: towards improved functional
558 annotations of α-amylase-related proteins. Protein Engineering, Design & Selection 19
559 (12):555-562.

Sullivan, J.C., A.M. Reitzel, and J.R. Finnerty, 2006 A high percentage of introns in human genes were present early in animal evolution: evidence from the basal metazoan *Nematostella vectensis*. Genome Informatics 17 (1):219-229.

Svensson, B., 1994 Protein engineering in the α-amylase family: catalytic mechanism, substrate specificity, and stability. Plant Molecular Biology 25:141-157.

Tajima, F., 1993 Simple methods for testing the molecular evolutionary clock hypothesis. Genetics 135:599-607.

Uribe, J., Y. Kano, J. Templado, and R. Zardoya, 2016 Mitogenomics of Vetigastropoda: insights into the evolution of pallial symmetry. Zoologica Scripta 45:145-159.

van der Kaaij, R.M., Š. Janeček, M.J.E.C. van der Maarel, and L. Dijkhuizen, 2007 Phylogenetic and biochemical characterization of a novel cluster of intracellular fungal α-amylase enzymes. Microbiology 153:4003-4015.

Whelan, N.V., K.M. Kocot, T.P. Moroz, K. Mukherjee, P. Williams *et al.*, 2017 Ctenophore relationships and their placement as the sister group to all other animals. Nature Ecology and Evolution 1 (11):1737-1746.

Whelan, S., and N. Goldman, 2001 A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Molecular Biology and Evolution 18 (5):691-699.

Wybouv, N., Y. Pauchet, D.G. Heckel, and T. Van Leuuwen, 2016 Horizontal gene transfer contributes to the evolution of arthropod herbivory. Genome Biology and Evolution 8 (6):3594-3613.

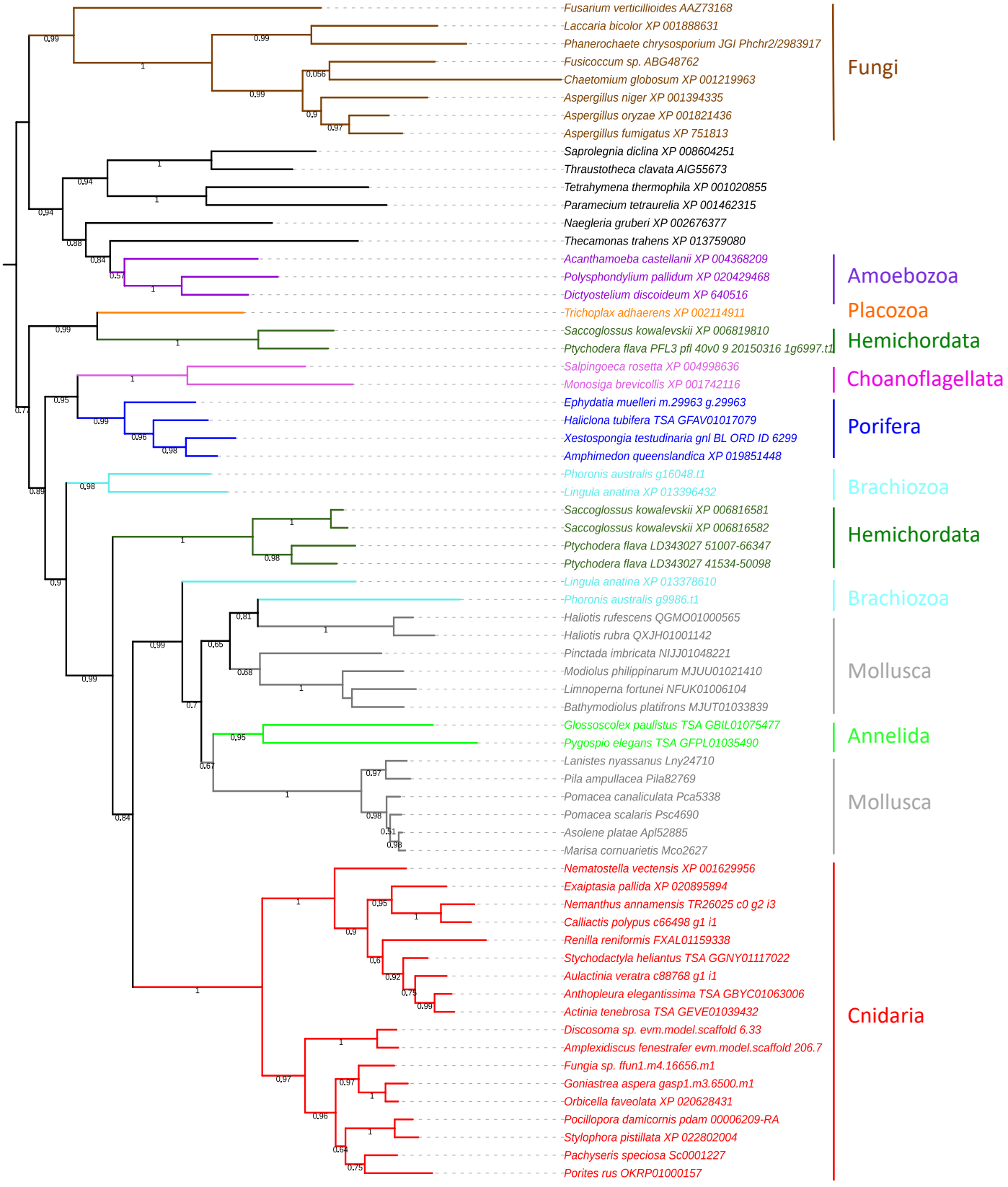
Zapata, F., F.E. Goetz, S.A. Smith, M. Howison, S. Siebert *et al.*, 2015 Phylogenomic Analyses Support Traditional Relationships within Cnidaria. PLoS one 10:e0139068.

Legends of figures :

Figure 1: ML tree of GH13_1 protein sequences of metazoan and non-metazoan species. The tree was rooted by placing fungi and unicellular organisms, except choanoflagellates, as outgroups. The numbers at the nodes are the aLRT supports. Dark green: hemichordates; light blue: brachiozoans; red: cnidarians, dark blue: sponges; orange: placozoans; pink: choanoflagellates; purple: amoebozoans; brown: fungi; grey, molluscs; bright green: annelids; black: other protists.

Figure 2: Intron positions compared across the sampled GH13_1 genes. The intron positions found in the studied parts of the sequences were numbered from 1 to 56. Pink: phase zero introns; green: phase 1 introns; blue: phase 2 introns. The black horizontal bar separates bilaterians from species where GH13_1 alpha-amylases are considered native. The color code for species is the same as in Figure 1.

Figure 3: Two scenarii of HGT/gene losses of the GH13_1 genes. HGT or gene loss events were plotted on one of the proposed phylogenies of Bilateria, adapted from Plazzi *et al.* (2011); Kocot (2015); Kocot *et al.* (2017); Luo *et al.* (2015); Luo *et al.* (2018); Uribe *et al.* (2016). Fractions after the lineage names are the number of species showing GH13_1 sequences over the total number of species investigated. A: HGT hypothesis. Black diamonds represent the HGT events, crosses indicate subsequent GH13_1 loss events. B: Gene loss hypothesis. Crosses indicate GH13_1 loss events. Dashed crosses indicate lineages for which only a fraction of the available reliable genome or transcriptome data were found to contain a GH13_1 sequence. Divergence times are from Kumar *et al.* (2017).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Fungi
Saprolegnia diclina XP 008604251
Thraustotheca clavata AIG55673
Tetrahymena thermophila XP 001020855
Paramecium tetraurelia XP 001462315
Naegleria gruberi XP 002676377
Thecamonas trahens XP 013759080
Acanthamoeba castellanii XP 004368209
Polysphondylium pallidum XP 020429468
Dictyostelium discoideum XP 640516
Amoebozoa
Trichoplax adhaerens XP 002114911
Placozoa
Saccoglossus kowalevskii XP 006819810
Ptychodera flava PFL3 pfl 40v0 9 20150316 1g6997.t1
Hemichordata
Salpingoeca rosetta XP 004998636
Monosiga brevicollis XP 001742116
Choanoflagellata
Ephydatia muelleri m.29963 g.29963
Haliclona tubifera TSA GFAV01017079
Xestospongia testudinaria gnl BL ORD ID 6299
Amphimedon queenslandica XP 019851448
Porifera
Phoronis australis g16048.t1
Lingula anatina XP 013396432
Brachiozoa
Saccoglossus kowalevskii XP 006816581
Saccoglossus kowalevskii XP 006816582
Ptychodera flava LD343027 51007-66347
Ptychodera flava LD343027 41534-50098
Hemichordata
Lingula anatina XP 013378610
Phoronis australis g9986.t1
Brachiozoa
Haliotis rufescens QGMO01000565
Haliotis rubra QXJH01001142
Pinctada imbricata NIJJ01048221
Modiolus philippinarum MJUU01021410
Limnoperna fortunei NFUK01006104
Bathymodiolus platifrons MJUT01033839
Mollusca
Glossoscolex paulistus TSA GBIL01075477
Pygospio elegans TSA GFPL01035490
Annelida
Lanistes nyassanus Lny24710
Pila ampullacea Pila82769
Pomacea canaliculata Pca5338
Pomacea scalaris Psc4690
Asolene platae Apl52885
Marisa cornuarietis Mco2627
Mollusca
Nematostella vectensis XP 001629956
Exaiptasia pallida XP 020895894
Nemanthus annamensis TR26025 c0 g2 i3
Calliactis polypus c66498 g1 i1
Renilla reniformis FXAL01159338
Stychodactyla heliantus TSA GGNY01117022
Aulactinia veratra c88768 g1 i1
Anthopleura elegantissima TSA GBYC01063006
Actinia tenebrosa TSA GEVE01039432
Discosoma sp. evm.model.scaffold 6.33
Amplexidiscus fenestrafer evm.model.scaffold 206.7
Fungia sp. ffun1.m4.16656.m1
Goniastrea aspera gasp1.m3.6500.m1
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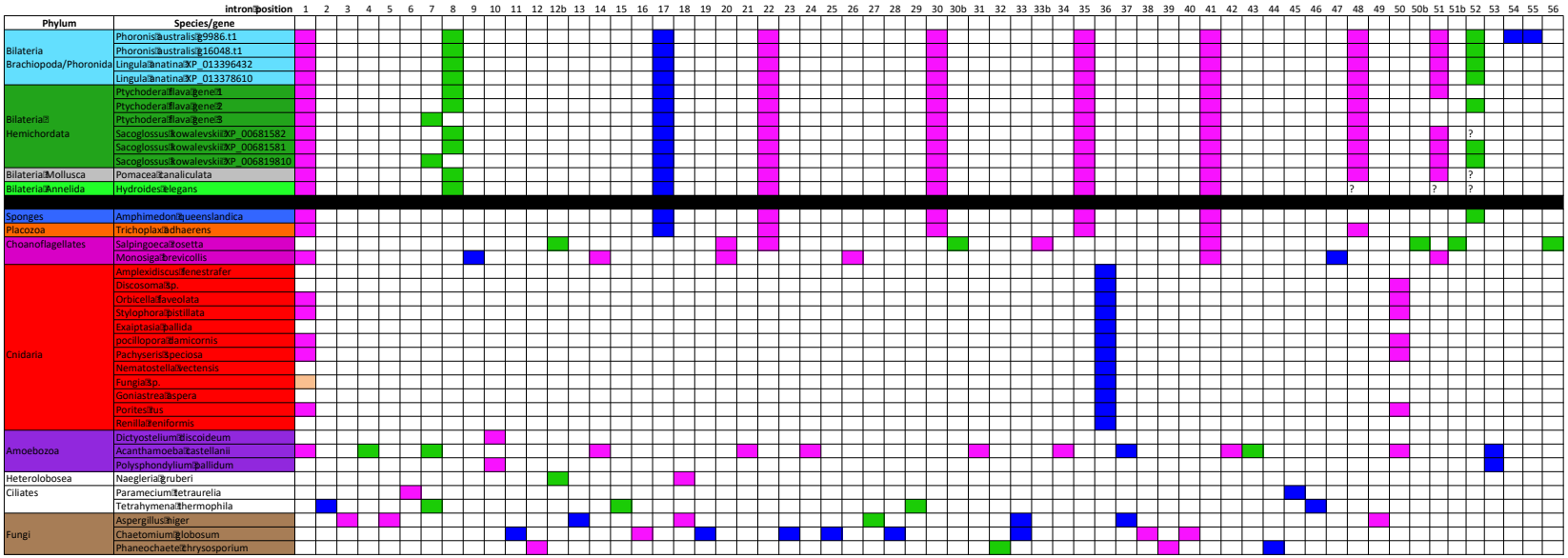
Cnidaria


intron position
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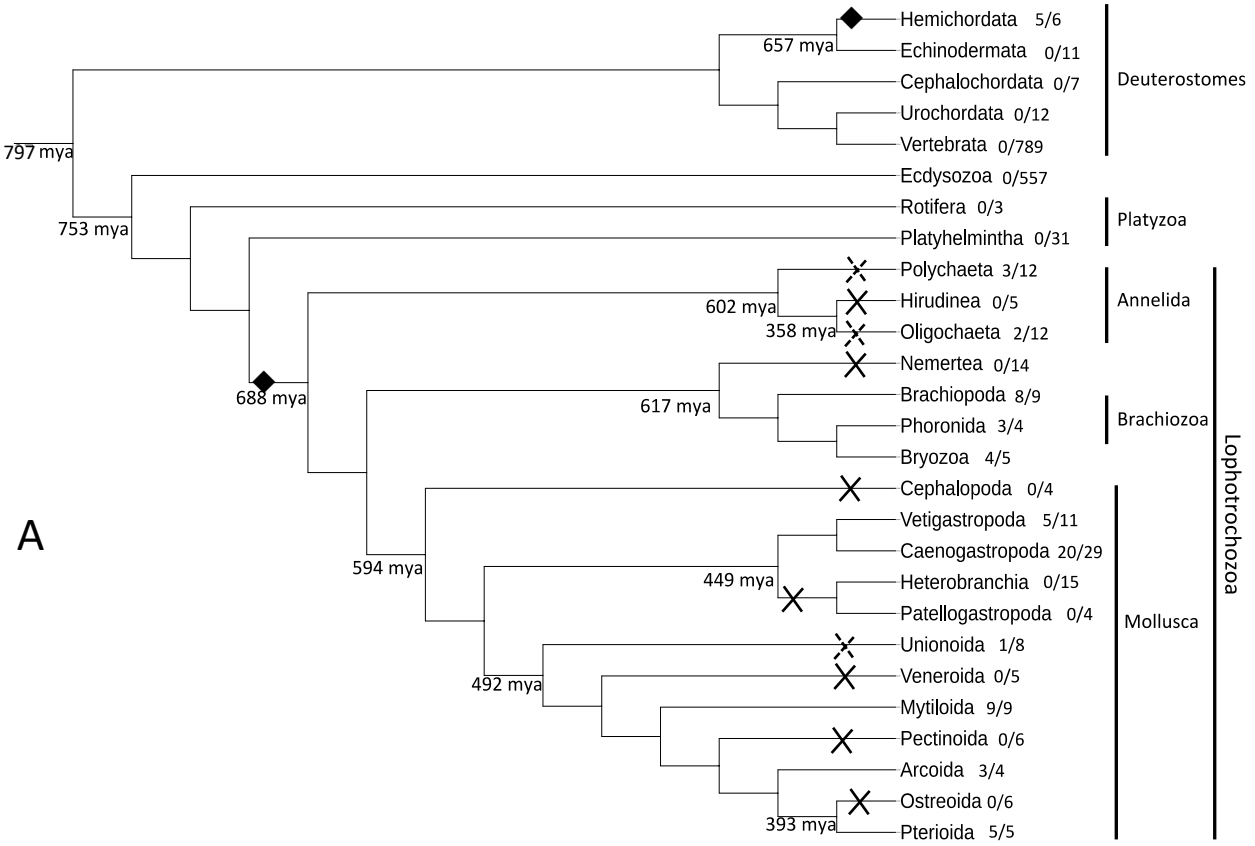
Phylum
Species/gene
Bilateria
Brachiopoda/Phoronida
Phoronis australis g9986.t1
Phoronis australis g16048.t1
Lingula anatina XP_013396432
Lingula anatina XP_013378610
Bilateria
Hemichordata
Ptychodera flava gene 1
Ptychodera flava gene 2
Ptychodera flava gene 3
Saccoglossus kowalevskii XP_00681582
Saccoglossus kowalevskii XP_00681581
Saccoglossus kowalevskii XP_006819810
Bilateria
Mollusca
Pomacea canaliculata
Bilateria
Annelida
Hydroides elegans
Sponges
Amphimedon queenslandica
Placozoa
Trichoplax adhaerens
Choanoflagellates
Salpingoeca rosetta
Monosiga brevicollis
Cnidaria
Amplexidiscus fenestrafer
Discosoma sp.
Orbicella faveolata
Stylophora pistillata
Exaiptasia pallida
pocillopora damicornis
Pachyseris speciosa
Nematostella vectensis
Fungia sp.
Goniastrea aspera
Porites lutus
Renilla reniformis
Amoebozoa
Dictyostelium discoideum
Acanthamoeba castellanii
Polysphondylium pallidum
Heterolobosea
Naegleria gruberi
Ciliates
Paramecium tetraurelia
Tetrahymena thermophila
Fungi
Aspergillus niger
Chaetomium globosum
Phaneochaete chrysosporium


A
Hemichordata 5/6
Echinodermata 0/11
Cephalochordata 0/7
Urochordata 0/12
Vertebrata 0/789
Deuterostomes
Ecdysozoa 0/557
Rotifera 0/3
Platyhelmintha 0/31
Platyzoa
Polychaeta 3/12
Hirudinea 0/5
Oligochaeta 2/12
Annelida
Nemertea 0/14
Brachiopoda 8/9
Phoronida 3/4
Bryozoa 4/5
Brachiozoa
Cephalopoda 0/4
Vetigastropoda 5/11
Caenogastropoda 20/29
Heterobranchia 0/15
Patellogastropoda 0/4
Unionoida 1/8
Veneroida 0/5
Mytiloida 9/9
Pectinoida 0/6
Arcoida 3/4
Ostreoida 0/6
Pterioida 5/5
Mollusca
Lophotrochozoa
657 mya
797 mya
753 mya
602 mya
358 mya
688 mya
617 mya
594 mya
449 mya
492 mya
393 mya

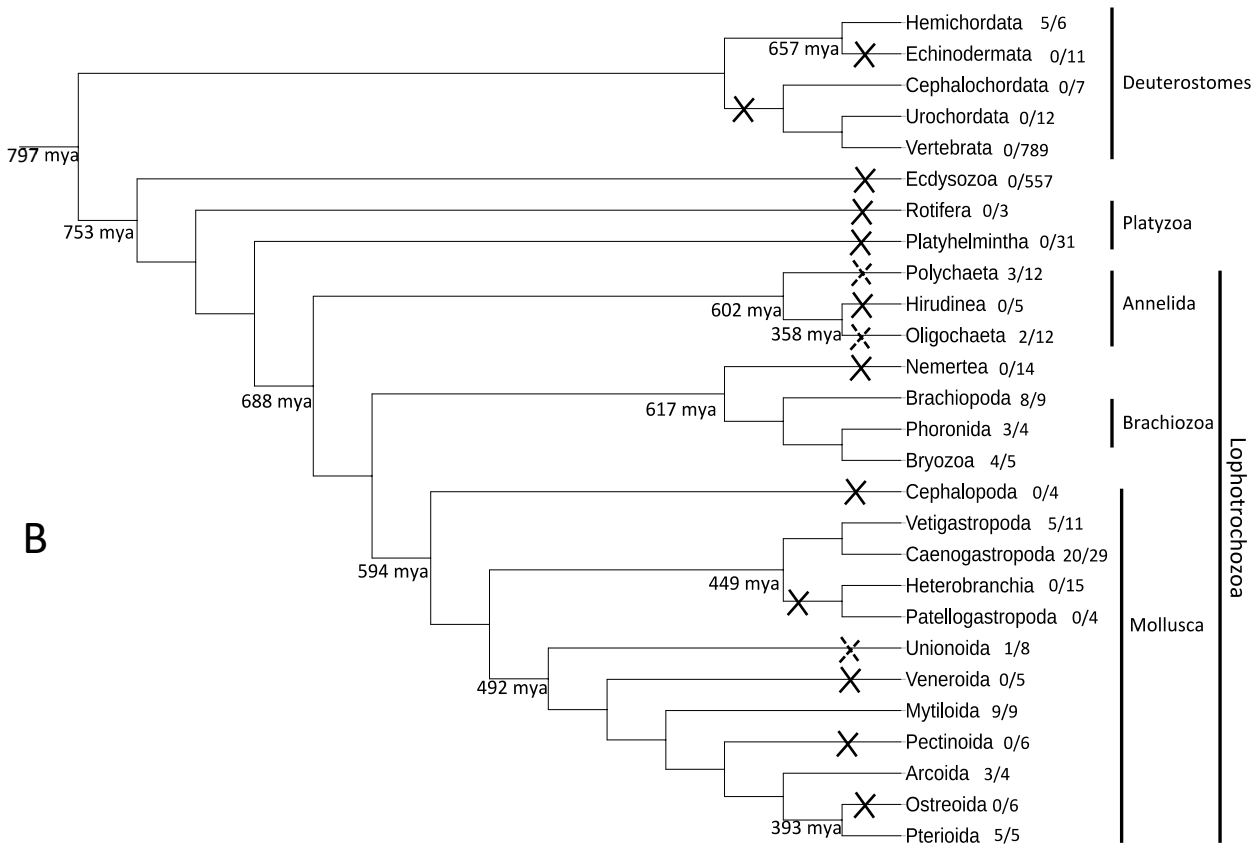

B
Hemichordata 5/6
Echinodermata 0/11
Cephalochordata 0/7
Urochordata 0/12
Vertebrata 0/789
Deuterostomes
Ecdysozoa 0/557
Rotifera 0/3
Platyhelmintha 0/31
Platyzoa
Polychaeta 3/12
Hirudinea 0/5
Oligochaeta 2/12
Annelida
Nemertea 0/14
Brachiopoda 8/9
Phoronida 3/4
Bryozoa 4/5
Brachiozoa
Cephalopoda 0/4
Vetigastropoda 5/11
Caenogastropoda 20/29
Heterobranchia 0/15
Patellogastropoda 0/4
Unionoida 1/8
Veneroida 0/5
Mytiloida 9/9
Pectinoida 0/6
Arcoida 3/4
Ostreoida 0/6
Pterioida 5/5
Mollusca
Lophotrochozoa
657 mya
797 mya
753 mya
602 mya
358 mya
688 mya
617 mya
594 mya
449 mya
492 mya
393 mya

Table 1: GH13_1-like sequences found after BLAST searches in online databases (not comprehensive for unicellars, without the Fungi). * : sequences which have not been characterized as protein-coding, in sequenced genomes with long contigs ; (1) : from short DNA sequences (except Sequence reads archive) ; ** : reported as GH13_1 in CAZy. Most of the SRA data are from transcriptome studies ; see Tables S1 and S2.

Phylum	**Species**	**Database**	**Accession**
NON BILATERIAN METAZOA			
Porifera Demospongiae Heteroscleromorpha	*Amphimedon queenslandica*	GenBank proteins	XP_019851448
Porifera Demospongiae Heteroscleromorpha	*Ephydatia muelleri (1)*	Compagen.org	m.29963 g.29963
Porifera Demospongiae Heteroscleromorpha	*Haliclona tubifera*	GenBank TSA	GFAV01017079
Porifera Demospongiae Heteroscleromorpha	*Spongilla lacustris*	GenBank SRA	SRX470277
Porifera Demospongiae Heteroscleromorpha	*Xestospongia testudinaria (1)*	Reefgenomics.org	gnl|BL_ORD_ID|6299
Cnidaria Hexacorallia Actiniaria	*Actinia tenebrosa*	GenBank TSA	GEVE01039432
Cnidaria Hexacorallia Actiniaria	*Anthopleura elegantissima*	GenBank TSA	GBYC01063006
Cnidaria Hexacorallia Actiniaria	*Anthopleura buddemeieri (1)*	Reefgenomics.org	c117986_g2_i1
Cnidaria Hexacorallia Actiniaria	*Aulactinia veratra (1)*	Reefgenomics.org	c88768_g1_i1
Cnidaria Hexacorallia Actiniaria	*Calliactis polypus (1)*	Reefgenomics.org	c66498_g1_i1
Cnidaria Hexacorallia Actiniaria	*Exaiptasia pallida*	GenBank proteins	XP_020895894
Cnidaria Hexacorallia Actiniaria	*Nematostella vectensis*	GenBank proteins	XP_001629956
Cnidaria Hexacorallia Actiniaria	*Stychodactyla heliantus*	GenBank TSA	GGNY01117022
Cnidaria Hexacorallia Actiniaria	*Telmatactis sp.*	Reefgenomics.org	C36117_g1_i2
Cnidaria Hexacorallia Corallimorpharia	*Amplexidiscus fenestrafer**	Reefgenomics.org	evm.model.scaffold_206.7
Cnidaria Hexacorallia Corallimorpharia	*Discosoma sp.**	Reefgenomics.org	evm.model.scaffold_6.33
Cnidaria Hexacorallia Scleratinia	*Acropora digitifera*	GenBank proteins	XP_015760547 partial
Cnidaria Hexacorallia Scleratinia	*Acropora millepora*	GenBank proteins	XP_029201467
Cnidaria Hexacorallia Scleratinia	*Acropora tenuis**	Reefgenomics.org	aten_0.1.m1.10359.m1
Cnidaria Hexacorallia Scleratinia	*Fungia sp.**	Reefgenomics.org	ffun1.m4.16656.m1

Cnidaria Hexacorallia Scleratinia	*Goniastrea aspera**	Reefgenomics.org	gasp1.m3.6500.m1
Cnidaria Hexacorallia Scleratinia	*Nemanthus annamensis (1)*	Reefgenomics.org	TR26025|c0_g2_i3
Cnidaria Hexacorallia Scleratinia	*Orbicella faveolata*	GenBank proteins	XP_020628431
Cnidaria Hexacorallia Scleratinia	*Pachyseris speciosa**	Reefgenomics.org	Sc0001227 74283-80000
Cnidaria Hexacorallia Scleratinia	*Pocillopora damicornis*	GenBank genomes	XP_027058081
Cnidaria Hexacorallia Scleratinia	*Porites lutea**	Reefgenomics.org	plut2.m8.18618.m1
Cnidaria Hexacorallia Scleratinia	*Porites rus*	GenBank genomes	OKRP01000157
Cnidaria Hexacorallia Scleratinia	*Stylophora pistillata*	GenBank proteins	XP_022802004
Cnidaria Octocorallia Pennatulacea	*Renilla koellikeri*	GenBank SRA	SRX4364609
Cnidaria Octocorallia Pennatulacea	*Renilla muelleri*	GenBank SRA	SRX4717871
Cnidaria Octocorallia Pennatulacea	*Renilla reniformis**	GenBank genomes	FXAL01159338
Placozoa	*Trichoplax adhaerens*	GenBank proteins	XP_002114911
BILATERIA			
Brachiopoda Linguliformea	*Glottidia pyramidata*	GenBank SRA	SRX731468
Brachiopoda Linguliformea	*Lingula anatina*	GenBank proteins	XP_013396432
Brachiopoda Linguliformea	*Lingula anatina*	GenBank proteins	XP_013378610
Brachiopoda Craniiformea	*Novocrania anomala*	GenBank SRA	SRX731472
Brachiopoda Rhynchonelliformea	*Kraussina rubra*	GenBank SRA	SRX112037
Brachiopoda Rhynchonelliformea	*Macandrevia cranium*	GenBank SRA	SRX731471
Brachiopoda Rhynchonelliformea	*Hemithiris psittacea*	GenBank SRA	SRX731469
Brachiopoda Rhynchonelliformea	*Terebratalia transversa*	GenBank SRA	SRX1307070
Brachiopoda Phoroniformea or Phoronida	*Phoronis australis*	marinegenomics	g9986.t1
Brachiopoda Phoroniformea or Phoronida	*Phoronis australis*	marinegenomics	g16048.t1
Brachiopoda Phoroniformea or Phoronida	*Phoronopsis harmeri*	GenBank SRA	SRX1121914
Bryozoa Flustrina	*Bugula neritina*	GenBank SRA	SRX2112329
Bryozoa Flustrina	*Bugulina stolonifera*	GenBank SRA	SRX6428326
Bryozoa Ctenostomatida	*Flustellidra corniculata*	GenBank SRA	SRX6428327
Bryozoa Cheilostomatida	*Membranipora membranacea*	GenBank SRA	SRX1121923
Hemichordata Enteropneusta	*Ptychodera flava*	Marinegenomics	pfl_40v0_9_20150316_1g2314.t1
		GenBank WGS	LD343027 41534-50098
Hemichordata Enteropneusta	*Ptychodera flava*	GenBank WGS	LD343027 51007-66347

Hemichordata Enteropneusta	*Ptychodera flava*	Marinegenomics	pfl_40v0_9_20150316_1g6997.t1
		GenBank WGS	BCFJ01022326 32811-41459
Hemichordata Enteropneusta	*Saccoglossus kowalevskii*	GenBank proteins	XP_006816582
Hemichordata Enteropneusta	*Saccoglossus kowalevskii*	GenBank proteins	XP_006816581
Hemichordata Enteropneusta	*Saccoglossus kowalevskii*	GenBank proteins	XP_006819810
Hemichordata Enteropneusta	*Schizocardium californicum*	GenBank SRA	SRX1436000
Hemichordata Enteropneusta	*Torquaratoridae antarctica*	GenBank SRA	SRX798197
Hemichordata Pterobranchia	*Rhabdopleura sp.*	GenBank SRA	SRX879690
Mollusca Gastropoda Caenogastropoda	*Asolene platae*	AmpuBase	Apl52885
Mollusca Gastropoda Caenogastropoda	*Batillaria attramentaria*	GenBank SRA	SRX2957288
Mollusca Gastropoda Caenogastropoda	*Charonia tritonis*	GenBank SRA	SRX2753455
Mollusca Gastropoda Caenogastropoda	*Conus tribblei (1)*	GenBank WGS	LFLW010536118
Mollusca Gastropoda Caenogastropoda	*Crepidula novicella*	GenBank TSA	GELE01086894
Mollusca Gastropoda Caenogastropoda	*Glaussolax didyma*	GenBank SRA	SRX5277776
Mollusca Gastropoda Caenogastropoda	*Hemifusus tuba*	GenBank SRA	ERX3138276
Mollusca Gastropoda Caenogastropoda	*Lanistes nyassanus*	AmpuBase	Lny24710
Mollusca Gastropoda Caenogastropoda	*Marisa cornuarietes*	AmpuBase	Mco2627
Mollusca Gastropoda Caenogastropoda	*Melanoides tuberculata*	GenBank SRA	SRX5832309
Mollusca Gastropoda Caenogastropoda	*Neverita didyma*	GenBank TSA	GHHQ01002371
Mollusca Gastropoda Caenogastropoda	*Nucella lapillus*	GenBank SRA	SRX4378318
Mollusca Gastropoda Caenogastropoda	*Oncomelania hupensis*	GenBank SRA	SRX2739536
Mollusca Gastropoda Caenogastropoda	*Pila ampullacea*	AmpuBase	Pila82769
Mollusca Gastropoda Caenogastropoda	*Pomacea canaliculata*	GenBank proteins	XP_025109323 (incomplete)
		AmpuBase	Pca5338
Mollusca Gastropoda Caenogastropoda	*Pomacea diffusa*	AmpuBase	Pdi16479 (partial)
Mollusca Gastropoda Caenogastropoda	*Pomacea maculata*	AmpuBase	Pma33988 (partial)
Mollusca Gastropoda Caenogastropoda	*Pomacea scalaris*	AmpuBase	Psc4690
Mollusca Gastropoda Caenogastropoda	*Rapana venosa*	GenBank TSA	GDIA01047641
Mollusca Gastropoda Caenogastropoda	*Semisulcospira coreana*	GenBank TSA	GGNX01073707
Mollusca Gastropoda Vetigastropoda	*Haliotis laevigata*	GenBank TSA	GFTT01038064
Mollusca Gastropoda Vetigastropoda	*Haliotis rubra**	GenBank WGS	QXJH01001142
Mollusca Gastropoda Vetigastropoda	*Haliotis rufescens**	GenBank WGS	QGMO01000565

Mollusca Gastropoda Vetigastropoda	*Tegula atra*	GenBank SRA	SRX958768
Mollusca Bivalvia Mytiloida	*Bathymodiolus platifrons**	GenBank Assembly	MJUT01033839
Mollusca Bivalvia Mytiloida	*Limnoperna fortunei (1)*	GenBank Assemby	NFUK01006104
Mollusca Bivalvia Mytiloida	*Lithophaga lithophaga*	GenBank SRA	SRX1940727
	*Modiolus philippinarum**	GenBank Assembly	MJUU01021410
Mollusca Bivalvia Mytiloida			
Mollusca Bivalvia Mytiloida	*Mytilus galloprovincialis (1)*	GenBank Assembly	APJB011511270
Mollusca Bivalvia Mytiloida	*Mytilus galloprovincialis*	GenBank TSA	GHIK01025031
Mollusca Bivalvia Mytiloida	*Perna canaliculus*	GenBank TSA	GGLA01150624
Mollusca Bivalvia Mytiloida	*Septifer virgatus*	GenBank TSA	GFKS01035611
Mollusca Bivalvia Mytiloida	*Perumytilus purpuratus*	GenBank SRA	SRX2210805
Mollusca Bivalvia Mytiloida	*Xenostrobus securis*	GenBank SRA	SRX4058936
Mollusca Bivalvia Pterioida	*Malleus candeanus*	GenBank SRA	SRX1688295
Mollusca Bivalvia Pterioida	*Pinctada martensi**	GenBank Assembly	CM008066
Mollusca Bivalvia Pterioida	*Pinctada fucata*	Marinegenomics	pfu_aug1.0_4142.1_01638
Mollusca Bivalvia Pterioida	*Pteria penguin*	GeneBank TSA	GEMO01011007
Mollusca Bivalvia Arcoida	*Anadara trapeza*	GenBank SRA	SRX323049
Mollusca Bivalvia Arcoida	*Scapharca broughtoni*	GenBank TSA	GEXI01046152
Mollusca Bivalvia Arcoida	*Tegillarca granosa*	GenBank SRA	SRX1334524
Mollusca Bivalvia Unionoida	*Cristaria plicata*	GenBank SRA	SRX1153631
Annelida Oligochaeta	*Drawida calebi*	GenBank SRA	SRX6596293
Annelida Oligochaeta	*Glossoscolex paulistus*	GenBank TSA	GBIL01075477
Annelida Polychaeta	*Hydroides elegans**	GenBank Assembly	LQRL01141559 LQRL01153670 LQRL01157410
Annelida Polychaeta	*Pygospio elegans*	GenBank TSA	GFPL01035490
Annelida Polychaeta	*Spirobranchus lamarcki*	GenBank TSA	GGGS01192599

UNICELLULAR EUKARYOTES

Amoebozoa Mycetozoa	*Cavendaria fasciculata*	GenBank proteins	XP_004351949
Amoebozoa Mycetozoa	*Dictyostellium discoideum*	GenBank proteins	XP_640516**
Amoebozoa Mycetozoa	*Polysphondylium pallidum*	GenBank proteins	XP_020429468
Amoebozoa Discosea	*Acanthamoeba castellanii*	GenBank proteins	XP_004368209
Choanoflagellida Salpingoecidae	*Monosiga brevicollis*	GenBank proteins	XP_001742116
Choanoflagellida Salpingoecidae	*Salpingoeca rosetta*	GenBank proteins	XP_004998636
Ciliata	*Ichthyophthirius multifiliis*	GenBank proteins	XP_004027176
Ciliata	*Euplotes focardii*	GenBank proteins	AGU13046**
Ciliata	*Moneuplotes crassus*	GenBank proteins	AGU13047**
Ciliata	*Paramecium tetraurelia*	GenBank proteins	XP_001462315
Ciliata	*Stentor coeruleus*	GenBank proteins	OMJ70617
Ciliata	*Stylonychia lemnae*	GenBank proteins	CDW84776
Ciliata	*Tetrahymena thermophila*	GenBank proteins	XP_001020855**
Heterolobosea	*Naegleria gruberi*	GenBank proteins	XP_002676377
Apusozoa	*Thecamonas trahens*	GenBank proteins	XP_013759080
Oomycetes	*Achlya hypogyna*	GenBank proteins	AIG56379**
Oomycetes	*Saprolegnia diclina*	GenBank proteins	XP_008604251
Oomycetes	*Thraustotheca clavata*	GenBank proteins	AIG55673**