

**The evolution of genes encoding for green fluorescent proteins:
insights from cephalochordates (amphioxus)**

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Supporting Information Online:

Supplementary file 1: The CDS nucleotide sequences of the 49 cephalochordate GFP-encoding genes used in this study (in FASTA format).

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Supplementary file 2: The protein sequences of the 49 cephalochordate GFP-encoding genes used in this study (in FASTA format).

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Supplementary file 3: The trimmed protein sequence alignment used in the phylogenetic analysis for Fig. 3 (in FASTA format).

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Supplementary file 4: The trimmed protein sequence alignment used in the phylogenetic analysis for Fig. 5 (in FASTA format).

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>gi|19982609|gb|AAK71337.1||Scolymia_cubensis|Cnidarian-Anthozoa
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>gi|257221243|gb|ACV52387.1||Scleractinia_sp._Lizard_Island_39|Cnidarian-Anthozoa
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>gi|268612455|pdb|3KCS|A|Discosoma_sp.|Cnidarian-Anthozoa
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YNVNIKLDITSHNYTIVEQYERAEGRHSTG
>gi|662702316|pdb|4Q7R|A|Discosoma_sp.|Cnidarian-Anthozoa
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>gi|315022045|pdb|3NEZ|B|Discosoma_sp.|Cnidarian-Anthozoa
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>gi|114794216|pdb|2H5P|A|Discosoma_sp.|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTMFP
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>gi|114794217|pdb|2H5Q|A|Discosoma_sp.|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTMFP
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>gi|308387883|pdb|3NF0|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTMFP
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>gi|409974060|pdb|4H3M|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTMFP
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>gi|409974062|pdb|4H3N|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTMFP
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>gi|410563140|pdb|4H3L|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTMFP
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>gi|315022058|pdb|3NED|A|Discosoma_sp.|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTMFP
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>gi|55792807|gb|AAV65486.1||Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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>gi|194368491|pdb|2QLG|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTMFP
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>gi|194368493|pdb|2QLH|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa

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>gi|194368495|pdb|2QLI|A|Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTNFP
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>gi|55792809|gb|AAV65487.1||Discosoma_sp._LW-2004|Cnidarian-Anthozoa
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 AYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKVRGTNFP
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 YKTDIKLDITSHNYTIVEQYERAEGRHSTG

>gi|160286149|pdb|2VAE|A|Discosoma_sp.|Cnidarian-Anthozoa
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>gi|114794265|pdb|2H8Q|A|Discosoma_sp.|Cnidarian-Anthozoa
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 VYVKHPADIPDYMKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGCFIYKVKFIGVNF
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>gi|73535927|pdb|1ZGP|A|Discosoma_sp.|Cnidarian-Anthozoa
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 VYVKHPADIPDYKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGCFIYKVKFIGVNF
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>gi|12084491|pdb|1G7K|A|Discosoma_sp.|Cnidarian-Anthozoa
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 VYVKHPADIPDYKLSFPEGFKWERVXNFEDGGVVTVTQDSSLQDGCFIYKVKFIGVNF
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>gi|12084722|pdb|1GGX|A|Discosoma_sp.|Cnidarian-Anthozoa
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 VYVKHPADIPDYKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGCFIYKVKFIGVNF
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>gi|55976617|sp|Q9U6Y8.1|RFP_DISSP|Discosoma_sp.|Cnidarian-Anthozoa

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 >gi|211938970|pdb|2V4E|A|Discosoma_sp.|Cnidarian-Anthozoa
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 >gi|290790214|pdb|3LF4|A|Discosoma_sp.|Cnidarian-Anthozoa
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 >gi|33333764|gb|AAQ11987.1||Discosoma_sp._JW-2002|Cnidarian-Anthozoa
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 >gi|51472047|gb|AAU04444.1||Discosoma_sp._RC-2004|Cnidarian-Anthozoa
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 >gi|85002146|gb|ABC68474.1||Discosoma_sp._RC-2004|Cnidarian-Anthozoa
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>gi|91754017|dbj|BAE93224.1||Montipora_sp._20|Cnidarian-Anthozoa
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>gi|383502329|dbj|BAM10197.1||Acropora_digitifera|Cnidarian-Anthozoa
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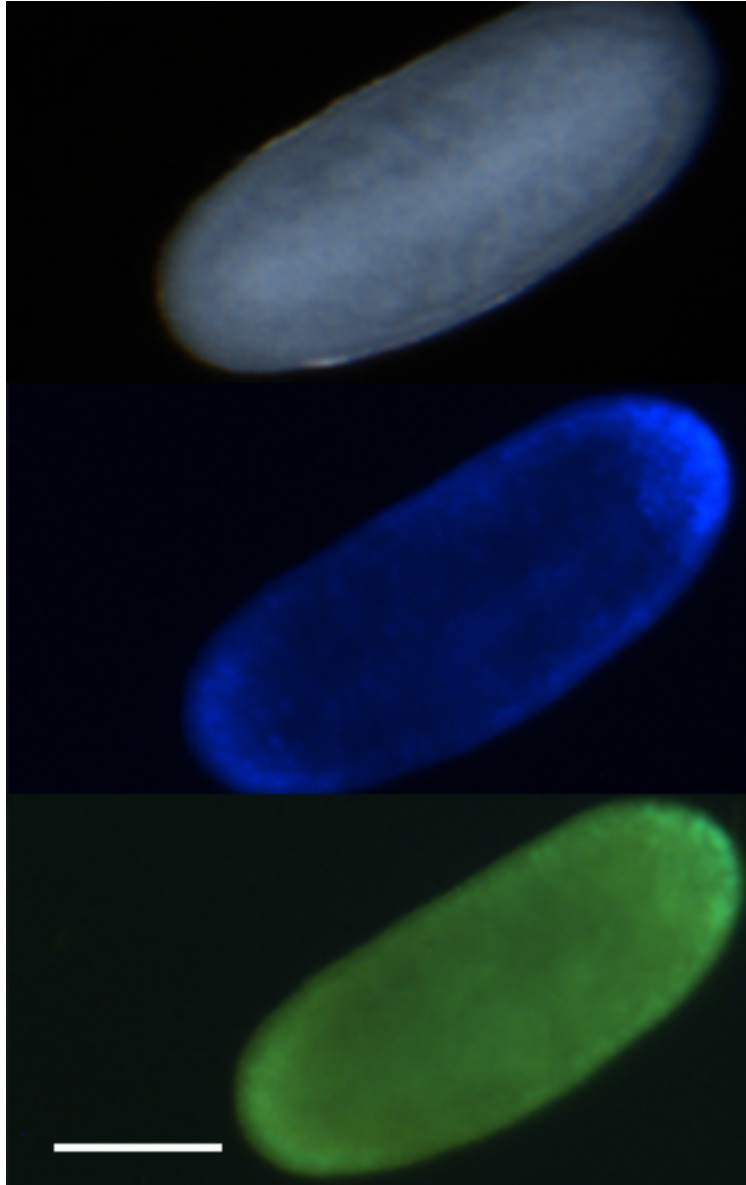
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 EGSVLLQAGSSSPSSSSIEKAIPFGLKNYCQFFYPIKDGDVIIATQMTQNHPINKSIPPA
 HWKRVECKQFKDPKDHIVQDEI--NKYYEF

Supplementary file 5

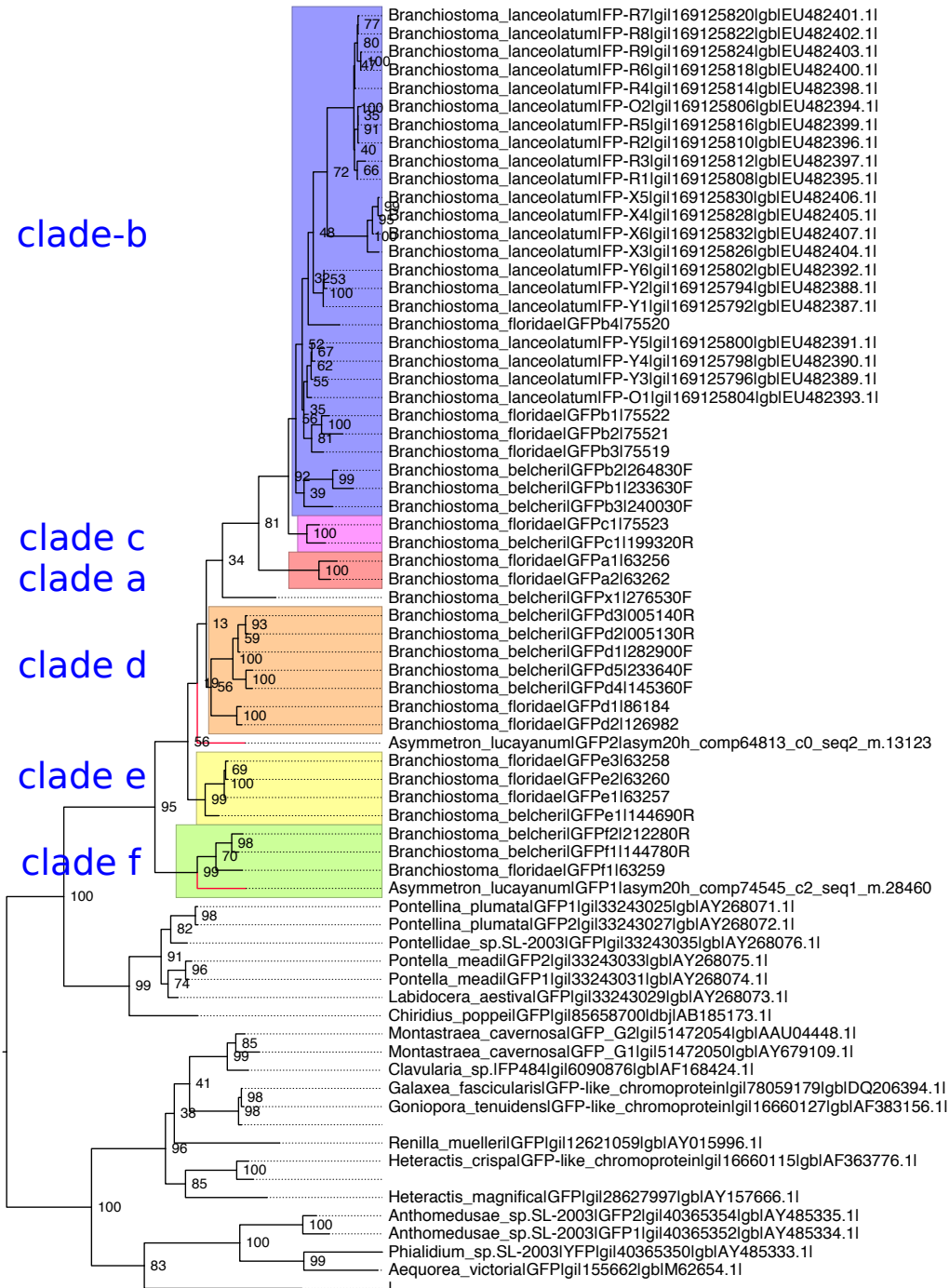
Fig. S1. Early stage (about 24 hrs) larvae of *Asymmetron lucayanum* in bright field reflectance, and in fluorescence when excited at 390nm, and at 470nm (from top to bottom). Scale bar: 70 μm (this larval stage is about 250-300 μm in length). Excitation at 355nm also triggers blue fluorescence, but with lesser intensity of fluorescence.



Supplementary file 6

Fig. S2. The full version of the phylogenetic tree of cephalochordate GFP-encoding genes without branch collapsing, with cnidarian and copepod GFP-encoding genes as outgroups. Branches representing the two GFP-encoding genes from *A. lucayanum* are highlighted in red. Branches corresponding to *B. lanceolatum* GFP-encoding sequences were collapsed and the collapsed nodes were represented by triangles in the tree. For each internal node, the local support value was calculated by 100 rapid bootstrapping via RAxML. The clades are highlighted with colors previously designating *B. floridae* GFP clades (following our earlier study²⁵).

- 25 Bomati, E. K., Manning, G. & Deheyn, D. D. Amphioxus encodes the largest known family of green fluorescent proteins, which have diversified into distinct functional classes. *BMC Evol. Biol.* **9**, 1-11, doi:10.1186/1471-2148-9-77 (2009).



0.3

Supplementary file 7

Table S1. Sequence information for all GFP-encoding genes used in Fig. 1 and Fig. S1.

Species name	Gene model ID	Gene name	GenBank accession number
<i>Asymmetron lucayanum</i>	asym20h_comp74545_c2_seq1_m.28460	GFP1	
<i>Asymmetron lucayanum</i>	asym20h_comp64813_c0_seq2_m.13123	GFP2	
<i>Branchiostoma belcheri</i>	005130R	GFPd2	
<i>Branchiostoma belcheri</i>	005140R	GFPd3	
<i>Branchiostoma belcheri</i>	144690R	GFPe1	
<i>Branchiostoma belcheri</i>	144780R	GFPf1	
<i>Branchiostoma belcheri</i>	145360F	GFPd4	
<i>Branchiostoma belcheri</i>	199320R	GFPc1	
<i>Branchiostoma belcheri</i>	212280R	GFPf2	
<i>Branchiostoma belcheri</i>	233630F	GFPb1	
<i>Branchiostoma belcheri</i>	233640F	GFPd5	
<i>Branchiostoma belcheri</i>	240030F	GFPb3	
<i>Branchiostoma belcheri</i>	264830F	GFPb2	
<i>Branchiostoma belcheri</i>	276530F	GFPa1	
<i>Branchiostoma belcheri</i>	282900F	GFPd1	
<i>Branchiostoma floridae</i>	126982	GFPd2	
<i>Branchiostoma floridae</i>	63256	GFPa1	
<i>Branchiostoma floridae</i>	63257	GFPe1	
<i>Branchiostoma floridae</i>	63258	GFPe3	
<i>Branchiostoma floridae</i>	63259	GFPf1	
<i>Branchiostoma floridae</i>	63260	GFPe2	
<i>Branchiostoma floridae</i>	63262	GFPa2	

<i>Branchiostoma floridae</i>	75519	GFPb3	
<i>Branchiostoma floridae</i>	75520	GFPb4	
<i>Branchiostoma floridae</i>	75521	GFPb2	
<i>Branchiostoma floridae</i>	75522	GFPb1	
<i>Branchiostoma floridae</i>	75523	GFPc1	
<i>Branchiostoma floridae</i>	86184	GFPd1	
<i>Branchiostoma lanceolatum</i>		FP-O1	gi 169125804 gb EU482393.1
<i>Branchiostoma lanceolatum</i>		FP-O2	gi 169125806 gb EU482394.1
<i>Branchiostoma lanceolatum</i>		FP-R1	gi 169125808 gb EU482395.1
<i>Branchiostoma lanceolatum</i>		FP-R2	gi 169125810 gb EU482396.1
<i>Branchiostoma lanceolatum</i>		FP-R3	gi 169125812 gb EU482397.1
<i>Branchiostoma lanceolatum</i>		FP-R4	gi 169125814 gb EU482398.1
<i>Branchiostoma lanceolatum</i>		FP-R5	gi 169125816 gb EU482399.1
<i>Branchiostoma lanceolatum</i>		FP-R6	gi 169125818 gb EU482400.1
<i>Branchiostoma lanceolatum</i>		FP-R7	gi 169125820 gb EU482401.1
<i>Branchiostoma lanceolatum</i>		FP-R8	gi 169125822 gb EU482402.1
<i>Branchiostoma lanceolatum</i>		FP-R9	gi 169125824 gb EU482403.1
<i>Branchiostoma lanceolatum</i>		FP-X3	gi 169125826 gb EU482404.1
<i>Branchiostoma lanceolatum</i>		FP-X4	gi 169125828 gb EU482405.1
<i>Branchiostoma lanceolatum</i>		FP-X5	gi 169125830 gb EU482406.1
<i>Branchiostoma lanceolatum</i>		FP-X6	gi 169125832 gb EU482407.1
<i>Branchiostoma lanceolatum</i>		FP-Y1	gi 169125792 gb EU482387.1
<i>Branchiostoma lanceolatum</i>		FP-Y2	gi 169125794 gb EU482388.1
<i>Branchiostoma lanceolatum</i>		FP-Y3	gi 169125796 gb EU482389.1
<i>Branchiostoma lanceolatum</i>		FP-Y4	gi 169125798 gb EU482390.1
<i>Branchiostoma lanceolatum</i>		FP-Y5	gi 169125800 gb EU482391.1
<i>Branchiostoma lanceolatum</i>		FP-Y6	gi 169125802 gb EU482392.1
<i>Aequorea victoria</i>		GFP	gi 155662 gb M62654.1

<i>Anthomedusae sp.SL-2003</i>	GFP- like_chro	
<i>Anthomedusae sp.SL-2003</i>	moprotein	gi 40365356 gb AY485336.1
<i>Anthomedusae sp.SL-2003</i>	GFP1	gi 40365352 gb AY485334.1
<i>Chiridius poppei</i>	GFP2	gi 40365354 gb AY485335.1
<i>Clavularia sp.</i>	GFP	gi 85658700 dbj AB185173.1
	FP484	gi 6090876 gb AF168424.1
	GFP- like_chro	
<i>Condylactis passiflora</i>	moprotein	gi 16660124 gb AF383155.1
	GFP- like_chro	
<i>Galaxea fascicularis</i>	moprotein	gi 78059179 gb DQ206394.1
	GFP- like_chro	
<i>Goniopora tenuidens</i>	moprotein	gi 16660127 gb AF383156.1
	GFP- like_chro	
<i>Heteractis crispa</i>	moprotein	gi 16660115 gb AF363776.1
<i>Heteractis magnifica</i>	GFP	gi 28627997 gb AY157666.1
<i>Labidocera aestiva</i>	GFP	gi 33243029 gb AY268073.1
<i>Montastraea cavernosa</i>	GFP_G1	gi 51472050 gb AY679109.1
<i>Montastraea cavernosa</i>	GFP_G2	gi 51472054 gb AAU04448.1
	GFP- like_chro	
<i>Montipora efflorescens</i>	moprotein	gi 78059145 gb DQ206377.1
<i>Phialidium sp.SL-2003</i>	YFP	gi 40365350 gb AY485333.1
<i>Pontella meadi</i>	GFP1	gi 33243031 gb AY268074.1
<i>Pontella meadi</i>	GFP2	gi 33243033 gb AY268075.1

<i>Pontellidae sp.SL-2003</i>	GFP	gi 33243035 gb AY268076.1
<i>Pontellina plumata</i>	GFP1	gi 33243025 gb AY268071.1
<i>Pontellina plumata</i>	GFP2	gi 33243027 gb AY268072.1
<i>Renilla muelleri</i>	GFP	gi 12621059 gb AY015996.1
