

Supplementary Table 1. Next generation sequencing data of choanoflagellates and early-diverging metazoans and number of cystatin homologues mined for each species and used for phylogenetic analyses. The life cycle stage sequenced is specified (underlined> for *Edwardsiella* spp. and *Polypodium hydriforme*.

Taxonomic group/Species	GenBank identifier/Type of database	Reference	Classical stefins	Atypical stefins	Type 2 cystatins	Multi-domain cystatins
CHOANOFLAGELLATA						
<i>Codosiga hollandica</i>	GGOV01000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Hartaetosiga balthica</i>	GGOO00000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Hartaetosiga gracilis</i>	GGOU01000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Choanoeca perplexa</i>	GGOP01000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Microstomoeca roanoka</i>	GGON01000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Monosiga brevicollis</i>	ABFJ01000000/WGS	(King, et al. 2008)	1	0	0	?
<i>Mylnosiga fluctuans</i> ^x	GGOI00000000/TSA	(Richter, et al. 2018)	1	0	0	1
<i>Salpingoeca dolichothecata</i>	GGOK01000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Salpingoeca helianthica</i>	GGOJ01000000/TSA	(Richter, et al. 2018)	1	0	0	1
<i>Salpingoeca infusionum</i>	GGOW00000000/TSA	(Richter, et al. 2018)	1	0	0	?
<i>Salpingoeca rosetta</i>	ACSY01000000/WGS	(Fairclough, et al. 2013)	1	0	0	?
PLACOZOA						
<i>Trichoplax adhaerens</i>	ABGP01000000/WGS GFSG00000000/TSA	(Srivastava, et al. 2008) (Kamm, et al. 2018)	0	0	0	0
<i>Trichoplax</i> sp. H2 strain Panama	NOWV00000000/WGS GFSF01000000/TSA	(Kamm, et al. 2018) (Kamm, et al. 2018)	0	0	0	0
<i>Trichoplax</i> sp. strain H4 isolate TR1	SRR8193748/TSA	(Nosenko, et al. 2013)	0	0	0	0
<i>Trichoplax</i> sp. strain H4 isolate TR2	SRR8193747/TSA	(Nosenko, et al. 2013)	0	0	0	0
<i>Trichoplax</i> sp. strain H4 isolate TR3	SRR8193749/TSA	(Nosenko, et al. 2013)	0	0	0	0
<i>Hoilungia hongkongensis</i>	SRR5311040, SRR5311137/ WGS* SRR5311041/TSA**	(Eitel, et al. 2018)	0	0	0	0
PORIFERA						
<i>Amphimedon queenslandica</i>	ACUQ00000000/WGS LIBEST_025611/EST	(Srivastava, et al. 2010); DOE Joint Genome Institute A. queenslandica EST project	6	0	0	0
<i>Haliclona tubifera</i>	GFAV00000000/TSA	(Guzman and Conaco 2016)	1	0	0	0

<i>Halisarca caerulea</i>	GFSI00000000/TSA	(Kenny, et al. 2018)	1	0	0	0
CTENOPHORA						
<i>Mnemiopsis leidyi</i>	GFAT00000000/TSA AGCP00000000/WGS	(Moroz, et al. 2014) (Ryan, et al. 2013)	1	0	1	0
<i>Hormiphora californensis</i>	GGLO00000000/TSA	(Francis, et al. 2015)	1	0	1	0
<i>Pleurobranchia bachei</i>	AVPN00000000/WGS	(Moroz, et al. 2014)	0	0	1	0
<i>Pleurobranchia pileus</i>	LIBEST_023403/EST LIBEST_022668/EST SRR3417589/EST SRR6074514/TSA	(Alie, et al. 2011) (Philippe, et al. 2009) (Simion, et al. 2017) Moroz Lab	0	0	1	0
<i>Beroe ovata</i>	UOYG00000000/WGS	(Babonis, et al. 2018)	2	0	1	0
<i>Beroe</i> sp.	ERR216194/EST	(Nosenko, et al. 2013)	1	0	1	0
CNIDARIA						
Staurozoa						
<i>Calvadosia cruxmelitensis</i>	HAHC00000000/TSA OFHS00000000/WGS	(Kayal, et al. 2018) (Ohdera, et al. 2019)	1	0	0	0
<i>Craterolophus convolvulus</i>	HAGZ00000000/TSA	(Kayal, et al. 2018)	1	0	0	0
<i>Haliclystus auricula</i>	HAHA00000000/TSA	(Kayal, et al. 2018)	1	0	0	0
<i>Haliclystus sanjuanensis</i>	HAHB00000000/TSA	(Kayal, et al. 2018)	1	0	0	0
Anthozoa						
<i>Exaiptasia pallida</i>	LJWW00000000/WGS JV124988, JV096676, JV089924/TSA	(Baumgarten, et al. 2015) (Lehnert, et al. 2012)	1	0	1	0
<i>Nematostella vectensis</i>	ABAV00000000/WGS HADN00000000/TSA	(Putnam, et al. 2007) (Babonis, et al. 2016)	2	0	1	0
<i>Edwardsiella lineata</i> - <u>assembly of all life cycle stages</u>	WGS/TSA in EdwardsiellaBase	(Stefanik, et al. 2014)	1	0	1	0
<i>Edwardsiella carnea</i> - Swedish isolate, <u>parasite stage</u>	GGGB00000000/TSA	(Dnyansagar, et al. 2018)	1	0	1	0
<i>Edwardsiella carnea</i> - Swedish isolate, <u>free-living stage</u>	GGGD00000000/TSA	(Dnyansagar, et al. 2018)	2	0	1	0
<i>Acropora digitifera</i>	BACK00000000/WGS GIAQ01000000/TSA	(Shinzato, et al. 2015) (Gajigan, et al. 2017)	3	0	1	0

<i>Clytia hemisphaerica</i>	ERP112953/WGS HAMV0100000/TSA	(Leclere, et al. 2019) (Leclere, et al. 2019)	1	0	1	0
<i>Porpita porpita</i>	GHBA00000000/TSA	(Khalturin, et al. 2019)	0	0	1	0
<i>Velella velella</i>	GHAZ00000000/TSA	(Khalturin, et al. 2019)	5	0	3	0
<i>Physalia physalis</i>	GHBB00000000/TSA	(Khalturin, et al. 2019)	1	0	1	0
<i>Hydra vulgaris</i> (syn. <i>H. magnipapillata</i>)	ACZU00000000/WGS	(Chapman, et al. 2010)	2	0	6	0
	ABRM00000000/WGS	(Chapman, et al. 2010)	2	0	6	0
	HAAD00000000/TSA	(Wenger and Galliot 2013)	1	0	0	0
	GGKH00000000/TSA	(Wenger, et al. 2019)	1	0	4	0
	HAAC00000000/TSA	(Wenger and Galliot 2013)	1	0	6	0
	GEVZ01000000 /TSA	(Rodrigues, et al. 2016)	0	0	4	0
	GAOL00000000/TSA	(Juliano, et al. 2014)	2	0	4	0
<i>Millepora alcicornis</i>	SRX323169-SRR935384/WGS GFAS00000000/TSA	(Ruiz-Ramos and Baums 2014) (Ortiz-Gonzalez, et al. 2017)	2	0	0	0
<i>Podocoryna carnea</i>	GBEH00000000/TSA	(Hensel, et al. 2014)	2	0	2	0
	GCHV00000000/TSA	(Sanders and Cartwright 2015)				
<i>Turritopsis</i> sp. SK-2016	IAAF00000000	(Hasegawa, et al. 2016)	1	0	0	0
Polypodiozoa						
<i>Polypodium hydriforme</i> - <u>parasite stage</u>	SRX687102- SRR1558173/WGS GBGH00000000/TSA	(Chang, et al. 2015) (Shpirer, et al. 2014)	1	0	3	0
MYXOZOA						
Malacosporea						
<i>Tetracapsuloides bryosalmonae</i>	LIBEST_027541/EST LIBEST_028454/EST LIBEST_028455/EST figshare***/TSA	Holland group (Kumar, et al. 2015) (Kumar, et al. 2015) (Faber et al., 2021)	0	1	0	0
<i>Buddenbrockia plumatellae</i>	LIBEST_021265/EST	(Jiménez-Guri, et al. 2007)	0	1	0	0
Myxosporea						
<i>Ceratonova shasta</i>	Illumina HiSeq 2000/WGS SRR6782113/TSA	Bartholomew group (unpublished) (Alama-Bermejo, et al. 2020)	0	1	0	0
<i>Enteromyxum leei</i>	LDNA00000000/WGS	(Shpirer, et al. 2014; Chang, et al. 2015)	0	4	0	0

	Illumina NovaSeq6000 and Oxford Nanopore (FLO-MIN106)/WGS	Palenzuela group (unpublished)				
	Illumina NovaSeq6000/TSA	Palenzuela group (unpublished)				
<i>Enteromyxum scopthalmi</i>	Illumina NovaSeq6000 and Oxford Nanopore (FLO-MIN106)/WGS	Palenzuela group (unpublished)	0	3	0	0
	Illumina HiSeq2000 and Illumina NovaSeq6000/ TSA	Palenzuela group (unpublished)				
<i>Kudoa ivatai</i>	JRUX000000000/WGS	(Chang, et al. 2015)				
	JRUY000000000/WGS	(Chang, et al. 2015)	0	3	0	0
	GBGI000000000/TSA	(Chang, et al. 2015)				
<i>Myxidium lieberkuehni</i>	SRX9268956/TSA	(Kyslík, et al, In Press)	0	5	0	0
<i>Nephrocystidium pickii</i>	SRX9268957/TSA	(Kyslík, et al. In Press)	0	2	0	0
<i>Myxobolus cerebralis</i>	SRR628302/WGS	(Nesnidal, et al. 2013)	0	2	0	0
	GBKL000000000/TSA	(Chang, et al. 2015)				
<i>Myxobolus pendula</i>	SRX1269149 - SRR2472984, SRR2472987, SRR2472989/TSA	(Foux, et al. 2015)	0	2	0	0
<i>Sphaeromyxa zaharoni</i>	LDMZ000000000/WGS	(Shpirer, et al. 2014; Chang, et al. 2015)	0	3	0	0
<i>Sphaerospora molnari</i>	Illumina NovaSeq 6000 and Oxford Nanopore PromethION/WGS	Holzer group (unpublished)	0	2	0	0
	PRJNA522909/TSA	(Hartigan, et al. 2020)				
<i>Thelohanellus kitauei</i>	JWZT010000000/WGS	(Yang, et al. 2014)	0	7	0	0
	SRR1103279/TSA	(Yang, et al. 2014)				

^x previously identified as *Monosiga ovata* (Carr et al. 2017); ^{*} assembly at <https://bitbucket.org/molpalmuc/hoilungia-genome>; ^{**} assembly at <https://bitbucket.org/molpalmuc/hoilungia-genome>; ^{***} the intersect transcriptome available from www.figshare.com, 10.6084/m9.figshare.11889672.

Abbreviations: TSA = Transcriptome Shotgun Assembly; WGS = Whole Genome Shotgun; EST = Expressed Sequence Tag.

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