SUPPLEMENTAL INFORMATION

A taxogenomics approach uncovers a new genus in the phylum Placozoa

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NOTE RELATING TO TAXONOMIC RULES: According to the <u>International Code</u> of <u>Zoological Nomenclature</u> preprint publication of taxonomic names is discouraged. Consequently, the "*Xxxxxxx yyyyyyyyyyyy/X. yyyyyyyyyyy*" given here is a dummy only. The valid name will be available upon formal journal publication.



Figure S1. Lavalamp plot of kmer coverage.

The color code denotes the number of reads with a specific %GC and 31-bp kmer coverage. Heterozygous and homozygous coverage clouds show high counts at roughly 100x and 200x coverage, respectively.



Figure S2. Per base genome coverage.

The grey area (81% of the assembly) marks bases of the reference assembly that are in the merged stage with a peak at 260x coverage.



Figure S3. SNP histogram of genomic fractions.

Plotted are SNP counts in the exonic, intronic and intergenic genome fractions against the genome mapping coverage. The histogram shows that most of the genic (exonic and intronic) fraction were merged (peak at ~250x coverage) and further indicates a very low number of false gene duplications caused by genome misassembly.





Plotted are fractions of the full protein length for unchanged codons as well as synonymous and nonsynonymous sites for the 2,720 orthologs set. dN1, dN2, dN3 refer to non-synonymous sites with single, double, and triple base change, respectively. Orthologs are sorted by increasing dN/dS ratio. The percentage of unchanged sites is mostly above 40% (mean 45.8% \pm 8.5) and never drops below 14.2%. Third codon positions are thus never saturated and the three orthologs with dN/dS >1 are truly positively selected.



Figure S5. Length of syntenic blocks.

Shown are numbers of genes in detected syntenic blocks between a reduced set of T. *adhaerens* scaffolds and X. *yyyyyyyyyyy* contigs (blue circles; same set as used for collinearity analyses, see also Figure 2 & Table S5) as well as between both whole genomes (red rectangles). Numbers of genes within blocks as well as numbers of blocks are in the same order of magnitude indicating that the reduced set is representative for full genomes.



Figure S6. 18S genetic distances in non-bilateria.



Figure S7. 28S genetic distances in non-bilateria.



Figure S8. 16S genetic distances in non-bilateria.



Figure S9. CO1 genetic distances in non-bilateria.



Figure S10. ND1 genetic distances in non-bilateria.



Figure S11. Summary of genetic distances of all markers.

Shown are the means of all mean group distances for different taxonomic ranks in the non-bilaterian phyla Cnidaria, Ctenophora and Porifera. Interspecific genetic distance between placozoans are shown on the right (yellow).



Figure S12. PhyloBayes tree of the dataset 2 protein matrix.



Figure S13. PhyloBayes tree of the dataset 2 Dayhoff-6 recoded matrix.

Table S1. Genome assembly statistics.

		Xxxxxxxxx yyyyyyyyyyyy	Trichoplax adhaerens
Contigs	assembly size	87.19Mb	94.75Mb
	numbers	669	3,217
	N50	407.8Kb	204.2Kb
	mean length	130.3kb	29.5kb
	longest	2.06Mb	803.8Kb
Scaffolds	assembly size	-	105.6Mb
	numbers	-	1,414
	N50	-	5.98Mb
	mean length	-	74.65kb
	longest	-	13.26Mb
	gaps	-	10.86Mb
mt genome size		36.5Kb	43.1Kb
GC content		31.30%	32.70%
JGI best gene models		-	11,520
BRAKER1 gene models	ab initio genes	12,010	12,521
	ab initio transcripts	12,575	26,796
	unexpressed ab initio genes	422 (3.5%)	-
Trinity transcripts	total transcripts	124,155	-
	N50	2,550	
	protein coding	58,838	-
StringTie transcripts	total transcripts	25,266	-
	protein coding	19,795	-

Table S2. Summary of SNP counts.

source	total SNP count	exonic SNP count	total exonic SNPs per 1kb CDS	unique exonic SNPs per 1kb CDS
RNA	302,430	138,302	6.4	1.0
DNA	1,397,488	202,901	9.5	4.0

Shown are summaries from the genomic and transcriptomic datasets.

data type	reads or transcripts	mapped	mapping %
DNA reads	206,777,776	172,868,338	83.6
Moleculo (long) reads	79,974	74,626	93.3
RNAseq reads	114,475,046	92,154,755	80.5
Trinity mRNA	124,155	110,509	89.0
Trinity transdecoder mRNA	64,753	59,137	91.3
Trinity transdecoder CDS	64,753	58,838	90.9

protein set	complete (total)	complete (single)	complete (duplicate)	fragmented	missing
BRAKER1	92.4%	86.7%	5.7%	1.0%	6.6%
StringtTie	89.5%	70.1%	19.4%	1.0%	9.5%
Trinity	93.0%	41.5%	51.5%	2.5%	4.5%

Table S4. Results of the BUSCO v2 gene set searches.

Table S5. Collinearity analyses.

<i>Trichoplax</i> scaffold ID	scaffold size [Mbp]	gene number in <i>T.a</i> . scaffold	collinear <i>X.y.</i> genes	percentage of collinear genes
ta1	13.2	1,581	1,199	75.8
ta2	9.7	1,168	887	75.9
ta3	9.6	1,109	812	73.2
ta4	8.6	1,027	789	76.8
ta5	8.2	955	664	69.5
ta6	5.9	757	592	78.2
ta7	5.8	669	527	78.8
ta8	3.4	342	205	59.9
ta9	3.2	373	278	74.5
ta10	2.4	279	206	73.8
total	70.0	8,260	6,159	73.6 (±5.5)

Collinearity between the ten largest *Trichoplax adhaerens* (*T.a.*) scaffolds and the associated 144 *Xxxxxxxx yyyyyyyyyyy (X.y.*) supercontigs larger than 100Kb.

Table S6. Transcriptomic and genomic data resources.

Used data for genetic distance calculations and phylogenetic inferences. A Trinity transcriptome assembly has been generated for each species with a given accession number. Otherwise transcriptomes were used from the reference.

phylum	species	Genbank accession	reference
Porifera	Amphimedon queenslandica		[1]
	Aphrocallistes vastus		[2,3]
	Chondrilla nucula		[2,3]
	Cliona varians	SRP028615	[4]
	Corticium candelabrum	SRP012620	[2,3]
	Crambe crambe	ERP008520	[5]
	Crella elegans	SRP017286	[6]
	Ephydatia fluviatilis		[7]
	Ephydatia muelleri		Nichols lab; through www.compagen.org
	Halisarca dujardini		[8]
	Hyalonema populiferum	SRP056162	[9]
	Ircinia fasciculata		[2,3]
	Kirkpatrickia variolosa	SRP056162	[9]
	Latrunculia apicalis	SRP056162	[9]
	Lendenfeldia sp.		Vargas et al., unpubl.
	Leucosolenia complicata		Adamska lab; through www.compagen.org
	Mycale phyllophila	SRP051131	[10]
	Oscarella carmela		Nichols lab; through www.compagen.org

	Oscarella sp.		Nichols lab; through www.compagen.org
	Petrosia ficiformis	SRP012619	[2,3]
	Pseudospongosorites suberitoides	SRP037540	[2,3]
	Rossella fibulata	SRP056162	[9]
	Scopalina sp.	SRP061923	[11]
	Spongilla lacustris		[2,3]
	Stylissa carteri	SRP051559	[12]
	Sycon ciliatum		Adamska lab; through www.compagen.org
	Sycon coactum		[2,3]
	Sympagella nux	SRP056162	[9]
	Tedania anhelans	SRP061923	[11]
	Tethya wilhelma		[13]
	Xestospongia testudinaria	SRP051561	[12]
Cnidaria	Abylopsis tetragona		[14,15]
	Acropora digitifera		[16]
	Acropora millepora		[17,18]
	Agalma elegans		[14,15]
	Alatina alata	SRP056878	[15]
	Anthopleura elegantissima		[19]
	Astreopora sp		[19]
	Aurelia aurita		[15,20]
	Chironex fleckeri	SRP055535	[21]
	Chrysaora fuscescens	SRP070629	[22]
	Clytia hemisphaerica		www.compagen.org
	Corallium rubrum		[23]

Craseoa lathetica		[14,15]
Craspedacusta sowerbyi		[15,24]
Cyanea capillata	SRP056566	[25]
Dendrogramma enigmatica		[26]
Ectopleura larynx		[27]
Eunicella cavolinii	SRP042651	[28]
Eunicella verrucosa	SRP042651	[28]
Exaiptasia pallida		[15,29–31]
Fungia scutaria		[19]
Gorgonia ventalina		[32]
Hydra magnipapillata		[19,33]
Hydra vulgaris		[34]
Hydractinia symbiolongicarpus		[15,35,36]
Madracis auretenra		[19]
Montastraea cavernosa		[19]
Nanomia bijuga		[15,37]
Nematostella vectensis		[38,39]
Physalia physalis		[14,15]
Pocillopora damicornis		[40]
Porites astreoides		[19]
Porites australiensis		[19]
Pseudodiploria strigosa		[19]
Rhopilema esculentum	SRP073155	[41]
Seriatopora hystrix		[19]
Seriatopora sp		[19]
Siderastrea siderea		[42]
Stomolophus meleagris	SRP037742	[43]

	Stylophora pistillata		[44]
Ctenophora	Beroe abyssicola		[45]
	Beroe sp		[46]
	Bolinopsis infundibulum		[45]
	Coeloplana atericola		[45]
	Dryodora glandiformis		[45]
	Euplokamis dunlapae		[45]
	Hormiphora californensis	SRP057600	[47]
	Mertensiidae sp		[45]
	Mnemiopsis leidyi		[45,47]
	Pleurobrachia bachei		[45]
	Pleurobrachia pileus	SRP001155	[45]
	Vallicula multiformis		[45]
Placozoa	Trichoplax adhaerens		[48]

Table S7. Created OTUs.

Operational Taxonomic Units (OUTs) used for distance calculations (dataset 1, all) and phylogenetic analyses (dataset 2, as specified). The first species each participated most.

OTU	used species	in dataset 2
Acropora	Acropora digitifera, Acropora millepora	+
Ephydatia	Ephydatia fluviatilis, Ephydatia muelleri	+
Hydra	Hydra vulgaris, Hydra magnipapillata	+
Porites	Porites astreoides, Porites australiensis	+
Seriatopora	Seriatopora hystrix, Seriatopora sp	-
Beroe	Beroe abyssicola, Beroe sp	-
Eunicella	Eunicella cavolinii, Eunicella verrucosa	+
Oscarella	Oscarella carmela, Oscarella sp	+

Table S8. Datasets used for distance calculation and phylogenetic inferences.

Summary of protein datasets used for distance calculations (dataset 1) and phylogenetic inferences (dataset 2). Matrix length is 38,869 amino acid characters (dataset 1) and 35,799 (dataset 2), respectively.

data- set #	description	total taxa	outgroup taxa	non- bilaterian taxa	% missing characters
1	full dataset	139	4	77	29.0
2	100% gene content in outgroup taxa; overall gene coverage >50%	58	2	32	10.8

Table S9. Genetic distances (%) between the two placozoan species in comparison to Cnidaria.

Shown are averages of all mean genetic distances (\pm SD) between genera within families and families within orders for the Cnidaria as well as genetic distances between *X*. *yyyyyyyyyy (X.y.)* and *T. adhaerens (T.a.)*. Number of all mean pairwise distances used to calculate the average are given in parentheses.

taxonomic level	212 nuclear proteins	185	288	168	CO1	ND1
Cnidarian genera	5.9±2.7	3.8±4.3	1.8±1.9	5.1±3.4	2.2±1.5	4.4±5.1
	(10)	(8)	(9)	(9)	(9)	(9)
Cnidarian families	11.0±4.2	5.9±7.4	8.2±7.2	19.7±9.4	8.5±3.8	17.8±10.5
	(41)	(41)	(41)	(26)	(37)	(37)
X.y. / T.a.	9.8	1.2	2.9	17.8	3.0	8.6

phylum	class	species	accession
Porifera	Calcarea	Leucosolenia complicata	KT997975.1, KT997966.1
		Sycon ciliatum	KU244276.1, KU244278.1
		Sycon coactum	-
	Demospongiae	Amphimedon queenslandica	NC_008944.1
		Chondrilla nucula	NC_010208
		Cliona varians	-
		Crambe crambe	-
		Crella elegans	NC_027520.1
		Ephydatia muelleri	NC_010202.1
		Halisarca dujardini	NC_010212.1
		Ircinia fasciculata	-
		Kirkpatrickia variolosa	-
		Latrunculia apicalis	-
		Lendenfeldia sp.	-
		Mycale phyllophila	-
		Petrosia ficiformis	NC_027519.1
		Pseudospongosorites suberitoides	-
		Scopalina sp.	-
		Spongilla lacustris	LT158503.1, KU759841.1
		Stylissa carteri	-
		Tedania anhelans	-
		Tethya wilhelma	-
		Xestospongia testudinaria	-

Table S10. Accession numbers of used mitochondrial genomes.

	Hexactinellida	Aphrocallistes vastus	NC_010769.1
		Hyalonema populiferum	-
		Rossella fibulata	-
		Sympagella nux	EF537577.1
	Homoscleromorpha	Corticium candelabrum	NC_014872.1
		Oscarella carmela	NC_009090.1
Cnidaria	Anthozoa	Acropora digitifera	NC_022830.1
		Anthopleura elegantissima	-
		Corallium rubrum	-
		Eunicella verrucosa	-
		Exaiptasia pallida	-
		Fungia scutaria	-
		Gorgonia ventalina	-
		Madracis auretenra	-
		Montastraea cavernosa	-
		Nematostella vectensis	NC_008164.1
		Pocillopora damicornis	NC_009797.1
		Porites astreoides	-
		Pseudodiploria strigosa	
		Seriatopora hystrix	NC_010244.1
		Siderastrea siderea	-
		Stylophora pistillata	NC_011162.1
	Cubozoa	Alatina alata	-
		Chironex fleckeri	-
	Hydrozoa	Abylopsis tetragona	-
		Agalma elegans	-
		Clytia hemisphaerica	-

		Craseoa lathetica	-
		Craspedacusta sowerbyi	NC_018537.1
		Dendrogramma enigmatica	-
		Ectopleura larynx	-
		Hydra vulgaris	NC_011221.1
		Hydractinia symbiolongicarpus	LN901197.1
		Nanomia bijuga	-
		Physalia physalis	KT809328.1
	Scyphozoa	Aurelia aurita	NC_008446.1
		Chrysaora fuscescens	-
		Cyanea capillata	JN700937.1
		Rhopilema esculentum	
		Stomolophus meleagris	
Ctenophora	Tentaculata	Bolinopsis infundibulum	-
		Coeloplana atericola	-
		Dryodora glandiformis	-
		Euplokamis dunlapae	-
		Hormiphora californensis	-
		Mertensiidae sp	-
		Mnemiopsis leidyi	NC_016117.1
		Pleurobrachia bachei	NC_016697.1
		Pleuronrachia pileus	-
		Vallicula multiformis	-
Placozoa		Trichoplax adhaerens	NC_008151.2
		Placozoa sp. H4	NC_008833.2
		Placozoa sp. H8	NC_008832.2

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