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1 **Genomic resources and annotations for a colonial ascidian, the light-bulb sea squirt *Clavelina***
2 ***lepadiformis***

3
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8
9 **Abstract**

10 Ascidian embryos have been studied since the birth of experimental embryology at the end of
11 the 19th century. They represent textbook examples of mosaic development characterized by a
12 fast development with very few cells and invariant cleavage patterns and lineages. Ascidi-
13 ans belong to tunicates, the vertebrate sister group, and their study is essential to shed light on the
14 emergence of vertebrates. Importantly, deciphering developmental gene regulatory networks
15 has been carried out mostly in two of the three ascidian orders, Phlebobranchia and
16 Stolidobranchia. To infer ancestral developmental programs in ascidians, it is thus essential to
17 carry out molecular embryology in the third ascidian order, the Aplousobranchia. Here, we
18 present genomic resources for the colonial aplousobranch *Clavelina lepadiformis*: a
19 transcriptome produced from various embryonic stages, and an annotated genome. The
20 assembly consists of 184 contigs making a total of 233.6 Mb with a N50 of 8.5 Mb and a L50 of
21 11. The 32,318 predicted genes capture 96.3% of BUSCO orthologs. We further show that these

1 resources are suitable to study developmental gene expression and regulation in a comparative
2 framework within ascidians. Additionally, they will prove valuable for evolutionary and
3 ecological studies.

4
5 **Key words:** *Clavelina lepadiformis*, colonial ascidian, Aplousobranchia, tunicate, genome,
6 transcriptome, evo-devo

7 8 **Significance**

9 *Clavelina lepadiformis* belongs to Aplousobranchia, one of the three ascidian orders, that
10 includes only colonial animals and that has been under-explored at the molecular level. This
11 species is a promising model for developmental, evolutionary and ecological studies. We present
12 a transcriptome and an annotated genome, and show how these resources are immediately
13 useful for comparative analysis of embryonic development in ascidians.

14 15 **Introduction**

16 Ascidians belong to the tunicates, the vertebrate sister group. These marine filter-feeding
17 animals share with vertebrates and cephalochordates (amphioxus) a typical chordate body plan
18 during embryonic life (most prominently visible by the presence of a notochord and a dorsal
19 neural tube). Ascidians have a simple and stereotyped invariant embryonic development with
20 very few cells (100 at gastrulation and 2,500 in the tadpole larva) allowing deciphering
21 developmental mechanisms at cellular resolution. In addition, these externally developing
22 embryos are easily amenable to experimentation, and are particularly well suited for functional

1 genomics (Sato 2014; Lemaire 2011). These classical features of ascidians actually correspond
2 to a few species that have been used as laboratory animals. Since the advent of molecular
3 approaches, *Ciona* (represented by two closely related species: *C. robusta* (or *C. intestinalis* type
4 A) from the Pacific Ocean and the Mediterranean Sea, and *C. intestinalis* (or *C. intestinalis* type
5 B) from the Atlantic Ocean) has been the best studied and became the reference organism. In
6 recent years, the progress of sequencing technologies and the generally small size of ascidian
7 genomes has led to whole genome sequencing for a number of species (Dardaillon et al. 2020).
8 Our current understanding is that ascidian genomes have been extensively rearranged in this
9 fast-evolving lineage. Consequently, synteny is overall absent and DNA sequence conservation is
10 limited to the coding parts of the genomes. This drastic divergence of the genomes appears to
11 be contradictory to the fact that embryogenesis is remarkably conserved in distantly related
12 species. This raises the question of whether the molecular control of embryonic development is
13 the same in different species. Since ascidians have extensively diversified into around 3,000
14 species (Shenkar & Swalla 2011), they offer a great opportunity to evaluate the evolution of
15 developmental mechanisms. Current ascidian phylogenetics support a traditional classification
16 into 3 orders: the Phlebobranchia, the Aplousobranchia and the Stolidobranchia (Delsuc et al.
17 2018; Kocot et al. 2018). Developmental biology research has largely focused on the embryonic
18 and non-embryonic (asexual reproduction and regeneration) development in Phlebobranchia
19 and Stolidobranchia. Aplousobranchia make a group of strictly colonial ascidians that have been
20 so far overlooked. The few studies dedicated to their development are limited to rather ancient
21 and descriptive literature. Some genomic resources are available for three species: a very
22 fragmented but annotated assembly for *Didemnum vexillum* (Parra-Rincón et al. 2021; Velandia-

1 Huerto et al. 2016), and non-annotated chromosome level assemblies for *Aplidium turbinatum*
2 (Bishop et al. 2022) and *Clavelina lepadiformis*
3 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB57668>). RNA-seq data are also available for a
4 specific period in the life cycle of the latter species, the dormancy (Hiebert et al. 2022). Here, we
5 describe complementary transcriptomic and genomic resources for *C. lepadiformis*, and show
6 that they are immediately valuable for comparative developmental biology of ascidians.

8 **Results and discussion**

10 **Genome assembly**

11 The Cvlepa_BANY2021 assembly of the PacBio reads led to an estimated genome size of 233.6
12 Mb allocated to 184 contigs, the largest one being 15.4 Mb (Table 1). The completeness of the
13 genome seems quite high with a BUSCO score of 96.3%, and a mapping of 95.4% of the RNA-seq
14 reads that we have generated (Table S1). Interestingly, 90% of the genome was found in the 30
15 longest contigs. This number is higher than the anticipated number of chromosomes taking
16 other ascidian species as references (14 chromosomes in *Ciona robusta* and *Ciona intestinalis*
17 (Satou et al. 2021), and 16 in *Styela clava* and *Aplidium turbinatum* (Zhang et al. 2021; Bishop et
18 al. 2022)), or the karyotype that indicated 9 chromosomes (Colombera 1971). At the time we
19 planned to reach chromosome level assembly using HiC, a 210.1 Mb assembly for *C.*
20 *lepadiformis* that includes 9 chromosomes was released at ENA (kaClaLepa1.1 assembly.
21 <https://www.ebi.ac.uk/ena/browser/view/PRJEB58329>). The sequenced animal was collected
22 from the Atlantic Ocean while we sequenced an animal from the Mediterranean Sea. We thus

1 wondered how close these two samples were. We first extracted *Cox1* sequences and compared
2 them to available sequences for *Clavelina* species (Fig S1). As previously demonstrated, there are
3 two *C. lepadiformis* clades suggestive of separate species, Atlantic vs Mediterranean (Turon et al.
4 2003). Our sample that was collected in a harbor falls in the Atlantic clade as other similar
5 Mediterranean samples. This likely corresponds to a recent colonization of Mediterranean
6 marinas by the Atlantic type (Turon et al. 2003). Furthermore, the alignment of the 14,478 bp
7 mitogenomes from the kaClaLepa1.1 and the Cvlepa_BANY2021 assemblies displayed an
8 identity of 99.7%, confirming that the two specimen belong to the same species (Table S2).
9 The overall comparison of the two assemblies indicated that the 9 chromosomes of the
10 kaClaLepa1.1 assembly match to 30 scaffolds of the Cvlepa_BANY2021 assembly, with 2 to 6
11 scaffolds for 1 chromosome (Fig S2, Table S2). This fraction of the genomes corresponded to
12 approximately 206 Mb and captured 98.6% of the RNA-seq reads that map to our assembly
13 (Table S2,S3). They could be aligned over 173 Mb with an average identity of 67.3%. The parts
14 that did not align correspond most likely to centromeric regions: they are central in the
15 chromosomes, and have a low gene density and a low RNA-seq coverage (Table S2,S3,S4). The
16 remaining fraction of the assemblies were 3 Mb (kaClaLepa1.1) and 28 Mb (Cvlepa_BANY2021),
17 and did not match to each other. The rather large size of this fraction in our assembly suggests
18 that it might be artifactual, may correspond to repeated regions or unresolved haplotypes.
19 Accordingly, RNA-seq coverage was overall very low with the exception of a few scaffolds. In
20 conclusion, for the Atlantic type of *C. lepadiformis*, genome assemblies from two individuals
21 (one from the Mediterranean sea and one from the Atlantic ocean) are now available and
22 should be useful for studying recent genome evolution and adaptation to different

1 environments. Such a line of research would be beneficially complemented by sequencing the
2 genome of *C. lepadiformis* of endogenous Mediterranean type.

3

4 **Transcriptome assembly and genome annotation**

5 With a focus on embryonic development, we performed RNA-seq using Illumina on three classes
6 of embryonic stages (egg to neurula, tailbud stages, and larval stages) (Fig 1). To complement
7 this data set, we also sequenced adult pharyngeal tissue. The DRAP-assembled transcriptome
8 contains 31,035 transcripts of which 22,048 are coding (a large fraction of them, 87.4% have a
9 hit against *C. robusta* proteome). The completeness of this dataset is lower (93% BUSCO score)
10 than the genome, probably owing to the non-extensive sampling of tissues/life cycle.

11 The genome annotation indicates 32,318 genes, 25,067 of which are coding for proteins.

12 Surprisingly, only 62.2% of these coding genes had a hit on Swissprot using blastp (Table 1, Table
13 S5). This percentage increased to 79.4% when considering the proteome of the best annotated
14 ascidian genome *Ciona robusta* (Satou et al. 2022). Yet, almost 20% of the predicted genes had
15 no equivalent, suggesting that the prediction was partly inaccurate, and/or some novel genes
16 have to be found in the aplousobranch ascidian lineage. However, similar numbers were found
17 when the latest version of the *C. robusta* proteome was analyzed in the same manner (Table S5).

18

19 **Applications of genomic resources for comparative developmental biology**

20 ***Comparisons of developmental gene expression patterns***

21 *Ciona* is the reference ascidian species for developmental studies with extensive information on
22 gene expression and gene function during embryogenesis that are accessible in dedicated

1 databases such as Aniseed and Ghost (Dardaillon et al. 2020; Satou et al. 2005). To compare the
2 expression of developmental regulators, we performed *in situ* hybridization for the transcription
3 factor coding genes, *Dmrta*, *Foxn1/4*, *Klf1/2/4/17*, *Isl*, and *Sp6/7/8/9*, and for the neural marker
4 *Celf3/4/5/6* (Fig S3A). For each gene, phylogenetic analysis indicated the presence of a single
5 ortholog in *Clavelina* (File S1). These genes displayed similar patterns as their *Ciona*
6 counterparts: early neural precursors in gastrulae for *Dmrta*, tail epidermis midlines for
7 *Klf1/2/4/17*, adhesive papillae for *Isl* and *Sp6/7/8/9*, and neural cells for *Celf3/4/5/6*. *Foxn1/4*
8 expression has not been described in *Ciona*. It seemed to be expressed in a pattern reminiscent
9 of *Ciona* presumptive germ cells.

11 **Testing cis-regulatory activity**

12 Transcriptional regulation is an essential aspect of developmental gene networks. *In vivo*
13 evaluation of transcriptional activity is readily feasible in different ascidian species through
14 plasmid DNA introduction in the fertilized egg by electroporation (Lemaire 2011; Darras 2021;
15 Coulcher et al. 2020). Since *Clavelina* embryos are not yet amenable to experimentation, we
16 aimed at testing a candidate regulatory region in *Ciona* embryos. We focused on the *CesA* gene
17 that codes for a cellulose synthase, a gene acquired by horizontal transfer that gives tunicates
18 their unique capacity of synthesizing cellulose (Matthysse et al. 2004; Sasakura et al. 2005). In
19 *Ciona*, this gene is expressed in the entire epidermis under the control of the transcription factor
20 Tfap2-r.b, a determinant of epidermal fate (Sasakura et al. 2016). In *Clavelina*, we identified a
21 single *CesA* gene harboring 3 putative Tfap2 binding sites within 1 kb upstream of the start
22 codon (Fig S3B). When this region was placed upstream of a minimal promoter and *LacZ* as a

1 reporter, and tested in *Ciona intestinalis*, it showed activity in the epidermis (32% of the
2 embryos, n=820, results from 3 independent experiments). We obtained similar results when
3 the construct was tested in another phlebobranch ascidian species, *Phallusia mammillata* (29%
4 of the embryos with activity in the epidermis, n=558, results from 3 independent experiments).
5 These results strongly suggests that *CesA* has a conserved expression and regulation between
6 *Clavelina* and Phlebobranchia. Furthermore, it constitutes a proof of concept for the study of *cis*-
7 regulatory elements of *Clavelina*.

8

9 **Conclusion**

10 The resources that we have presented in this study will be directly beneficial for comparative
11 analyses of embryonic development in ascidians in order to study the evolution of
12 developmental mechanisms. They will complement other resources that are being generated for
13 a number of tunicate species. In addition, they will be very useful in other fields, such as the
14 study of non-embryonic development (Alié et al. 2020), adaptation and evolution.

15

16 **Material and methods**

17 *Sample collection, nucleic acid extraction and sequencing*

18 Colonies of *Clavelina lepadiformis* were collected on ropes in the harbor of Saint-Cyprien, France
19 (42°36'56.2"N 3°02'12.0"E) (Fig 1B). The gonad of a single sexually mature adult was dissected
20 for genomic DNA extraction (Fig 1C,E). The gonad was dissociated in sea water with the help of a
21 disposable plastic pestle, and by using a combination of pipetting and vortexing. The cells were
22 washed and collected by repeated centrifugation (6000 rpm at 4°C for 2 min) and resuspension

1 in sea water. High molecular weight genomic DNA was extracted using the Monarch HMW DNA
2 Extraction Kit for Cells & Blood (T3050, New England Biolabs) following manufacturer's protocol.
3 DNA quantity and quality was evaluated by agarose gel electrophoresis, spectrophotometry
4 (Nanodrop, Thermo Fisher Scientific) and fluorometry (Quantus, Promega). Genome sequencing
5 was performed by the GENTYANE platform (INRAe, Clermont-Ferrand) using PacBio Sequel II.
6 Circular consensus sequencing (CCS) protocol was used in order to obtain highly accurate long
7 read sequences that can be accessed through the BioProject PRJEB64590.

8 *C. lepadiformis* is a viviparous ascidian, and embryos at staggered stages can be found in the
9 atrial cavity of mature zooids (Fig 1C,D,F). Embryos from several zooids were released from the
10 adults by dissection, and separated into 4 samples according to stages: early (egg to neurula
11 stages, from 10 zooids), intermediate stages (initial tailbud to late tailbud stages, from 10
12 zooids), and late stages (late tailbud to larva stages, from 4 zooids). These 3 samples and an
13 adult sample (pharynx from 4 zooids) were immediately flash frozen using liquid nitrogen. Total
14 RNA was extracted using the NucleoSpin Tissue kit (Macherey-Nagel) following the provided
15 classical tissue protocol, except for the early embryo samples where the modifications from the
16 'difficult-to-lyse tissue' (Rev. 01) were applied (the classical protocol was fully inefficient on such
17 types of samples). RNA quality and concentration was determined using a Bioanalyzer 2100
18 (Agilent Technologies). The RNAs had a RIN>9.8 and were sequenced using the Illumina
19 technology (paired ends 2x150 bp on NextSeq550) by the BioEnvironnement facility (UPVD,
20 Perpignan). 39 to 41 million reads were produced for each of the 4 samples, and can be
21 accessed through the BioProject PRJEB64590.

22

1 *De novo transcriptome and genome assemblies*

2 We used the RNA-seq data to perform a *de novo* transcriptome assembly using the DRAP
3 pipeline (v1.91) (Cabau et al. 2017), with Oases as an assembler with kmers 37, 47, 57 and 63.
4 PacBio sequences were assembled with hifiasm assembler (v0.15.4-r342) with default
5 parameters (Cheng et al. 2021, 2022).

6 The mitogenome was assembled and annotated with MitoHiFi (v 3.0.0q 1.4.1) (Uliano-Silva et al.
7 2023; Allio et al. 2020) using *Ciona robusta* mitogenome as reference. This 14,478 bp
8 mitogenome was used in the final assembly to replace a duplicated version (scaffold S176) that
9 was present in the initial hifiasm assembly.

10 RNA-seq reads were mapped to the genome using STAR (2.7.5a) (Dobin et al. 2013).

11

12 *Annotation*

13 The genome was annotated with the funannotate pipeline (v1.8.14)
14 (<https://github.com/nextgenusfs/funannotate>). As a first step, 17 repetitive contigs were
15 removed from the primary hifiasm assembly using the funannotate *clean* script. The resulting
16 assembly is the current version of the genome that we named Cvlepa_BANY2021 and that we
17 deposited at ENA (BioProject PRJEB64590). The annotation process involved three main stages:
18 (1) funannotate *train* script (`--max_intronlen 8000 --busco_db metazoa`) was used to perform a
19 *de novo* genome-guided transcriptome assembly for RNA-seq data with HISAT (v2.2.1) (Kim et al.
20 2019), Trinity (v2.8.5) (Grabherr et al. 2011), StringTie (v.2.2.1) (Shumate et al. 2022), PASA
21 (v2.4.1) (Haas et al. 2003) and Kallisto (v0.46.1) (Bray et al. 2016) to identify the best probable
22 transcript at each locus. (2) Gene prediction was performed with funannotate *predict* script (--

1 organism other --repeats2evm --busco_db metazoa --ploidy 2 --optimize_augustus). This script
2 uses Evidence Modeler (v1.1.1) to select consensus gene models from Augustus (v3.3.2),
3 GlimmerHMM (v3.0.4), snap (v2006-07-28) predictions. The prediction yielded a total of 32,318
4 genes, among which 25,067 are protein-coding genes, exhibiting an average gene length of
5 3,188 bp. (3) Finally the funnannotate *annotate* script was used to assign functional annotation
6 to the protein coding gene models using evidence from InterProScan5 (v5.52-86) and UniProt DB
7 (v2022_05) databases. This procedure yielded 19,055 InterPro annotations, 16,229 PFAM
8 annotations, 13,862 GO terms, and 1,012 MEROPS annotations (Table S6). The quality of the
9 assembly was assessed with Quast-LG (v5.0.2) (Mikheenko et al. 2018) and BUSCO (v5.1.2)
10 (Manni et al. 2021) with the metazoan lineage orthologs dataset. Genetic elements were named
11 following the nomenclature of the tunicate community (Stolfi et al. 2015).
12 Repeated regions were identified using RepeatModeler (v2.0.4) (Flynn et al. 2020) and
13 RepeatMasker (v4.1.5) (<https://www.repeatmasker.org/RepeatMasker/>). They correspond to
14 43.9% of the assembly and their description is available in File S2.

16 *Sequence analyses and phylogenies*

17 Whole genome assemblies were aligned using minimap2 through the D-genies web interface
18 (<https://dgenies.toulouse.inra.fr/>) (Cabanettes & Klopp 2018)

19 Predicted proteins from the genome annotation were compared with proteins from *Ciona*
20 *robusta* (KY21) (Satou et al. 2022), *Branchiostoma lanceolatum* (BraLan3, NCBI) (Brasó-Vives et
21 al. 2022), *Corella inflata* (DeBiasse et al. 2020), *Styela clava* (ASM1312258v2, NCBI) (Wei et al.
22 2020), *Homo sapiens* (GRCh38.p13, NCBI) and Swiss-Prot dataset (Release 2023_03) (The

1 UniProt Consortium 2023) using blastp (v2.11.0), or with the whole genome assemblies of
2 *Aplidium turbinatum* (kaAplTurb1.1) (Bishop et al. 2022) using tblastn (hits were considered
3 positive for e value $<5.10^{-5}$). Similarly, coding transcripts from the DRAP transcriptome assembly
4 were assessed against *C. robusta* and Swiss-Prot datasets using blastx (hits were considered
5 positive for e value $<5.10^{-5}$).

6 We used blastp against whole proteomes to recover sequences of potential orthologs from
7 *Homo sapiens* (GRCh38.p13, NCBI), *Danio rerio* (GRCz11, NCBI), *Xenopus tropicalis*
8 (UCB_Xtro_10.0, NCBI), *Scyliorhinus canicula* (sScyCan1.1, NCBI), *Callorhinchus milii*
9 (IMCB_Cmil_1.0, NCBI), *Ciona robusta* (KH, NCBI), *Phallusia mammillata* (MTP2014, Aniseed;
10 and nr from NCBI), *Halocynthia roretzi* (MTP2014, Aniseed), *Styela clava* (ASM1312258v2, NCBI),
11 *Molgula occidentalis* (ELv1-2, Aniseed), *Branchiostoma lanceolatum* (BraLan3, NCBI),
12 *Branchiostoma belcheri* (Haploidv18h27, NCBI), *Strongylocentrotus purpuratus* (Spur_5.0, NCBI)
13 and *Saccoglossus kowalevskii* (Skow_1.1, NCBI). All sequences were aligned with the MUSCLE
14 program using the EMBL-EBI website (Edgar 2004; Madeira et al. 2022). Maximum-likelihood
15 phylogenies were inferred using IQ-TREE (<http://iqtree.cibiv.univie.ac.at/>) (Trifinopoulos et al.
16 2016).

17 A similar approach was used for *C. lepadiformis* and *C. oblongata* partial *Cox1* sequences that
18 were retrieved from NCBI, together with the ones extracted from the kaClaLepa1.1 and
19 Cvlepa_BANY2021 genomic assemblies.

20

21

22

1 *In situ hybridization*

2 We used the same method that prove efficient in several ascidian species (*Ciona intestinalis*,
3 *Phallusia mammillata*, *Molgula appendiculata* and *Halocynthia roretzi*) (Coulcher et al. 2020).
4 The main modification was as follows. Embryos were released from the adult by dissection with
5 scissors and tweezers. The protective chorion that surrounds the embryos was removed by
6 chemical digestion with 0.1% trypsin in sea water (15 to 30 min depending on the stage).
7 Antisense digoxigenin-labeled RNA probes were synthesized from plasmids (RT-PCR-
8 amplification and cloning in pGEM-T) or synthetic double-stranded DNA (eBlock, Integrated DNA
9 Technologies, Leuven, Belgium) (Table S7) as described previously (Chowdhury et al. 2022).

10

11 *Cvlepa.CesA locus analysis and transcriptional assay*

12 *Cvlepa.CesA* was identified from our data by blast using various tunicate CesA protein sequences
13 as queries. Actually, 3 neighboring gene models, Cvlepa.CG.BANY2021.S15.g019272,
14 Cvlepa.CG.BANY2021.S15.g019273 and Cvlepa.CG.BANY2021.S15.g019274, represented
15 significant hits. But only Cvlepa.CG.BANY2021.S15.g019272 coded for the 2 domains GT2 and
16 GH6 present in tunicate CesA, and was further considered (File S1). We examined the local
17 synteny of this locus (10 genes on each side of *CesA*). As expected from the study of other
18 tunicate genomes (Dardaillon et al. 2020), it was poorly preserved with a possible better synteny
19 with *Aplidium turbinatum*, another aplousobranch ascidian, than with *Ciona robusta*, a
20 phlebobranch ascidian (Fig S4). Putative Tfp2 binding sites were mapped on the *CesA* locus
21 using FIMO (<http://meme-suite.org/tools/fimo>) (Grant et al. 2011) with matrices collected from
22 the Jaspar database (Fornes et al. 2020) and the GCCN_{3/4}GGC motif (Eckert et al. 2005). A 993 bp

1 fragment was amplified from genomic DNA using PCR with Fwd ACTTCCCAGCGGTACAGTCA and
2 Rev TGTGACACGGTTCTTTCACCG, placed upstream of the *Ciinte.Fog* basal promoter and *LacZ*
3 using the Gateway technology (Invitrogen) (Roure et al. 2007; Coulcher et al. 2020).
4 Transcriptional assay was performed using *Ciona intestinalis* and *Phallusia mammillata* embryos
5 as previously described (Coulcher et al. 2020; Darras 2021).

6

7

8 **Conflict of interest.**

9 The authors declare that they have no conflict of interest.

10

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20

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3

4 **Authors' contributions**

5 VD and SD designed the project and wrote the manuscript. VD and HM performed the
6 bioinformatic analysis work. ML, CL and SD performed the experimental work. SD obtained
7 funding and supervised the project. All authors edited the manuscript, read and approved the
8 final version.

9

10 **Data availability**

11 RNA-seq data, PacBio data and the genomic assembly *Cvlepa_BANY2021* are available under the
12 BioProject accession PRJEB64590. All other data generated or analyzed during this study are
13 included in the manuscript and supporting files.

14

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26

1 **Table and figure legend**

2

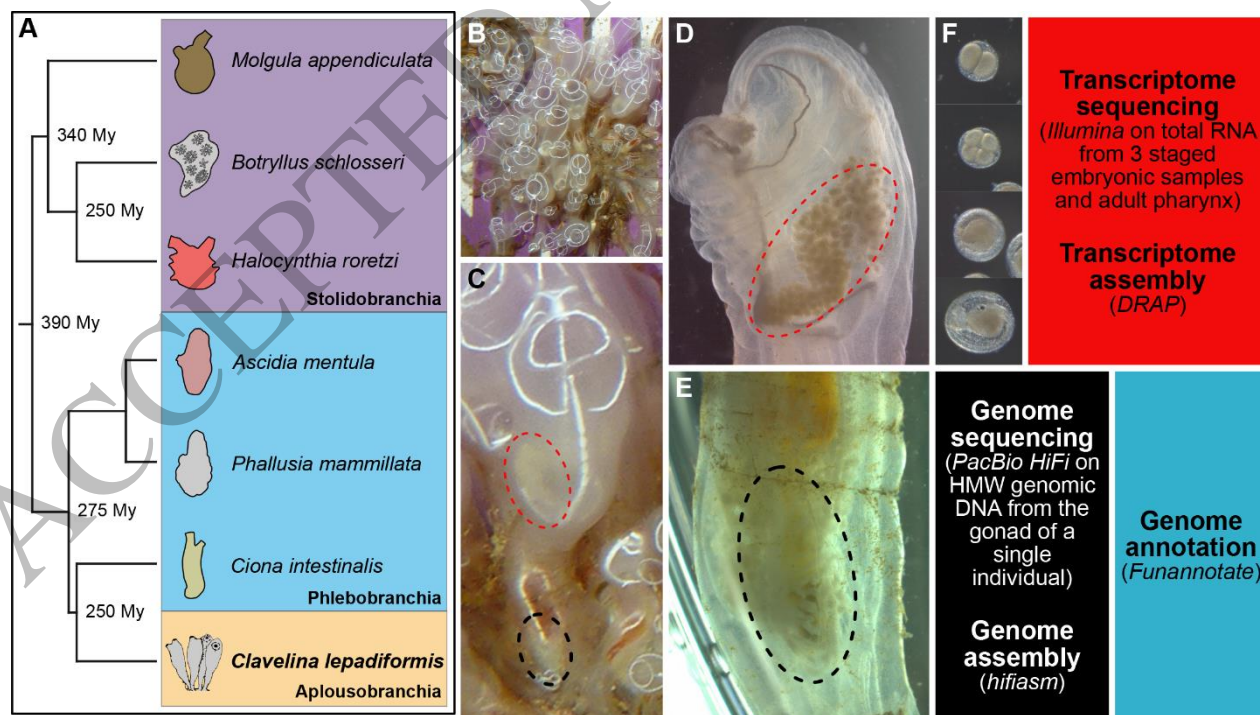
3 **Figure 1. Overall strategy and results.** (A) Simplified phylogenetic tree for ascidian species used
 4 in developmental biology with the three orders highlighted in colored boxes (based on (Delsuc
 5 et al. 2018)). Pictures of *Clavelina lepadiformis* biological samples: a colony (B), close up of a
 6 mature zooid (C) where the embryos developing in the pharynx are circled in red (D) and the
 7 gonad circled in black (E). Note that *C. lepadiformis* is hermaphrodite, the gonad is thus made of
 8 an ovary and a testis. Embryos at the 2-cell, 4-cell, late tailbud and early larva stages (F). RNA
 9 from embryos and pharynx was sequenced using Illumina and assembled using *DRAP* (red
 10 panel). Genomic DNA was sequenced using PacBio HiFi and assembled with *hifiasm* (black
 11 panel). The assembled genome was annotated using RNA-seq data with *Funannotate* (blue
 12 panel).

Genome assembly	Cvlepa_BANY2021
Genome size	233.6 Mb
Number of contigs	184
Mean length	1.3 Mb
Largest contig	15.4 Mb
N50	8.5 Mb
N90	1.7 Mb
L50	11
L90	30
GC	35.6%
BUSCO (Metazoa n=95)	
Complete single cc	91.1%
Complete duplicate	2.3%
Fragmented	2.9%
Missing	3.7%
Transcriptome	
Transcripts	31 035
Protein coding transcript	22 048

with <i>Cirobu</i> hit	19 263 (87.4%)
with SwissProt hit	16 818 (76.3%)
BUSCO (Metazoa n=95)	
Complete single cc	59.0%
Complete duplicate	31.2%
Fragmented	2.7%
Missing	7.0%
Genome annotation	
Genes	32 318
Transcripts	35 178
Protein coding genes	25 067
with <i>Cirobu</i> hit	19 894 (79.4%)
with SwissProt hit	15 599 (62.2%)
BUSCO (Metazoa n=95)	
Complete single cc	86.8%
Complete duplicate	6.4%
Fragmented	1.6%
Missing	5.2%

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2
3

Table 1. Statistics for the assemblies and annotation.



4

Figure 1
198x111 mm (DPI)