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An Annotated Draft Genome for *Radix auricularia* (Gastropoda, Mollusca)

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Abstract

Molluscs are the second most species-rich phylum in the animal kingdom, yet only 11 genomes of this group have been published so far. Here, we present the draft genome sequence of the pulmonate freshwater snail *Radix auricularia*. Six whole genome shotgun libraries with different layouts were sequenced. The resulting assembly comprises 4,823 scaffolds with a cumulative length of 910 Mb and an overall read coverage of 72×. The assembly contains 94.6% of a metazoan core gene collection, indicating an almost complete coverage of the coding fraction. The discrepancy of ~690 Mb compared with the estimated genome size of *R. auricularia* (1.6 Gb) results from a high repeat content of 70% mainly comprising DNA transposons. The annotation of 17,338 protein coding genes was supported by the use of publicly available transcriptome data. This draft will serve as starting point for further genomic and population genetic research in this scientifically important phylum.

Key words: de novo assembly, genome size, repeats.

Introduction

Gastropods are one of the broadest distributed eukaryotic taxa, being present across ecosystems worldwide. They occupy a maximally diverse set of habitats ranging from the deep sea to the highest mountains and from deserts to the Arctic, and have evolved to a range of specific adaptions (Romero et al. 2015, 2016). However, as for molluscs in general, whose species richness is second only to the arthropods (Dunn and Ryan 2015), gastropods are highly underrepresented among publicly available genomes (fig. 1). To date, only eleven mollusc genome sequences—of which six are from gastropods—exist with varying qualities concerning contiguity and completeness (table 1). Any additional genome sequence has therefore the potential to substantially increase the knowledge about molluscs in particular and animal genomics in general.
2011), local adaptation (Quintela et al. 2014; Johansson et al. 2016), hybridization (Patel et al. 2015) and biodiversity (Albrecht et al. 2012). Despite this broad range of interests, genomic resources, are scarce and limited to transcriptomes (Feldmeyer et al. 2011, 2015; Tills et al. 2015) and mitochondrial genomes (Feldmeyer et al. 2010).

Here, we present the annotated draft genome sequence for *Radix auricularia* L. (fig. 2). This serves as an important foundation for future genomic and applied research in this scientifically important genus.

### Materials and Methods

#### Sample Collection and Sequencing
Snails were collected from a pond in the Taunus, Germany, identified with COI barcoding (Pfenninger et al. 2006) and kept under laboratory conditions for at least five generations of inbreeding by full-sib mating. Three specimens of *R. auricularia* (fig. 2) were used for DNA extraction. Pooled DNA was used for preparation of three paired end and three mate pair (2, 5, and 10 kbp insert size) libraries, that were sequenced on an Illumina HiSeq 2000 and 2500 at Beijing Genomics Institute, Hong Kong (supplementary note 1 and table 1, Supplementary Material online). Reads were cleaned of adapter sequences using Trimmomatic 0.33 (Bolger et al. 2014; supplementary note 7, Supplementary Material online) and screened for contaminations with FastqScreen 0.5.2 (http://www.bioinformatics.babraham.ac.uk/projects/fastq_screen; last accessed February 22, 2017; supplementary note 8 and fig. 7, Supplementary Material online). Raw reads have been deposited under NCBI BioProject PRJNA350764.

#### Genome Size Estimation

Genome size was estimated by flow cytometry based on a modified protocol of (Otto 1990; supplementary note 9, Supplementary Material online). Additionally, we estimated the genome size from our sequence data by dividing the total sum of nucleotides used for the assembly by the peak coverage from mapping back the assembly reads with the *bwa mem* algorithm from BWA 0.5.10 (Li 2013;...
supplementary note 4, Supplementary Material online). Remappings were also used to estimate the repeat content of the genome (supplementary note 5, Supplementary Material online).

### Assembly Strategy

Reads were assembled using the Platanus 1.2.1 pipeline (Kajitani et al. 2014) with k-mers sizes ranging from 63 to 88 and a step size of 2. All other assembly parameters were kept at the default value. The output of the Platanus pipeline was filtered for sequences < 500 bp. Afterwards, scaffolding was performed using SSPACE 3.0 (Boetzer et al. 2011) with “contig extension” turned on. To further increase the contiguity of the draft genome, we applied a third scaffolding step, making use of the cDNA sequence data. Transcriptome contig sequences of *R. auricularia* and three closely related species (supplementary note 10, Supplementary Material online) were mapped sequentially according to phylogeny (Feldmeyer et al. 2015).

### Table 1

Available Mollusc Genomes

| Species | Assembly Length/Estimated Genome Size | % Assembled | #sequences/N50 (contigs) | Coverage/Technology | Gap [%] | BUSCOs Present | Number of Annotated Proteins |
|---------|--------------------------------------|-------------|--------------------------|---------------------|---------|----------------|-------------------------------|
| *Octopus bimaculoides* | 2.4 Gb/2.7 Gb = 89% | 151,674/475 kb | 92/Illumina | 15.1 | 73.8 | 23,994 |
| *Dreissena polymorpha* | 906 kb/1.7 Gb = 0.06% | * 1,057/855 bp | 3/Roche 454 | 0 | 0 | — |
| *Corbicula fluminea* | 663 kb? | * 778/849 bp | 3/Roche 454 | 0 | 0 | — |
| *Crassostrea gigas* | 558 Mbp/890 Mbp = 62.7% | 7,659/402 kb | 100/Illumina | 11.8 | 82 | 45,406 |
| *Mytilus galloprovincialis* | 1.6 Gb/1.9 Gb = 86% | * 2,315,965/1067 bp | 17/Illumina | 0 | 1.6 | — |
| *Lottia gigantea* | 360 Mbp/421 Mbp = 85% | 4,469/1870 kb | 8.87/Sanger | 16.9 | 97.0 | 23,822 |
| *Patella vulgata* | 579 Mbp/1,460 Mbp = 39.7% | 295,348/3160 bp | 25.6/Illumina | 0.00062 | 16.6 | — |
| *Conus tribblei* | 2,160 Mbp/2,757 Mbp = 78% | 1,126,156/2681 bp | 28.5/Illumina | 0 | 44 | — |
| *Aplysia californica* | 927 Mbp/1,760 Mbp resp. | 4,332/918 kb | 66/Illumina | 20.4 | 94.1 | 27,591 |
| *Biomphalaria glabrata* | 916 Mbp/929 Mbp = 99% | 331,401/48 kb | 27.5/Illumina | 1.9 | 89.1 | 36,675 |
| *Lymnaea stagnalis* | 833 Mbp/1,193 Mbp = 70% | * 328,378/8.5 kb | 0 | 88 | — |

**Notes.**—An overview from column 2 can be found in supplementary figure 4, Supplementary Material online. Column 5: Fraction of N’s in the assembly. Column 6: BUSCOs: (Benchmarking Universal Single-Copy Orthologs) NMetazoa = 843; Present = complete + fragmented.

References: Genome sizes are from the genome publications, if not cited separately.

*Albertin et al. 2015.*
*Penarrubia, Sanz, et al. 2015.*
*Gregory 2003.*
*Penarrubia, Araguas, et al. 2015.*
*Dong et al. 2012.*
*González-Tizón et al. 2000.*
*Nguyen et al. 2014.*
*Rodríguez-Juárez et al. 1996.*
*Simakov et al. 2013.*
*Hinegardner 1974.*
*Kenny et al. 2015.*
*Barghi et al. 2016.*
*Moroz et al.) GCF_000002075.1.
*Lasek & Dower 2013.*
*Matty Knight, Coen M. Adema, Nithya Raghavan, Eric S. Loker) GCF_000457365.1.
*Vinogradov 1998.*

**Fig. 2.**—Photograph of *Radix auricularia*. Picture by Markus Pfenninger.
2015) using BLAT 35 (Kent 2002), with -extendThroughN enabled apart from default settings, onto the scaffolds; the gapped alignments were then used for joining of sequences with L_RNA_scaffolder (Xue et al. 2013). Finally, all sequences with at least 1,000 bp were used as input for GapFiller 1.10 (Boetzer et al. 2012) to close extant gaps in the draft genome. Details of the assembly can be found in supplementary note 11, Supplementary Material online.

Annotation Strategy

Metazoan core orthologous genes were searched in the R. auricularia assembly and all other available mollusc genomes using BUSCO 1.2b (Simão et al. 2015).

The whole annotation process was performed using the MAKER2 2.31.8 pipeline and affiliated programs (Cantarel et al. 2008; Holt and Yandell 2011). Initially, we built a custom repeat library from the assembly using RepeatModeler 1.0.4 (Simit and Hubley 2015) and read data using dnaPipeTE 1.2 (Goubert et al. 2015) with 30 upstream trials on varying coverage depths and then 50 parallel runs on the best-fitting coverage of 0.025 (supplementary note 12, Supplementary Material online). The draft genome and transcriptome of R. auricularia (supplementary note 10, Supplementary Material online) in addition to the BUSCO 1.2b (Simão et al. 2015) annotations of core metazoan genes on the draft genome were used as input for the initial training at the Augustus webserver (Stanke et al. 2004; http://bioinf.uni-greifswald.de/webaugustus/; last accessed February 22, 2017). As additional input for MAKER2, we created two hidden Markov models on the gene structure of R. auricularia. One was generated by GeneMark 4.32 (Lomsadze et al. 2005) and another by SNAP 2006-07-28 (Korf 2004), using the output of CEGMA v2.5 (Parra et al. 2007; summarized results in supplementary table 9, Supplementary Material online). We ran three consecutive iterations of MAKER2 with the draft genome sequence, the transcriptomes (supplementary note 12, Supplementary Material online). The draft genome and transcriptome of R. auricularia (supplementary note 10, Supplementary Material online) in addition to the BUSCO 1.2b (Simão et al. 2015) annotations of core metazoan genes on the draft genome were used as input for the initial training at the Augustus webserver (Stanke et al. 2004; http://bioinf.uni-greifswald.de/webaugustus/; last accessed February 22, 2017). As additional input for MAKER2, we created two

| Parameter                                 | Value                     |
|-------------------------------------------|---------------------------|
| Haploid chromosome number                 | 17 (Garbar and Korniushin 2003) |
| Estimated genome length                   | 1.51 Gb (Vinogradov 1998)  |
| Flow cytometry                            | 1.58 Gb ± 21.5 Mb (this study) |
| Sequencing coverage                       | 1.60 Gb                   |
| Total assembly length                     | 0.91 Gb single copy or high complexity regions |
| #scaffolds                                | 4,823                     |
| N50                                       | 578,730 bp                |
| Gaps                                      | 6.4% N                    |
| Coverage                                  | 72x                       |
| Estimation of gene completeness           | 94.6% of BUSCO genes present |
| Gene prediction                           | 17,338 genes              |
| Gene space (UTR, Exons, Introns etc.)     | 200.6 Mb = 21.9% of assembly |
| Gene length (median)                      | 8.0 kb                    |
| Gene fragmentation                        | 147,195 exons             |
| exon space                                | 25.3 Mb = 2.8% of assembly (1.6% of total genome) |
| exon length (median)                      | 125 bp                    |
| Protein length (median)                   | 332 AA                    |

We created orthologous groups from protein sequences of all six annotated molluscs and 16 additional nonmollusc spiralian species with OrthoFinder 0.7.1 (Emms and Kelly 2015). All proteins were functionally annotated using InterProScan 5 (Zdobnov and Apweiler 2001; Quevillon et al. 2005). The enrichment analyses were performed in TopGO (Alexa and Rahnenfuhrer 2016), a bioconductor package for R (R Development Core Team 2008). We tested for significant enrichment of GO terms in proteins private to Radix and proteins found in all molluscs but Radix. We applied a Fischer’s exact test, FDR correction and filtered by q-values smaller than 0.05. Additional information can be found in supplementary note 6, Supplementary Material online.

Results and Discussion

Genome Assembly

A total of 1,000,372,010 raw reads (supplementary note 1and table 1, Supplementary Material online) were generated and assembled into 4,823 scaffolds (table 2; supplementary table 2, Supplementary Material online). The mitochondrial genome (13,744 bp) was fully reconstructed, evidenced by comparison to the previously published sequence (Feldmeyer et al. 2015). Re-mapping the preprocessed reads revealed that 97.6% could be unambiguously placed, resulting in a per position coverage distribution with its peak at 72x.
Additionally estimated insert sizes from mate pair libraries match their expected size (fig. 3B). The cumulative length of all scaffolds sums up to 910 Mb, which is about 665 Mb below the genome length estimates resulting from flow cytometric analyses (1,575 Mb; supplementary note 3, Supplementary Material online) and from a read-mapping analysis (1,603 Mb; supplementary note 4, Supplementary Material online). Both genome size estimates are consistent. This indicates an approximately uniform coverage of the nuclear genome in shotgun libraries without substantial bias introduced during library generation. This difference in length is most likely caused by a high repeat content in the *Radix* genome. Within scaffolds, 40.4% of the sequence content was annotated as repeats mostly at the ends of contigs (fig. 4). This, in combination with a pronounced increase of read coverage at contig ends (fig. 4) is typical for collapsed repeat stretches. The overall repeat content of the genome was estimated to be approximately 70% (supplementary note 5). The majority of repeats were either classified as Transposable Elements or as “unknown” (supplementary fig. 3, Supplementary Material online). The difference between genome size and assembly length of this *R. auricularia* draft assembly resembles that of other published mollusc genomes (supplementary fig. 4, Supplementary Material online). However, when considering contiguity reflected in the N50 value it ranks among the top mollusc genomes (tables 1 and 2). To evaluate completeness of the assembly’s gene space we used BUSCO (Simão et al. 2015) in combination with the provided metazoan set and recovered 94.6% of the subsumed genes. This suggests no conspicuous lack of gene information.

**Genome Annotation**

The annotation resulted in 17,338 protein coding genes (table 2) of which 70.4% show a significant sequence similarity to entries in the Swiss-Prot database (e-value < 10^-10, accessed on May 11, 2016). The number of identified genes is at the lower end compared with other annotated mollusc genomes (Min: *Lottia gigantea* 23,822; Max: *Crassostrea gigas* 45,406;...
Thus, predicted *Radix* proteins were screened for completeness regarding evolutionary conserved genes using HaMStR (Ebersberger et al. 2009). The analysis resulted in a recovery of 93.7% and is in line with the results from BUSCO. Extrapolating completeness estimates of both tools suggests that the annotation covers the majority of genes being present in the draft genome sequence. We then checked how the differences in protein numbers could be explained. The fraction of orthogroups (cluster of orthologous genes; see “Materials and Methods” section) containing only one sequence per species was highest in *Radix* (supplementary fig. 5, Supplementary Material online). Moreover, there was a negative correlation ($R^2 = 0.77; P = 0.02$) between the number of annotated proteins per species and fraction of orthogroups containing only one sequence per species (supplementary fig. 6, Supplementary Material online). One explanation for this observation could be lineage specific gene duplications in the other mollusc lineages. Additionally, artificial gene fissions in the course of genome annotation may be less common in *Radix*. This might be attributed to our use of comprehensive transcriptomic data of *Radix* for guiding gene prediction.

Next to the evolutionarily old genes represented in the BUSCO and HaMStR gene sets, *Radix* contains 1,481 genes for which we could find no orthologs in the other mollusc and additional nonmollusc spiralian gene sets (supplementary table 5 and note 6, Supplementary Material online). We tested for over-representation of functional categories in genes private to *Radix*, as well as in genes present in all molluscs but *Radix* (supplementary table 7 and note 6, Supplementary Material online). Among the categories found in all annotated molluscs but *Radix* (supplementary table 6, Supplementary Material online), the “G-protein coupled receptor signaling pathway” is the most prominent one. G-protein receptors are involved in reactions to “hormones, neurotransmitters, and environmental stimuli” (Rosenbaum et al. 2009). The loss of these genes could have led to reduced sensitivity to such stimuli in *Radix*. Whether the reduced number of G-coupled receptor pathway components is biologically meaningful, or a result of technical and analytical limitations, cannot be determined from the present data. Membrane proteins, for example, are generally more diverse than water soluble proteins in the tree of life (Sojo et al. 2016), so we hypothesize that its proteins could be highly modified and were thus not identified as such in *Radix*.

### Conclusion

Here we present a draft genome of the snail *Radix auriculara*. The genome is comparable in size to other mollusc genomes and also rich in repeats. This new genomic resource will allow conducting future studies on genome evolution, population genomics, and gene evolution within this genus and higher gastropod and mollusc taxa.

### Supplementary Material

Supplementary data are available at Genome Biology and Evolution online.
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