

1 **Gene content of the fish-hunting cone snail *Conus consors***

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15 **Abstract**

16 **Background.** *Conus consors* is a fish-hunting cone snail that lives in the tropical waters of the  
17 Indo-Pacific region. Cone snails have attracted scientific interest for the amazing potency of their  
18 venom, which consists of a complex mixture of small proteins known as conopeptides, many of  
19 which act as ion channel and receptor modulators with high selectivity.

20 **Results.** We have analysed publicly available transcriptomic sequences from 8 tissues of *Conus*  
21 *consors* and complemented the transcriptome data with the data from genomic DNA reads. We  
22 identified 17,715 full-length protein sequences from the transcriptome. In addition, we predicted  
23 168 full-length or partial conopeptide sequences and characterized gene structures of several  
24 conopeptide superfamilies.

25

26 **Introduction**

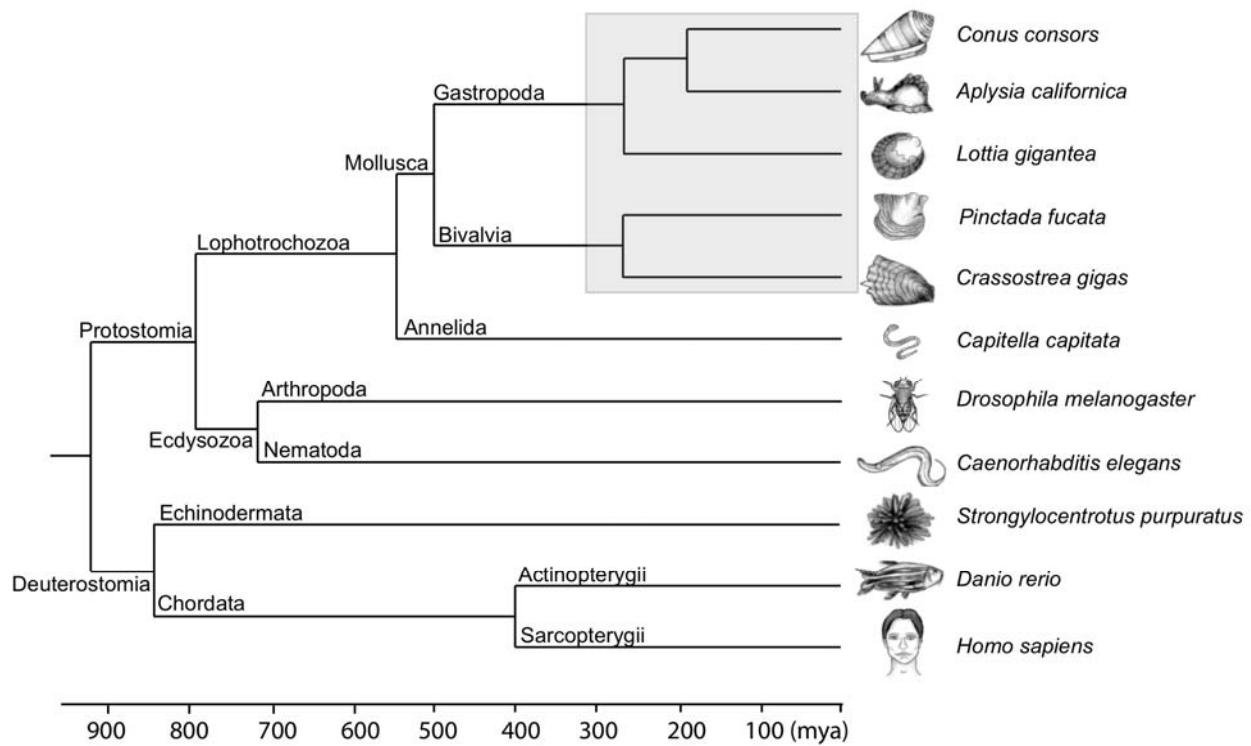
27 *Conus consors* is a marine gastropod of the species-rich and highly diverse Mollusca phylum and  
28 we present the first extensive study of this organism from a genomic point of view. The first few  
29 genomes from this phylum (California sea hare, pearl oyster, Pacific oyster, owl limpet, octopus,  
30 and a freshwater snail) have only recently been sequenced (Takeuchi et al., 2012; Zhang et al.,  
31 2012; Simakov et al., 2013; Albertin et al., 2015; Adema et al., 2017) The phylogenetic position  
32 of *C. consors* is provided in Figure 1, which was constructed with particular reference to the  
33 other mollusc species for which genomic data are available.

34 *C. consors* is a member of the *Conoidea* superfamily that consists of more than 700 species  
35 worldwide (Puillandre et al., 2014; Lavergne et al., 2015; Gao et al., 2017). *C. consors* lives in  
36 the tropical waters of the Indo-Pacific, inhabits sub-tidal coastlines, but is also found at depths of

37 up to 200 meters, where it buries itself under sand and silt for shelter

38 (<http://biology.burke.washington.edu/conus/>).

39



41 **Figure 1. Phylogenetic position of *C. cossors* in relation to some model organisms with**  
42 **sequenced genomes.** The divergence times were obtained from the "Timetree of life" project  
43 (Hedges et al., 2015). Phylogenetic relationships within the *Mollusca* phylum are based on  
44 (Smith et al., 2011b) and (Kocot et al., 2011). The nodes included in the grey box are not time-  
45 scaled.

46

47 The cone snails have attracted scientific interest because of their pharmacologically active  
48 venom, which may provide leads in the search for novel drugs. The venom is a complex mixture

49 of small peptides, termed conopeptides, that primarily act as ion channel modulators (Han et al.,  
50 2008; Favreau & Stöcklin, 2009; Lewis et al., 2012; Neves et al., 2015; Mir et al., 2016; Liu et  
51 al., 2018). When *C. consors* injects a fish with its venom, the fish is paralyzed within a few  
52 seconds and secured *via* a harpoon-like device. This “hook and line” strategy (Olivera, 1997) is  
53 unique to cone snails and makes up for their inability to chase prey.

54 Previous peptidomic and proteomic studies have revealed that the venom of cone snails is a  
55 complex mixture of several hundred peptides that shows both inter- and intra-species specific  
56 variability (Biass et al., 2009; Dutertre et al., 2010, 2013, 2014; Abdel-Rahman et al., 2011; Fu et  
57 al., 2018). Some variations in venom properties are linked to predation or defence stimuli  
58 (Dutertre et al., 2014).

59 To gain insight into the complexity of *C. consors*, we analysed transcriptome and genome  
60 sequences with the focus on gene content.

61

## 62 **Materials and Methods**

### 63 **Transcriptome assembly**

64 For assembly, we used publicly available sequencing reads generated by the CONCO consortium  
65 (Project #PRJNA271554 at NCBI SRA database). The transcriptome assembly included three  
66 steps: pre-processing of raw reads, separate assembly of tissue-specific transcriptomes from eight  
67 different tissues (venom duct, salivary gland, nerve ganglion, osphradium, mantle, foot,  
68 proboscis, and venom bulb) and combining transcriptomes into one non-redundant transcriptome  
69 set.

70 For pre-processing we trimmed the low quality 3'-ends of Illumina paired-end reads with the  
71 FASTQ Quality Trimmer from the FASTX Toolkit package version 0.0.13  
72 ([http://hannonlab.cshl.edu/fastx\\_toolkit/](http://hannonlab.cshl.edu/fastx_toolkit/)) using the quality cut-off (“-t”) at 30 and set the  
73 minimum length of the reads (“-l”) at 50 bp. We cleaned the reads with DeconSeq 0.4.1  
74 (Schmieder & Edwards, 2011) and almost 850 million reads remained (in total ~800 Gbps).

75 For assembly of the transcriptome, we used the Trinity assembler (version 2012-06-08)  
76 (Grabherr et al., 2011) to create *de novo* transcripts for each sample with a minimum assembled  
77 contig length (“--min\_contig\_length”) set to 201 nucleotides.

78 Finally, in order to obtain a non-redundant set of sequences, we clustered the transcripts with  
79 CD-HIT-EST (Li & Godzik, 2006) using a sequence identity threshold (“-c”) of 0.98. The  
80 clustered transcriptome set is called the TRINITY transcriptome.

81

## 82 **Genome assembly**

83 We have used publicly available sequencing reads generated by the CONCO consortium using a  
84 Roche 454 Genome Sequencer and an Illumina/Solexa GAII (Project #PRJNA267645 at NCBI  
85 SRA database). The average lengths of Roche 454 and Illumina reads were 354 bp and 104 bp,  
86 respectively. Four different types of data were used for the genome assembly: Roche 454  
87 shotgun-sequenced reads, artificial 454 reads from an Illumina preliminary assembly with  
88 SOAPdenovo, six libraries of Illumina paired-end reads (300 bp and 600 bp insert sizes), and  
89 three libraries of Illumina mate pair reads (1.2 kbp, 3 kbp, and 7 kbp insert sizes). Detailed  
90 specifications for these libraries are provided in Supplemental Article S1.

91 During pre-processing, low quality 3' ends of Roche 454 and Illumina reads were trimmed with  
92 the FASTQ Quality Trimmer. A quality cut-off ("‐t") was set to 30 and the minimum length of  
93 the reads ("‐l") was set to 50 bp. Consequently, reads were cleaned of human and bacterial  
94 contamination with DeconSeq 0.4.1. Identity ("‐i") and coverage ("‐c") cut-offs of 90% were  
95 used when scanning reads against human genome NCBI GRCh37 patch release 8 and 2,370  
96 different bacterial strains. For the third step, SeqClean (version 2011-02-22)  
97 (<https://sourceforge.net/projects/seqclean/>) was used to remove any vector contaminations,  
98 linkers or adapter sequences. Tool was executed with default parameters excepting a minimum  
99 length of valid reads ("‐l 50"), trimming of polyA/T tails, and low-complexity screening was  
100 disabled ("‐A ‐L"). Reads were scanned against UniVec database build 7.0  
101 (<http://www.ncbi.nlm.nih.gov/tools/vecscren/univec/>) to remove any vector sequences.

102 Assembly included two distinct steps. At first, SOAPdenovo 2.04 (Luo et al., 2012) was used to  
103 create the initial genome assembly with Illumina paired-end/mate-pair reads. The goal was to  
104 create 454 "pseudo-reads" from the Illumina assembly as additional input data for Newbler.  
105 SOAPdenovo was applied with a k-mer word size of 37. The SOAPdenovo assembly generated  
106 many scaffolds that contained unresolved gaps (strings of "N"s). These scaffolds were split into  
107 300 bp long sub-sequences with 200 bp overlaps to eliminate incorrect estimation of gap sizes  
108 using EMBOSS splitter (Rice, Longden & Bleasby, 2000). As a second step, Newbler 2.7  
109 (<https://sequencing.roche.com/>) was run with the parameters "‐large ‐rip ‐mi 98 ‐ml 100" to  
110 assemble all three types of reads – 454 (maximum read length 1,892 bp), "pseudo" 454 (300 bp)  
111 and Illumina (145 bp) – into one unique dataset. Contigs longer than 200 bp were reported in  
112 final assembly.

113

114 **Discovery of full-length genes from the transcriptome**

115 We compiled a list of full-length genes from the TRINITY transcriptome using the following  
116 criteria:

117 1. We selected transcripts that exhibit at least 95% of their length matched to the genome using a  
118 BLASTN (version 2.2.22) (Altschul et al., 1997) alignment search. We performed unique  
119 mapping by first finding pairwise alignments between a transcript and a genomic region where  
120 the given alignment had the highest homology bitscore for both the transcript and genomic  
121 regions (seeds). For each seed we added the alignments for which the same transcript had highest  
122 alignment bitscore with the given genomic regions.

123 2. We annotated these transcripts using a BLASTX homology search against the UniRef100  
124 database (Nov. 15, 2013) (Suzek et al., 2007). When homology to a given protein reached at  
125 least 75%, we annotated the transcript with its putative corresponding protein. In cases where  
126 there were multiple candidate proteins, we chose the one with highest cumulative alignment  
127 bitscore.

128 3. The cumulative bitscore of all transcript alignments with a given protein had to be greater than  
129 or equal to 100 bits.

130 4. All partial transcript homologies with a given protein had to be in the same translational  
131 frame.

132 5. The Open Reading Frame (ORF) had to be in one single translational frame, i.e. both the start  
133 and stop codons were present in the same frame.

134 6. The ORF start codon had to be located no more than 10 amino acids after the start of the first  
135 alignment and the stop codon not more than 10 amino acids before the end of the last alignment.

136 In cases where all of these criteria were met, we assigned the protein from the UniRef100  
137 database as the annotation of a given transcript and generated the predicted protein sequence  
138 from the ORF.

139

#### 140 **Annotation of conopeptides**

141 We used four approaches to annotate conopeptide sequences from the assembled genome: 1) a  
142 BLAST search against the UniProtKB/Swiss-Prot database (release 2012\_10) (The UniProt  
143 Consortium, 2015); 2) a HMM search using software HMMER 3.0 (<http://hmmer.org/>) (Eddy,  
144 2011) against conopeptide HMM profiles (Laht et al., 2012); 3) a BLAST search against peptide  
145 sequences from *C. consors* venom proteomic data (Violette et al., 2012); and 4) a BLAST search  
146 against conopeptide sequences predicted from the transcriptome data of *C. consors*. In all four  
147 cases we applied an E-value cut-off of  $10^{-5}$ . We ran the HMMER and BLAST searches with  
148 default parameter values, except that we turned off the BLAST filtering option (-F F). We  
149 discarded matches that covered less than 50% of the length of their respective HMM profiles.  
150 We manually assessed the alignments and domain boundaries for all predictions.

151

#### 152 **Data availability**

153 Draft genome assembly of the cone snail can be retrieved from the GenBank database with  
154 following assembly ID: GCA\_004193615. Gene and protein sequences predicted from  
155 transcriptome are included in Supplemental Data S4 (in FASTA format).

156

157 **Results and Discussion**

158 **Transcriptome and genome assembly**

159 Transcriptome assemblies were created with Trinity software using read libraries from eight  
160 different tissues (venom duct, salivary gland, nerve ganglion, osphradium, mantle, foot,  
161 proboscis, and venom bulb). The total number of transcripts (including isoforms) was 1,535,709  
162 and ranged from 85,807 (“Foot” sample) to 240,307 (“Mantle” sample) and contained around  
163 1,062 Gbp of sequence. The average length of the resulting transcripts for all samples was 692  
164 bp, N50 = 2,452 bp, and the longest sequence was 29,867 bp. After clustering the results from  
165 eight samples with CD-HIT-EST, the final dataset contains 587,852 transcripts (~324 Gbp in  
166 total). The transcriptome data was used to compile a full-length gene list and to predict  
167 conopeptide genes.

168 For genome assembly we used a strategy similar to the one employed to assemble the genome of  
169 the fire ant *Solenopsis invicta* (Wurm et al., 2011). Briefly, this strategy consisted of two major  
170 steps: (a) assembly of Illumina reads (9 libraries, overall 51 Gbp of raw data) into larger contigs  
171 using SOAPdenovo software and (b) combining the resulting Illumina contigs and original  
172 paired-end reads from the Illumina and unpaired reads from Roche 454 libraries (1 fragment  
173 library, overall 6 Gbp of raw data) into a final assembly using the software Newbler  
174 (Supplemental Article S1 Figure 1). The assembly of Illumina reads into longer artificial reads  
175 was required because Newbler is not optimized to work with short Illumina reads. In step (b), the  
176 original Illumina reads were also included to provide additional information about the distance  
177 between paired reads.

178 The final assembly of *Conus consors* genomic reads resulted in a 2,049 Mbp sequence consisting  
179 of 2,688,687 scaffolds and contigs with an N50 size of 1,128 bp. Newbler software is able to  
180 estimate the size of the entire genome based on k-mer frequency distribution. *C. consors* genome  
181 was estimated to be 3.025 Gbp, which is within the range of other cone snail genomes  
182 (<http://genomesize.com/>). The genomic DNA resulting from this assembly is fragmentary;  
183 nevertheless, the protein-coding exons are generally contiguous. Therefore, we were able to use  
184 it as an additional source of information in gene prediction process and for characterization of  
185 conopeptide gene structures.

186 The genome of *C. consors* is rich in repeats. Approximately 49% of the genome sequence  
187 contains repeated sequences, half of which are low-complexity (mononucleotide, dinucleotide,  
188 trinucleotide and tetranucleotide) repeat elements. Detailed analysis of repeat elements present in  
189 the genome is shown in Supplemental Article S1.

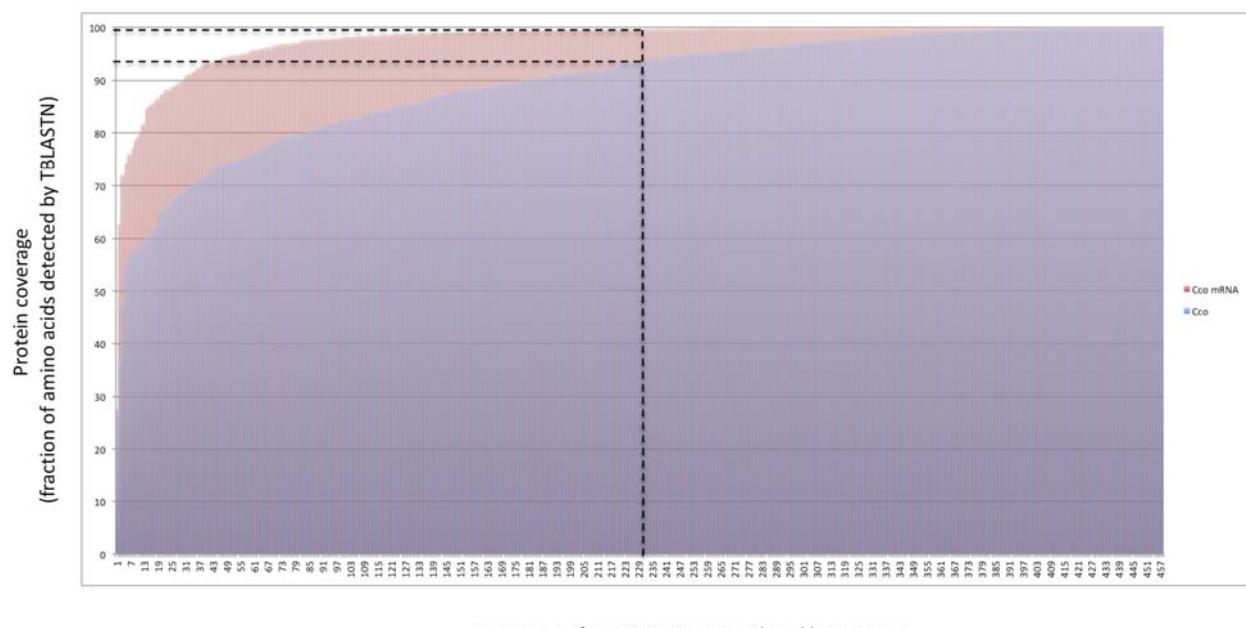
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### 191 **Coverage of core genes in transcriptome and genome**

192 To evaluate the completeness of our transcriptome and genome assemblies we calculated the  
193 length coverage of core genes from the Core Eukaryotic Genes Mapping Approach (CEGMA)  
194 dataset (Parra, Bradnam & Korf, 2007; Parra et al., 2009). This dataset consists of 458 core  
195 proteins that are universally present in 6 eukaryotic species: *Homo sapiens*, *Drosophila*  
196 *melanogaster*, *Arabidopsis thaliana*, *Caenorhabditis elegans*, *Saccharomyces cerevisiae* and  
197 *Schizosaccharomyces pombe*. A similar method has previously been used to evaluate the quality  
198 of two different ant genome assemblies (Smith et al. 2011; Wurm et al. 2011). Coverage  
199 (fraction of amino acids detected by TBLASTN search using core protein dataset as a query) of

200 core genes in our transcriptome and genome data is shown in Figure 2. The median coverage of  
201 core genes is 99.7% for transcriptome and 93.4% for the genome. Similar genome coverage was  
202 observed for other mollusc genomes (Supplemental Article S1). One has to take into account that  
203 TBLASTN is somewhat limited in finding short exons in genome, thus the coverage of core  
204 genes measured from genome will always be lower than coverage in transcriptome. An  
205 illustration of core gene alignment from *C. consors* genome is shown in Figure 3.

206



207 Core genes from CEGMA set, ordered by coverage

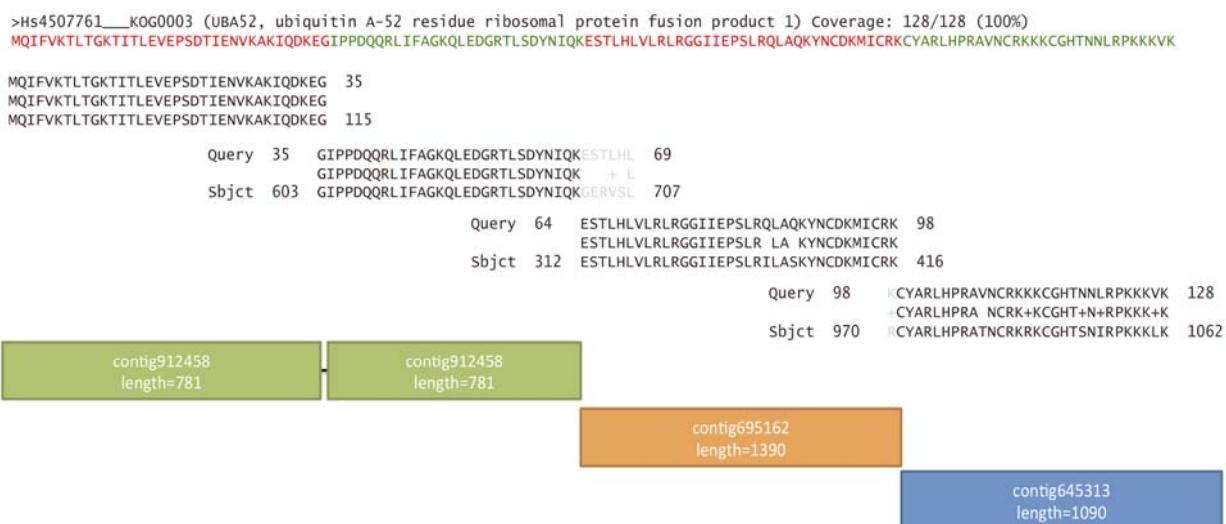
208 **Figure 2.** Coverage of 458 core proteins from the CEGMA dataset in *C. consors* transcriptome  
209 and genome. Coverage is defined as fraction of amino acids detected by TBLASTN search using  
210 core protein dataset as a query.

## 211 **Gene content of *C. consors***

212 We predicted full-length protein sequences from the transcriptome data using a reciprocal  
213 homology search between the transcriptome and the UniRef100 protein database. The genome

214 sequence was used to confirm the existence of genes predicted from transcriptome. We consider  
215 the resulting 17,715 full-length proteins to be a reliable prediction of protein-coding sequences of  
216 *C. consors*. The collection of mRNAs and translated protein sequences in FASTA format is  
217 available in Supplemental Data S4. It has to be kept in mind that the actual number of protein-  
218 coding genes is somewhat larger due to the fact that transcriptome analysis cannot reveal genes  
219 that are expressed at low levels, in other tissues or just temporarily.

220



221

222 **Figure 3. Example of gene content in the genome.** TBLASTN against the genome using  
223 CEGMA (core protein set present in all eukaryotes) protein Hs4507761 as a query. Red and  
224 green text denote location of alternating exons in the human gene *UBA52*. Red orange and blue  
225 boxes are matching regions from contigs or scaffolds of the *C. consors* genome. Alignment  
226 between Hs4507761 and translated genomic DNA is shown in the middle.

227

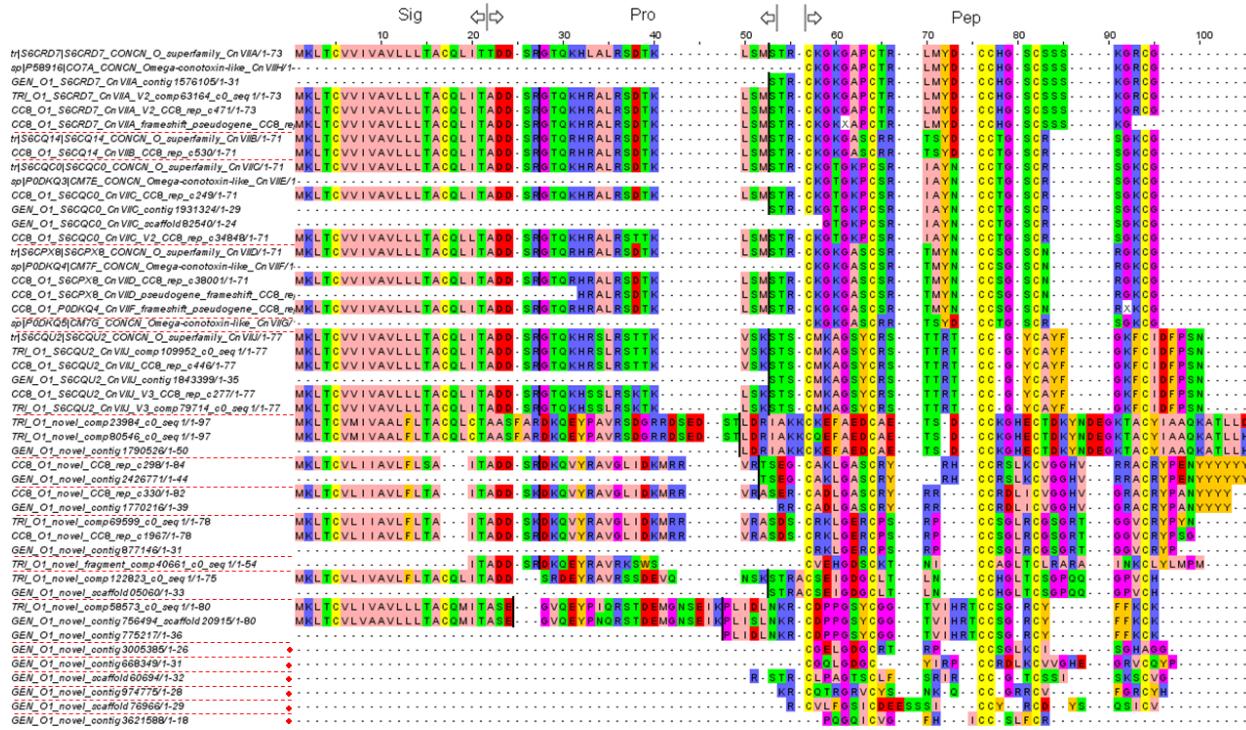
228 We analysed the number of tRNA, rRNA and of other non-protein-coding genes using  
229 tRNAscan-SE (Lowe & Eddy, 1997) and Infernal software (Nawrocki, Kolbe & Eddy, 2009).  
230 We detected a total of 761 different tRNA genes in the *C. consors* genome, 2500 miRNA genes  
231 and many other types of RNA genes. Detailed analysis of RNA genes present in the genome is  
232 shown in Supplemental Article S1 and full list of detected RNA genes is shown in Supplemental  
233 Article S1 Table 2.

234

### 235 **Conopeptide sequences**

236 To identify conopeptide sequences in the transcriptome and genome of *C. consors*, we used  
237 several sources of data with previously known conopeptide sequences or hidden Markov model  
238 (HMM) profiles. Conopeptide sequences available in the UniProtKB/Swiss-Prot database (975  
239 peptides from more than 30 different superfamilies), 64 conopeptide hidden Markov model  
240 (HMM) profiles from 20 different superfamilies (Laht et al., 2012), 126 peptide sequences from  
241 the *C. consors* proteome sequencing (Violette et al., 2012), and conopeptide precursor sequences  
242 predicted from the transcriptome data (135 distinct precursor sequences from 23 different  
243 superfamilies) were used. In addition to main transcriptome data we also used another dataset  
244 (CC8 transcriptome), sequenced earlier. This additional transcriptome data originated from two  
245 ESTs libraries constructed from venom duct and salivary gland tissues. The procedure for  
246 obtaining CC8 transcriptome sequences is described in (Terrat et al., 2012). The genome  
247 sequence was also checked for potential conopeptide genes in hope that it complements  
248 transcriptome-based data.

249 To estimate the overall number of conopeptides encoded by *C. consors*, we aligned predicted  
250 protein sequences obtained from the genome, transcriptome, and proteome into multiple  
251 alignments (Supplemental Data S3.). Sequences from different datasets exhibit clear clusters  
252 with slight variations between individual sequences. Closely related sequences were merged into  
253 clusters if the difference between sequences did not exceed 4 amino acids and the overall number  
254 of sequence clusters was counted. Example of multiple alignment of sequences from the O1-  
255 superfamily is shown in Figure 4. This way we estimated that *C. consors* could have at least 168  
256 conopeptides: 27 with previously known sequence and 141 novel sequences. In addition, we list  
257 46 dubious sequences, which were only detected in the genome and did not have any closely  
258 related sequence in databases. These might be products of pseudogenes, products of wrongly  
259 predicted genes or peptides with other functions. However, it is not excluded that some of these  
260 "dubious" clusters might represent novel conopeptides. The superfamilies M, O1, and A  
261 comprise about 42% of all identified conopeptides in the *C. consors* (Table 1), which is in  
262 concordance with previously published data (Puillandre et al., 2012).



263

264 **Figure 4. Example of conopeptide gene clusters.** A subset of O1 superfamily gene clusters is  
265 shown. Red lines denote boundaries of gene clusters. Red dots indicate "dubious" genes, which  
266 show some similarity with conopeptides, but are not counted as conopeptide genes.

267

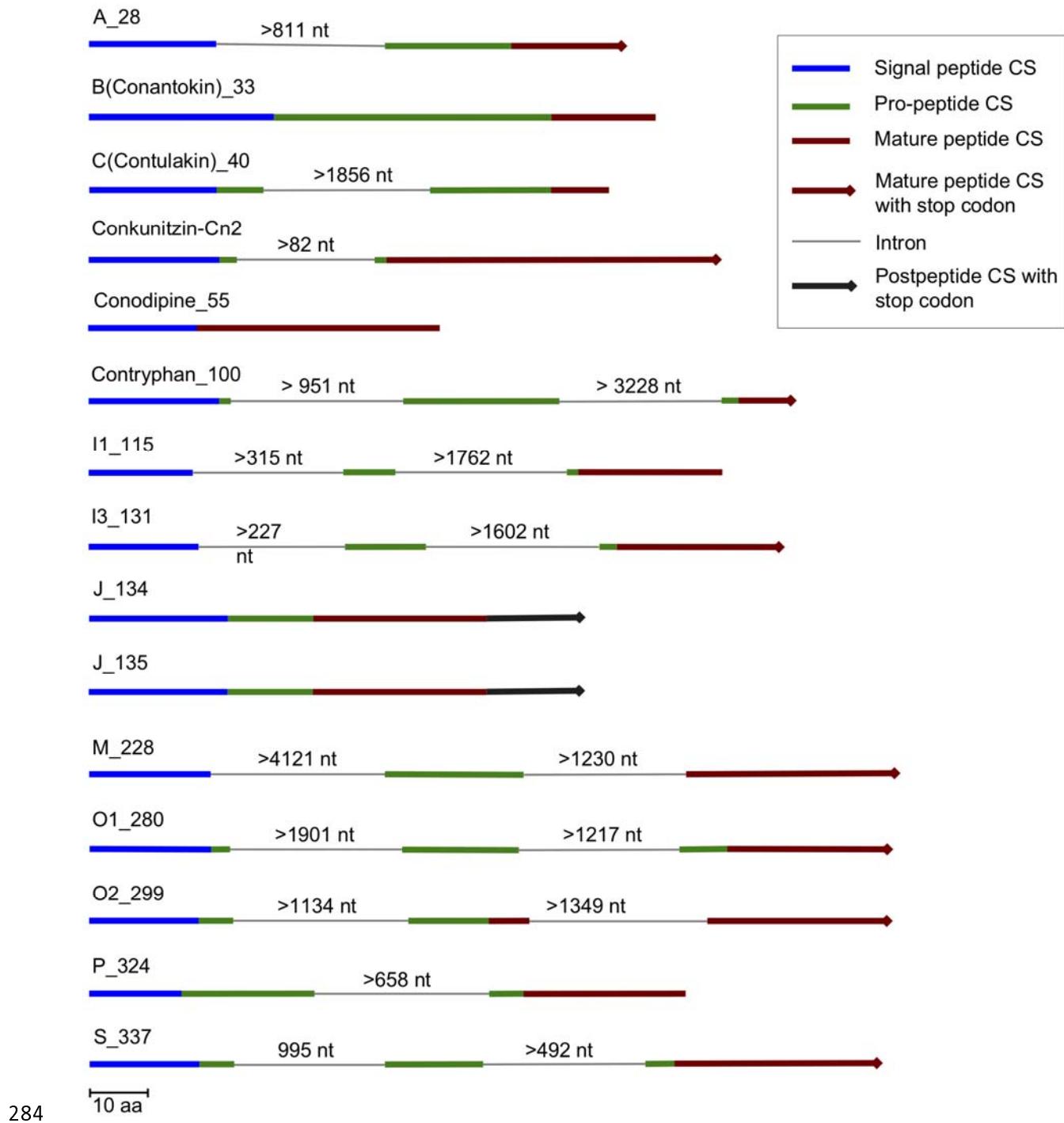
268 **Table 1.** Number of conopeptide genes predicted from the *C. consors* genome and  
269 transcriptomes, ordered by superfamilies.

Super-Family	Alignment	UniProt genes (present in our datasets)	Novel genes	Dubious genes	Total
A	A - CnI-like	5	0	0	5
	A - CcTx-like	2	10	0	12
	A - D4HPE6-like	1	3	8	12
B	B - Conantokin	0	2	1	3
	B - Linear conopeptide	1	1	0	2
C	C - Contulakin	0	2	3	5
	Conkunitzin	0	6	0	6
	ConoCAP	0	3	0	3
	Conodipine	1	3	2	6
	Conophysin	0	8	4	12
	Conoporin	1	13	0	14
I1	I1	0	5	0	5
I2	I2	0	3	0	3
I3	I3	0	2	1	3
J	J	0	4	1	5
K	K	0	3	2	5
M	M - CnIII-like	8	2	0	10
	M - Conomarphin-like	0	16	3	19
O1	O1 - CnVI-like	3	7	1	11
	O1 - CnVII-like	5	11	6	22
O2	O2	0	7	1	8
	O2 - Contryphan	0	1	3	4
O3	O3	0	7	2	9
P	P	0	5	1	6
S	S	0	9	1	10
T	T	0	7	5	12
V	V	0	1	1	2
<b>TOTAL:</b>		<b>27</b>	<b>141</b>	<b>46</b>	<b>214</b>

270

271 **Conopeptide genes in genome**

272 The majority of conopeptide superfamilies are known to contain introns that separate different  
273 functional domains (Olivera et al., 1999). The genome sequence allows us to identify the  
274 genomic structure of some conopeptide genes. Sequences that code for signal, propeptide, and  
275 mature peptide domains were retrieved for 15 conopeptides from 14 superfamilies (Figure 5). It  
276 is noteworthy that we can identify several different exon-intron organizations within the  
277 conopeptide genes. The first exon of the most abundant type encodes for the complete signal  
278 peptide sequence together with a variable length fragment of a pro-peptide, while the first exon  
279 of genes encoding type A, I1, I3, and M conopeptides encode the entire signal sequence. Pro-  
280 peptides appear to be encoded by one, two, or three different exons. Only conodipine genes are  
281 devoid of pro-peptide sequences. Finally, in the unique case of J-conopeptides, their genes  
282 appear to be made of a unique encoding exon containing, successively, a signal, an N-terminus  
283 pro- and a mature peptide, followed by a C-terminus pro-sequence.



284 **Figure 5. Conopeptide gene structures within the genome of *C. consors*.** Each sample  
285 represents one conopeptide gene. The peptide coding sequences (CS) for signal, pro- and mature  
286 peptides are represented by bold blue, green, and red lines. The length of each line is  
287

288 proportional to the number of amino acids. The introns are represented as thin grey lines and the  
289 length of the intron sequences is indicated in nucleotides above each line. The symbol '>'  
290 indicates that this gene was not assembled into a single contig and that the intron length is  
291 therefore not precisely known. Sequences of the conopeptide genes and additional information  
292 are available in Supplementary Data S3.

293

## 294 **Conclusions**

295 The annotation of a fish-hunting cone snail *C. consors* genome and transcriptome gives us a  
296 closer opportunity to peek into the complexity of its genes. The analysis of the combined eight  
297 different transcriptomic and genomic datasets resulted 17,715 full-length protein sequences. In  
298 addition, 168 conopeptide sequences were identified and in several cases the gene structures of  
299 conopeptide superfamilies were characterized. We have found several gene coding clusters that  
300 might represent novel conopeptides and are therefore good candidates for future studies.

301

## 302 **Supplemental information**

303 The following additional data are available with the online version of this paper. Supplemental  
304 Article S1 contains a detailed description of all supplementary analysis and methods.  
305 Supplemental Data S2 contains list of predicted RNA genes, clustered by RFam category.  
306 Supplemental Data S3 contains alignments of conopeptides from each superfamily. Gene and  
307 protein sequences predicted from transcriptome are included in Supplemental Data S4 as two  
308 separate FASTA format files.

309

310 **Authors' contributions**

311 MRe and RA were responsible for drafting the manuscript. MRo, LK, SL, TK, AB, VK, RA and  
312 MRe analyzed the sequencing data. All authors have read and approved the final manuscript.

313

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319

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327

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