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1 **Running title:** Local modulation in *R. prolixus* antennae

2 Transcriptomics supports local sensory regulation in the antenna of the kissing bug 3 *Rhodnius prolixus*

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11 **ABSTRACT**

12 *Rhodnius prolixus* has become a model for revealing the molecular bases of insect sensory biology due to
13 the publication of its genome, its well characterized behavioural repertoire and the advent of NGS
14 technologies. Gene expression modulation underlies behaviour-triggering processes at peripheral and
15 central levels. Still, the regulation of sensory-related gene transcription in sensory organs is poorly
16 understood. Here we study the genetic bases of plasticity in antennal sensory function, using *R. prolixus* as
17 an insect model. Antennal expression of neuromodulatory genes such as those coding for neuropeptides,
18 neurohormones and their receptors was characterized by means of RNA-Seq. New nuclear receptor and
19 *takeout* gene sequences were identified for this species, as well as those of enzymes involved in the
20 biosynthesis and processing of neuropeptides and biogenic amines. We report a broad repertoire of
21 neuromodulatory and endocrine genes expressed in antennae and suggest that they modulate sensory
22 neuron function locally. Diverse neuropeptide-coding genes showed consistent expression in the antennae
23 of all stages studied. Future studies should characterize the contribution of these modulatory components
24 acting over antennal sensory processes to assess the relative contribution of peripheral and central
25 regulatory systems on the plastic expression of insect behaviour.

26 Key words: antennae, transcriptomics, kissing-bugs, neuropeptides

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27 **1. INTRODUCTION**

28 *Rhodnius prolixus* has been an important insect model for neuroethological studies for many decades
29 (Barrozo et al., 2016; Wigglesworth and Gillett, 1934). Relevant aspects of its neuroethology, such as host
30 odour-mediated behaviour (Guerenstein and Lazzari, 2009; Manrique and Lorenzo, 2012), circadian
31 modulation (Lazzari, 1992), the action of biogenic amines and neuropeptides (Orchard, 2006) or the
32 expression of behavioural plasticity (Bodin et al., 2009a; Vinauger et al., 2016) have been thoroughly
33 studied. Recently, molecular processes related to sensory function have been characterized for *R. prolixus*,
34 such as the tissue-specific expression profiles of odorant receptor genes (Latorre-Estivalis et al., 2015a) and
35 related changes associated to development and nutrition (Latorre-Estivalis et al., 2015b). Additionally,
36 neuropeptide precursor genes were described for *R. prolixus* (Ons et al., 2009) and the dynamics of
37 neuropeptide expression or release at diverse physiological conditions were characterized for processes
38 such as feeding or ecdysis (Sterkel et al., 2011; Wulff et al., 2017). Based on the current knowledge on its
39 behaviour and physiology, and the recent publication of its genome sequence, it is reasonable to suggest
40 that *R. prolixus* has become an appropriate model for revealing the molecular bases of neuroethological
41 processes in insects. Furthermore, neuroethological research in kissing-bug insects is of medical interest
42 given their role as vectors of *Trypanosoma cruzi*, the causative agent of Chagas' disease, which is
43 considered a neglected disease affecting over 8 million people worldwide
44 (http://www.who.int/chagas/disease/en/).

45 Kissing-bug antennae are multimodal sensory organs dedicated to detect diverse stimuli associated to hosts
46 (Guerenstein and Lazzari, 2009), microenvironmental features and intraspecific communication (Barrozo et
47 al., 2016). The physiological bases of sensory processes sit on receptor neurons that express specific
48 membrane proteins that confer them an ability to react to specific stimuli present in the environment.
49 These neurons are mostly found in tiny hair-like structures called sensilla, which can house from one to
50 several dozen sensory cells (Carey and Carlson, 2011). In a recent study, the expression of sensory-receptor
51 coding genes was characterized in *R. prolixus* antennae by means of RNA-Seq (Latorre-Estivalis et al., 2017).
52 Therefore, the antennal expression of a large set of genes related to diverse stimulus transduction
53 processes was reported (chemoreceptors, odorant binding proteins - OBPs, chemosensory proteins - CSPs,

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54 transient receptor potential - TRP channels and pick pockets - PPKs receptors) (Latorre-Estivalis et al.,
55 2017).

56 The triggering of behaviors as a response to relevant external stimuli can be modulated at peripheral and
57 central levels (Gadenne et al., 2016). Insect behavior shows plasticity depending on age, physiological
58 status (i.e., phase of daily cycle, nutritional or reproductive status) and experience (Gadenne et al., 2016).
59 For instance, mature kissing-bugs seek host cues promptly, but they do not express proper host-seeking
60 behaviour during the first week after ecdysis (Bodin et al., 2009b) or after engorgement (Bodin et al.,
61 2009a). Electroantennography and single sensillum recordings performed on different insect species have
62 reported a high degree of physiological plasticity at sensory levels (Kromann et al., 2015; Qiu et al., 2006),
63 at least partially explaining behavioral changes triggered by feeding or development. Similar changes have
64 been documented at the molecular level, with altered gene expression associated to feeding (Bonizzoni et
65 al., 2011) or age (Bohbot et al., 2013). In fact, variations in gene expression depending on nutritional status
66 or development have been described for olfactory correceptors in the antennae of *R. prolixus* (Latorre-
67 Estivalis et al., 2015b). Nevertheless, information about elements regulating sensory gene transcription and
68 the abundance of the corresponding proteins in insect peripheral organs is very limited (Farhan et al., 2013;
69 Jung et al., 2013; Kwon et al., 2016; Lee et al., 2017). Physiological mechanisms modulating peripheral
70 responses to sensory stimuli involve signaling controlled by biogenic amines, hormones, and
71 neuropeptides, as well as their target G-protein coupled receptors (GPCRs) and nuclear receptors, overall
72 controlling the functional status of sensory processes (Gadenne et al., 2016). The main objective of this
73 study is to characterize modulatory components potentially involved in the local regulation of antennal
74 sensory function using *R. prolixus* as a model insect. For this purpose, we characterized the expression of a
75 diverse set of genes known for their neuromodulatory and endocrine roles in the antennae of 5th instar
76 larvae and adults of *R. prolixus* by means of RNA-Seq.

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77 **2. MATERIAL AND METHODS**

78 *2.1 Transcriptomic data analysis*

79 Read sequences and *de novo* assemblies were obtained from Latorre-Estivalis et al. (2017). In this study,
80 three antennal transcriptomes of unfed 21 day-old 5th instar larvae and female and male adults from *R.*
81 *prolixus* (colony originated from Honduras and held at the Centro de Pesquisas René Rachou – FIOCRUZ)
82 were obtained. A total of sixty antennae were collected per sample and used for RNA extraction for
83 subsequent RNA-Seq library preparation and sequencing as described in Latorre-Estivalis et al. (2017).
84 Briefly, sequencing was performed at the W. M. Keck Centre for Comparative and Functional Genomics
85 (University of Illinois at Urbana-Champaign, IL, USA) on an Illumina HiSeq2000 from both ends to a total
86 read length of 100 nucleotides. Read sequences were obtained from PRJNA281760/SRP057515 project at
87 NCBI, which contains data from the three conditions analysed: SRS923612/SRX1011796/SRR2001242
88 (antennal library from larvae); SRS923595/SRX1011769/SRR2001240 (antennal library from female adults);
89 and SRS923599/SRX1011778/SRR2001241 (antennal library from male adults). Reads were mapped to the
90 *R. prolixus* genome assembly (version RproC3) by means of STAR v.2.6.0 (Dobin et al., 2013) and an edited
91 genome GFF file. Raw read counts were used for differential expression analyses among stages and
92 between sexes using the edgeR (v3.6.8). The FDR adjusted p-value (False Discovery Rate) <0.1 was set as
93 threshold to define the significance level. Heat maps showing gene expression (expressed as Fragments Per
94 Kilobase Million - FPKM value +1 following by Log10 transformation) of the different protein families in the
95 conditions tested were prepared using the gplot package in R.

96 2.2 *Manual gene curation*

97 Manual curation of genome project databases by means of the inclusion and correction of gene models,
98 using transcriptomic data and published studies, is fundamental for increasing database quality. The use of
99 reliable genome databases, which need to be as complete and validated as possible, is especially relevant
100 for performing adequate quantitative transcriptomic and functional genetic studies. Most of the target
101 sequences curated herein were obtained from Ons et al. (2011); Ons et al. (2016); Ons (2017); Mesquita et
102 al. (2015); and Yeoh et al. (2017) (details in Supplementary Tables S1 and S2). Therefore, all sequences
103 were compared to the SOAPdenovo and Trinity generated antennal assemblies from Latorre-Estivalis et al.

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104 (2017). The discrepancies observed between target gene models from the *R. prolixus* genome (Gene set:
105 RproC3.3, available on 24 Oct 2017) and the transcripts from the *de novo* antennal assemblies are reported
106 in Supplementary Tables S1-S6. In the case of neuropeptide precursor and GPCRs genes that were manually
107 corrected/extended, new Generic Feature Format (GFF) files were created and included in the RproC3.3
108 version of the *R. prolixus* genome GFF file. In case of the other gene families, new gene models were
109 created only for those genes that were absent from the VectorBase gene prediction database or those
110 whose gene models were partially constructed. The modified GFF file of the genome was used for read
111 mapping. The protein sequences of all genes analysed and the edited GFF file are included in the
112 Supplementary Material (Database S1 and Database S2, respectively).

113 *2.3 Identification of new genes*

114 Orthologous sequences from *D. melanogaster* (Pauls et al., 2014; Velarde et al., 2006) were used in
115 tBLASTn searches in the *R. prolixus* genomic database (www.vectorbase.org) to identify nuclear receptor
116 genes and enzymes related to prepropeptide/preproprotein processing. Sequences of *takeout* (*to*) genes
117 previously annotated for *R. prolixus* (Mesquita et al., 2015) were used as query to search for new
118 sequences in the genome. Subsequently, all sequences were manually corrected/extended according to our
119 *de novo* antennal transcriptomes and annotated based on their phylogenetic relations to other insect
120 sequences. In addition, the structural characteristics of *to* genes, such as the presence of a signal peptide
121 (detected by means of SignalP 4.0 (Petersen et al., 2011)); of two conserved cysteine residues in the amino
122 terminal region implicated in disulfide bond formation and ligand binding (Touhara et al., 1993); and of two
123 conserved motifs (So et al., 2000) were confirmed in *R. prolixus to* sequences

124 *2.4 Phylogenetic analysis*

125 For building the phylogenetic trees, protein sequences of *R. prolixus* and other insect species were aligned
126 using G-INS-I strategy in MAFFT v.7 (mafft.cbrc.jp/alignment/server), and manually edited in Jalview v2.6.1.
127 Finally, maximum likelihood trees were built in PhyML v.3.0. Branch support was determined using the
128 approximate Likelihood Ratio Test (aLRT). Non-parametric branch support based on the Shimodaira-
129 Hasegawa-like (SH) procedure

130

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131 **3. RESULTS**

132 *3.1 Manual gene curation*

133 3.1.1 Neuropeptide and neurohormone precursor genes

134 A total of 17 neuropeptide precursor gene models that were absent from the RproC3.3 version of the *R.*
135 *prolixus* genome annotation were included in the genome GFF file (Supplementary Table S1). The long
136 neuropeptide F (LNPF) and orcokinin (OK) predictions were corrected according to Sedra and Lange (2016)
137 and Sterkel et al. (2012), respectively. The RYamide gene model was fixed based on our antennal
138 transcriptomes. Besides, IDLSRF-like peptide, glycoprotein hormones alpha-2 (GPA2) and beta-5 (GPB5),
139 and bursicon-beta (also known as partner of bursicon) genes were identified in the *R. prolixus* genome. A
140 new isoform of the *R. prolixus* adipokinetic hormone (AKH) gene, originated through alternative splicing,
141 was identified in the antennal assemblies (Supplementary Table S1). Both AKH isoforms share the signal
142 peptide and the active conserved peptide, but differ in the C-terminal region. Whereas the previously
143 reported isoform encodes the core peptide and a single spacer peptide, the isoform presented here
144 encodes the core peptide and two non-conserved spacer peptides. The gene models of eclosion hormone
145 (EH); ion transport peptide (ITP) isoform A; NVP-like; orcokinin-B; and orcokinin-C remained incomplete
146 because it was impossible to fix them due to problems in the genome assembly, e.g. some fragments were
147 located in the opposite strand or were absent from the genome assembly (Supplementary Table S1).

148 3.1.2 G-protein coupled receptors

149 Most of the biogenic amine-related GPCR gene models were edited (Supplementary Table S2). However,
150 many of these genes models are still incomplete. In the case of Family A neuropeptide receptor genes, a
151 total of 15 gene models based on Ons [40] were included in the GFF file of the *R. prolixus* genome
152 (Supplementary Table S2). Besides, 11 gene models of this receptor family were edited in the existing GFF
153 file of the genome. Two isoforms (alfa and beta) of the Corazonin (CZ) receptor gene were described
154 Hamoudi et al. (2016). Nevertheless, our antennal transcriptome only presented the alfa isoform (GenBank
155 Acc. N° AND99324). A second kinin receptor (previously described as an orphan receptor by Ons et al.
156 (2016)) and a Tachykinin 86C-like receptor were identified. Most of the Family B neuropeptide receptor
157 gene models were also fixed (Supplementary Table S2). A phylogenetic tree was built to annotate both the

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158 calcitonin-like (CT) and the corticotropin-releasing factor-related like (CRF) diuretic hormone (DH) receptors
159 (Supplementary Fig. 1S). Two CT/DH-like receptors were previously described in *R. prolixus* by Zandawala et
160 al. (2013): receptor 1 and receptor 2, the ortholog of *D. melanogaster hector* gene (FlyBase Acc. Number
161 CG4395). The resulting phylogenetic tree suggested that a third CT/DH like-receptor previously described
162 by Ons et al. (2016) seems to be exclusive of heteropteran insects (Supplementary Fig. S1). The CRF/DH-like
163 receptors 1 and 2 (including isoforms 2A and 2B) were grouped in a different clade as shown in Zandawala
164 et al. (2013).

165 3.1.3 Biogenic amine biosynthesis enzymes

166 All enzymes known to mediate biogenic amine biosynthesis in other insects were annotated in the last
167 version of the *R. prolixus* genome (Mesquita et al., 2015); however, minor changes would be needed to fix
168 some of them (Supplementary Table S3). These models include: 1) Tyrosine 3-monooxigenase (ple), which
169 synthesizes dopamine from L-tyrosine; 2) DOPA decarboxylase (Ddc), involved in the synthesis of dopamine
170 from L-DOPA; 3) Tyrosine decarboxylase-2 (Tdc2), which participates on synthesis of tyramine from L-
171 tyrosine and; 4) Tryptophan hydroxylase (Trh), which synthesizes serotonin from L-tryptophan.

172 3.1.4 Neuropeptide processing enzymes

173 The neuropeptide processing enzymes were not previously annotated in the *R. prolixus* genome (Mesquita
174 et al., 2015). Using sequences from *Drosophila* as queries, we were able to identify a total of 9 enzyme
175 genes that seem to correspond to *R. prolixus* orthologues (Supplementary Table S4). The processing of
176 neuropeptides involves the following enzymes: 1) signal peptidase (SP), which cleaves the signal peptides
177 from their N-terminals; 2) three members of the furin subfamily (dFUR1, dFUR2a and dFUR2b), which are
178 Subtilisin-like endoproteases that cleave the propeptide at monobasic (Arg) and dibasic (Arg-Arg/Lys-Arg)
179 sites; 3) prohormone convertase 2 (*amontillado* or PC2), which cleaves mono (Arg) and dibasic (Arg-Arg;
180 Lys-Arg; Arg-Lys; Lys-Lys) sites; 4) the carboxypeptidase M (two new isoforms were identified in the
181 antennal assemblies with differences in the 3´ region) and D (known as *silver*, which trims C-terminal Arg
182 and Lys after Furins/PC2 cleavage reaction); 5) the PHM (Peptidylglycine alfa-hydroxylating mono-
183 oxygenase) amidating enzyme, which is responsible for the alpha-amidation of the peptide C-terminal; 6) a
184 prolyl endoprotease belonging to the Peptidase 9 protein family, for which no functional information is

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185 available for insects (Supplementary Table S4); 7) the amidating enzymes, the peptidyl alfa-hydroxyglycine
186 alfa-amidating lyase (PAL) 1 and 2.

187 3.1.5 Nuclear receptors

188 The ecdysone receptor (*Eip75B*) gene was the only annotated nuclear receptor in the *R. prolixus* genome
189 so far (Mesquita et al., 2015); however, no information about isoforms was included in the annotation. In
190 the antennal assemblies, the sequence of the *RproEip75B* was identified using *DmelEip75B* and posteriorly
191 compared to the VectorBase prediction. This comparison allowed correcting the VectorBase prediction and
192 identifying it as isoform A (by means of the two distinctive exons in the N-terminal-region) and the antennal
193 sequence as isoform B (with the first exon located in the second intron of the A isoform)(Segraves and
194 Hogness, 1990). Besides *RproEi75B*, a total of 20 nuclear receptor genes were identified (Supplementary
195 Table S5) and annotated based on their phylogenetic relations to those of *Cimex lectularius*; *Pediculus*
196 *humanus*; and *D. melanogaster* nuclear receptor sequences (Supplementary Fig. S2). The orthologues of
197 *D. melanogaster eagle* and hormone receptor like-83 genes were not identified either in the *R. prolixus,*
198 *C. lectularius* or *P. humanus* genomes (Supplementary Fig. S2).

199 3.1.6 *takeout* genes

200 Three *takeout* (*to*) genes had been previously identified in the *R. prolixus* genome: *to1* (RPRC010098); *to2*
201 (RPRC002313); and *to3* (RPRC01009) (Mesquita et al., 2015). A total of 12 new *to* gene sequences were
202 identified in our assemblies (Supplementary Table S6) and annotated based on their phylogenetic relations
203 (Figure 4). Considering this analysis, RPRC002313 and RPRC010096 were annotated as *to6* and *to2*,
204 respectively. *R. prolixus to* genes were separated into two different clades: *to1-to9* and *to10-to15*. All the
205 structural characteristics of *to* genes were identified in *R. prolixus to* sequences: presence of signal peptide;
206 two conserved cysteine residues in the N-terminal region and two conserved motifs (So et al., 2000). As
207 expected, the length of all *to* sequences was close to 250 amino acids (Supplementary Fig. S3). Finally, it
208 was observed that 11 out of 15 *to* genes clustered in KQ034137 and KQO34102 supercontigs, with 8 and 3
209 genes each (Supplementary Fig. S4).

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210 *3.2 Antennal expression profiles*

211 3.2.1 Neuropeptide and neurohormone precursor genes

212 A total of 31 neuropeptide precursor genes were found to be expressed in *R. prolixus* antennae,
213 considering a value of >1 Fragments Per Kilobase Million (FPKM) in at least one library as an exclusion
214 threshold (see Supplementary Database 3). Fifteen out of 44 *R. prolixus* neuropeptide genes showed FPKM
215 values higher than 10 in at least one library. Allatostatin-CC (AstCC), allatostatin-CCC (AstCCC), ITG-like,
216 IDLSRF-like peptide and OK were the most highly expressed neuropeptide genes in the antennae of *R.*
217 *prolixus* (Fig. 1a and Supplementary Database 3). The gene encoding for AstCC was the one showing highest
218 expression in our database, especially in larval antennae (larvae FPKM value = 888; female FPKM value =
219 98.5 and male FPKM value = 55). Indeed, the lower expression of this gene in male antennae was
220 statistically significant (FDR<0.05) when compared to that observed in larval antennae (Table S7). For AstA
221 and myoinhibitory peptide (MIP), a significant lower expression (FDR<0.05) was also observed in the
222 antennae of both adult stages when compared to larvae (Table S7). The antennal expression of allatotropin
223 (AT); OK and IDLSRF-like peptide seems to increase after imaginal moult (Fig. 1a). The expression reported
224 for OK; Dh31; CAPA; AKH and ITP is the sum of their different isoforms or splicing variants.

225 3.2.2 GPCRs

226 Data suggest that more than half of Family A neuropeptide receptor genes (25 out of 38 genes) were
227 expressed in the antennae (FPKM values >1 in at least one library; Supplementary Database 3). Crustacean
228 cardioactive peptide (CCAP) receptor 1; NPF receptor 1; ITP; GPA2/GPB5 receptor and RFamide peptide
229 receptor were the most highly expressed Family A receptor-coding genes (Fig. 1b and Supplementary
230 Database 3). The expression of the AKH receptor was significantly lower (FDR=0.06) in females, as
231 compared to larval, antennae (Table S7). Interestingly, the expression of kinin receptor 2 increased
232 significantly in the antennae of adults (FDR=0.058 and FDR=0.014 for female and male, respectively; Fig.1b
233 and Table S7). The antennal expression reported for ACP/CZ related peptide, Capability (CAPA) and CZ
234 receptors, as well as for Pyrokinin receptor 2 was the sum of their different isoforms.

235 In the case of Family B neuropeptide receptor genes, only calcitonin-like diuretic hormone (CT-DH) receptor
236 2 showed FPKM values lower than 1 (Supplementary Database 3). Five out of seven receptor genes

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237 belonging to this family presented FPKM values higher than 10 in at least one library (Supplementary
238 Database 3). CT/DH receptor 3, which according to our phylogenetic analysis seems to be exclusive of
239 heteropterans, showed the highest expression for this family. In fact, its expression showed a significant
240 increase in the antennae of adults (FDR=0.0978 and FDR=0.041 for female and male, respectively) when
241 compared to those from larvae (Fig. 1b and Table S7). A similar expression pattern was observed for CT/DH
242 receptor 1 gene (isoforms B and C included) and for the corticotropin releasing factor like diuretic hormone
243 (CRF/DH) receptor 2 (isoforms A and B included) (Fig. 1b). Regarding opsin expression, transcripts of UV
244 opsin and long wave sensitive opsin 1 (LWS1) were detected in all three libraries (Fig. 1b).

245 3.2.3 Tyrosine kinase and guanylyl cyclase type receptors

246 The neuropeptide-like precursor 1 (NPLP1) putative receptor (tyrosine kinase-type) and the potential
247 neuroparsin (guanylyl cyclase receptor) seem to be expressed in the antennae of *R. prolixus* (Fig. 1b).

248 3.2.4 Neuropeptide processing enzymes

249 All enzymes involved in neuropeptide processing, except prohormone convertase 1, seem to be expressed
250 in the antennae of *R. prolixus*, presenting values higher than 10 FPKM in at least one library (Supplementary
251 Table S7). The peptidyl-amidating monooxigenase, signal peptidase and furin-like protease 1 genes showed
252 the highest expression (Fig. 1c).

253 3.2.5 Biogenic amine related genes

254 Expression of at least 16 out of 20 biogenic amine receptor genes was detected in the antennae of
255 *R. prolixus* (FPKM value >1 in at least one library). Dopamine ecdysone receptor, muscarinic acetylcholine
256 receptor type C; orphan receptor 1; serotonin receptors 1b and 2b presented the highest antennal
257 transcription within this group (Fig. 2a and Supplementary Database 3). The expression of the octopamine
258 (Oct) beta receptor 3 showed a significant increase (FDR=0.071) in male antennae compared to larvae (Fig.
259 2a), while octopamine beta receptors 1 and 2 showed a similar trend.

260 All genes encoding for enzymes involved in the biosynthetic pathway of biogenic amines were detected in
261 the antennae of *R. prolixus* (Fig. 2b). The gene that encodes for Tyrosine 3-monoxigenase, which
262 synthesizes DOPA from L-tyrosine, was the most highly expressed of this enzyme group (Fig. 2b).

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263 3.2.6 Nuclear receptor genes

264 Ecdysone-induced protein 75, hepatocyte nuclear factor 4, hormone receptor-like in 96 and *ultraspiracle*
265 were the genes with the highest expression, with FPKM values >10 in the three libraries (Fig. 3;
266 Supplementary Database 3). The expression of hormone receptor-like in 3 increased significantly after
267 imaginal moult in male antennae (FDR= 0.017; Table S7). Six nuclear receptor genes had no expression
268 (FPKM value < 1 in the three libraries) in the *R. prolixus* antennal transcriptomes, these were:
269 *Dissatisfaction*; Ecdysone-induced protein (EIP) 78C; Hormone receptor (HR) like in 51; *Knirps-like2*; *Tailless*
270 and *Seven up* (Fig. 3; Supplementary Database 3).

271 3.2.7 *takeout* genes

272 These genes were highly expressed in *R. prolixus* antennae (Fig. 4), 6 out 15 presenting FPKM values higher
273 than 1000 in at least one library (Supplementary Database 3). While most *to* genes tended to present an
274 increased expression in adult antennae, a few seemed to follow the opposite pattern. For example, *to11*
275 gen showed a significant decrease after imaginal molt (FDR <0.05 in both sexes; Table S7), while *to2*,
276 decreased its expression significantly only for male adults (FDR=0.012; Table S7). Nevertheless, the
277 expression of *to3* showed a significant increase in both adult stages after molting (FDR<0.05; Table S7), and
278 those of *to4*, *to7*, *to8*, *to10*, *to12*, *to14* and *to15* followed a similar profile. The genes included in the clade
279 of *to1-to9* tended to present higher expression level in antennae.

280 **4. DISCUSSION**

281 The molecular bases of sensory plasticity at the local antennal level have been sparsely analysed (revised by
282 Gadenne et al. 2016). Our study has characterized the expression profile of a diverse set of genes encoding
283 different modulatory elements (neuropeptides, GPCRs, nuclear receptors and *takeout* genes) in the
284 antenna of *R. prolixus*. The antennal transcription of a broad repertoire of these genes suggests that diverse
285 local systems may be dedicated to the modulation of antennal functions, such as the detection of host cues
286 and communication signals (Barrozo et al., 2016).

287 Our results have proven that neuropeptide gene transcripts are produced in the antennae of kissing-bugs (a
288 total of 31 neuropeptide genes seem to be expressed). The production of neuropeptide gene transcripts
289 has already been reported in the antennae of a few insect species (Jung et al., 2013; Matthews et al., 2016;

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290 Rinker et al., 2013). The expression of neuropeptide processing enzyme genes was also detected in bug
291 antennae, and as far as we know, this is the first report on the expression of this type of enzyme-coding
292 genes in insect antennae (Figure 1c). The results presented herein add evidence supporting the antennal
293 production of neuropeptides. However, immunohistochemistry and microscopy experiments would be
294 necessary to identify the types of cells producing neuropeptide transcripts in insect antennae. The
295 presence of neurosecretory cells in insect antennae has only been described for mosquitoes (Meola et al.,
296 2000). The authors showed that these cells form synaptoid sites on the dendrites of sensory neurons
297 (Meola and Sittertz-Bhatkar, 2002; Meola et al., 2000).

298 In recent years, modulatory action by different neuropeptides have been shown for both antennal and
299 labellar chemosensory neurons (Farhan et al., 2013; Jung et al., 2013; Kwon et al., 2016; Lee et al., 2017).
300 Nevertheless, the source of these neuropeptides, whether local or central, was not reported. The current
301 study shows that *R. prolixus* antennae produce a diversity of neuropeptide-coding transcripts, among them
302 high levels of AstCC and ITG-like peptide transcripts in all three libraries (Fig. 1a). Functional RNAi or
303 CRISPR/CAS9 studies should be performed in order to elucidate their role. Orcokinin and IDLSRF-like
304 peptide presented increased antennal expression after the imaginal moult, suggesting that these peptides
305 may modulate adult-specific sensory processes underlying dispersion by flight and mating in kissing-bugs.
306 On the other hand, the decreased antennal expression of AstA and MIP in adults, when compared to 5th
307 instar larvae, suggests an augmented role in immature instars. Instar-specific functional studies with both
308 allatostatins and orcokinin will be necessary in order to understand their antennal function. The
309 significantly lower expression of AstCC in male antennae may suggest a sex-specific antennal role.

310 The expression of 33 out of 49 neuropeptide and neurohormone receptor genes (FPKM value >1,
311 Supplementary Database 3), the other fundamental component of the neuropeptidergic system, suggests
312 that diverse local regulatory processes can react to a similarly complex set of modulatory signals. Indeed,
313 14 neuropeptides/neurohormones and their corresponding receptors presented expression higher than 1
314 FPKM value in at least two conditions (Table 1), reinforcing that parallel local regulatory systems may
315 modulate diverse components of antennal sensory function. The expression of neuropeptide receptor

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316 genes in antennae has been already described in other insects (Matthews et al., 2016; Rinker et al., 2013).
317 The high expression shown in all conditions by LNPF receptor 1, GPA2/GPB5 receptor (also known as
318 leucine-rich repeat-containing G protein-coupled receptor 1 - LGR1) and CT/DH receptor 1 (Fig. 1b),
319 suggests important regulatory roles on antennal function. Interestingly, a LNPF-based system modulates
320 responsiveness to food odours of a specific class of OSN in *D. melanogaster* (Lee et al., 2017). Whether this
321 could also be the case for OSNs in *R. prolixus* antennae deserves consideration. The significantly augmented
322 expression of CT/DH receptor 3 and Kinin receptor 2 observed in the antennae of adults (Fig. 1b and
323 Supplementary Table S7) suggests a regulatory function of adult-specific sensory processes. A similar
324 increased adult expression profile was previously observed in the antennae of *R. prolixus* for several
325 chemoreceptors (Latorre-Estivalis et al., 2017). Therefore, it would be interesting to study whether these
326 are functionally connected in the adult phase. The significant decrease observed on the expression of the
327 AKH receptor gene in female antennae may suggest a relation to the modulation of pheromone perception
328 and production as observed for *D. melanogaster* in a sex-specific and starvation dependent manner
329 (Lebreton et al., 2016). Again, it would be interesting to analyse its functional role in kissing-bugs.

330 Peripheral effects of biogenic amines and their antennal production in insects have been reviewed by
331 Zhukovskaya and Polyanovsky (2017). As observed for neuropeptides (Jung et al., 2013; Kwon et al., 2016;
332 Lee et al., 2017), the modulation of chemosensation and other sensory modalities by biogenic amines
333 (Andres et al., 2016; Inagaki et al., 2012) depends on their levels (Zhukovskaya and Polyanovsky, 2017), as
334 well as the abundance of their receptors (McQuillan et al., 2012). Actually, *in situ* hybridization allowed
335 detecting octopamine and tyramine receptor gene transcripts in the vicinity of sensory receptor neurons of
336 different insects (Jung et al., 2013; Kutsukake et al., 2000). Furthermore, the presence of dopamine
337 ecdysone receptor has been shown for the labellar cells expressing Gr5 in *D. melanogaster* (Inagaki et al.,
338 2012). This supports the existence of direct modulatory effects of biogenic amines on peripheral sensory
339 processes. Biogenic amines such as octopamine have been proposed to directly affect signal transduction
340 and spike generation on OSNs (Grosmaitre et al., 2001). Consistent with these findings, a diverse set of
341 transcripts of biogenic amine receptors was identified in the antennal transcriptome of *R. prolixus* (a total
342 of 16 biogenic amine receptor genes seem to be expressed in them) and in those from other insects

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343 (Farhan et al., 2013; Matthews et al., 2016; Rinker et al., 2013). As observed for neuropeptides, most of the
344 genes coding for enzymes involved in the biosynthesis of biogenic amines seem to be expressed in *R.*
345 *prolixus* antennae (Fig. 2b). Serotonergic nerve fibres innervate the antennae of mosquitoes (Siju et al.,
346 2008) which could relate to the high antennal expression observed for 5-HT receptors in *R. prolixus*, (Fig.
347 2a). The dopamine ecdysone receptor, which binds dopamine and ecdysone, showed a high expression on
348 adult antennae, especially in those from males (Fig. 2a). Interestingly, this receptor modulates sex
349 pheromone sensitivity in the antennal lobe of male moths (Abrieux et al., 2014). Our results suggest a
350 similar modulation could also occur at peripheral level in *R. prolixus* male antennae. Octopamine receptors
351 may also have a modulatory role on male sensory processes, as they showed increased expression, this
352 being significant in the case of beta receptor 3, in the antennae of male adults (Fig. 2a; Supplementary
353 Table S7). A role of octopamine receptors in the modulation of male sensory physiology was observed in
354 male moths in which this molecule enhances OSN sensitivity to specific sexual pheromone components
355 (Grosmaitre et al., 2001).

356 Hormonal regulation on insect sensory systems has been poorly studied at the peripheral organs (Bigot et
357 al., 2012). Here we show that most described nuclear receptors are expressed in the antennae of an insect
358 (Fig. 3 and Supplementary Database S3), suggesting that these organs have broad capacity to respond to
359 endocrine signals. It is worth mentioning that *Eip75B* and hepatocyte nuclear factor 4 (*Hnf4*) genes are the
360 most expressed nuclear receptor in *R. prolixus* antennae (Fig. 3). Considering ecdysteroid signalling, the
361 detection of *Eip75B* transcripts indicates a potential capacity of kissing-bug antennae to respond to the EcR-
362 *USP* complex (Ecdysone receptor + *Ultraspiracle*), as observed for *Spodoptera litoralis* (Bigot et al., 2012).
363 Besides, *Eip75B* and hormone receptor-like in 51 transcripts (also known as *unfulfilled*) have been identified
364 in central clock cells of *D. melanogaster* and control the expression of clock genes, playing an important
365 role in the maintenance of locomotor rhythms (Jaumouillé et al., 2015; Kumar et al., 2014). Therefore, we
366 suggest that these nuclear receptors may have a similar regulatory role at the periphery, considering that
367 the presence of a peripheral circadian clock has been reported for insect antennae (Tanoue et al., 2004).
368 The *Hnf4* gene, which induces the expression of enzymes that drive lipid mobilization and β-oxidation as a
369 response to starvation in *D. melanogaster* (Palanker et al., 2009), also showed high expression in antennae.

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370 The relatively low nutritional status of the insects used in our studies could relate to its high expression in
371 *R. prolixus* antennae. Functional studies would need to be performed in order to evaluate the potential role
372 of this gene as a nutritional sensor in insect antennae. An increased expression of the hormone receptor-
373 like in 3, which is the heterodimer partner of Eip75B, in male specimens suggest a sex-specific role in
374 antennae.

375 Fifteen *takeout* genes were identified in the *R. prolixus* genome, while Ribeiro et al. (2014) identified 18
376 potential takeout transcripts in a midgut transcriptome of this species and Marchant et al. (2016) identified
377 25 takeout transcripts in the transcriptome of the kissing-bug *Triatoma brasiliensis*. Consistently, these
378 numbers match the scale of those found in *Anopheles gambiae* (10); *Acyrthosiphon pisum* (17); and *Bombyx*
379 *mori* (14) genomes (Vanaphan et al., 2012). *R. prolixus to* genes present a cluster organization
380 (Supplementary Fig.4S), probably due to gene duplication events, as it was previously observed in other
381 insects (Vanaphan et al., 2012). The antennal expression of *takeout* genes has already been reported in
382 Dipterans (Bohbot and Vogt, 2005; Sarov-Blat et al., 2000). Furthermore, it has been shown that starvation
383 induces the expression of these genes (Sarov-Blat et al., 2000) that have also been related to foraging
384 activity (Meunier et al., 2007). This putative function could explain the high expression observed in the
385 three antennal libraries (Fig. 4), however, functional studies need to be performed to be able to confirm
386 these roles in the antennae of kissing-bugs. Two *to* genes presented significant differences between larval
387 and adult antennal transcriptomes (*to11* and *to3,* with an up and downregulation, respectively) and *to2* is
388 significantly down-regulated when male antennae are compared to those of larvae (Supplementary Table
389 S7). Results suggest that these *to* genes may be related to sex, as observed in *D. melanogaster* (Dauwalder
390 et al., 2002) but experiments are necessary to test this hypothesis.

391 Antennal cells are bathed by haemolymph but not so the dendrites of sensory neurons (bathed by sensillar
392 lymph).Therefore, it is certain that central signals, i.e., circulating hormones, biogenic amines and
393 neuropeptides can modulate the function of most cells in insect antennae (Gadenne et al., 2016). However,
394 the antennal detection of neuropeptide transcripts (and those of enzymes involved in their biosynthesis
395 and that of biogenic amines) suggests the existence of local regulatory systems that could represent
396 additional sources of modulation of the sensitivity of peripheral neurons. Future RNA-seq, peptidomics, in

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397 *situ* hybridisation and other functional genetic experiments should test whether these regulatory
398 components are also present in the antennae of other insects and unveil the interaction between central
399 and peripheral regulatory systems to understand their relative contribution to the control of antennal
400 sensory physiology.

401 **ACKNOWLEDGEMENTS**

402 The authors wish to thank the Program for Technological Development in Tools for Health-PDTIS-FIOCRUZ
403 for having facilitated the use of its facilities. We wish to thank Ivana Helena Rocha Oliveira for her help in
404 processing samples used in our experiments, Prof. Claudio Lazzari for kindly providing comments on an
405 earlier version of the manuscript, and Dr. Gabriel da Rocha Fernandes and Fausto Gonçalves dos Santos for
406 their help and support in the bioinformatic analysis.

407 **SPONSORSHIPS**

408 Authors are indebted to INCTEM (Project number: 573959/2008-0), FAPEMIG (Project number: APQ-01359-
409 11), PROEP-FIOCRUZ (Project number: 401973/2012-3), CNPq (Project number: 483805/2013-0), Le
410 Studium for a granting research fellowship to M.G.L (Short Term Contract of Employment N° 2017-2001-
411 179 - Y17F16), FIOCRUZ Visiting researcher fellowship program (fellowship to J.M.L.E 550017/2015-17),
412 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) postdoctoral fellowship (fellowship to
413 J.M.L.E 2015-2017) and Agencia Nacional de Promoción Científica y Tecnológica (Project number: PICT
414 2016-3103). J.M.L.E., M.S and S.O are researchers from CONICET.

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587 **LEGENDS**

588 **Figure 1 - Heat map comparing the expression levels of (a) neuropeptide precursor genes, (b) G protein-**
589 **coupled receptor genes, and (c) neuropeptide processing enzymes in the antennae of *R. prolixus* larvae**
590 **(L), female (F) and male (M) adults.**

591 Expression levels (displayed as Log10 FPKM +1) represented by means of a colour scale, in which blue/red
592 represent lowest/highest expression. Abbreviations: R, receptor; H, hormone. The complete names of
593 neuropeptide precursor genes, their receptors and enzymes are detailed in Supplementary Table S1-3.

594 **Figure 2 - Heat map comparing antennal expression levels of *R. prolixus* genes coding for putative (a) BA-**
595 **detecting GPCRs and for (b) enzymes involved in BA synthesis in the antennae of larvae (L), female (F)**
596 **and male (M) adults.**

597 Expression levels (displayed as Log10 FPKM +1) represented by means of a colour scale, in which red/red
598 represent lowest/highest expression. Abbreviations: AC, acetylcholine; R, receptor; Dop, Dopamine, M-Ach,
599 Muscarinic Acetylcholine; Oct, Octopamine; Tyr, Tyramine; Ser, Serotonine; AADC, Amino acid
600 decarboxylase. Complete names of biogenic amine receptors and enzymes are detailed in Supplementary
601 Table S4-5.

602 **Figure 3 - Heat map comparing the expression levels of *R. prolixus* nuclear receptors in the antennae of**
603 **larvae (L), female (F) and male (M) adults.**

604 Expression levels (displayed as Log10 FPKM +1) represented by means of a colour scale, in which blue/red
605 represent lowest/highest expression. Abbreviations: R, receptor; Eip, Ecdysone-induced protein; TF,
606 transcription factor; NF, nuclear factor; HR, hormone receptor; PNR, photoreceptor-specific nuclear
607 receptor. Complete names of these genes are detailed in Supplementary Table S6.

608 **Figure 4 - Heat map comparing the expression levels of *R. prolixus takeout* (*to*) genes in the antennae of**
609 **larvae (L), female (F) and male (M) adults.**

610 Expression levels (displayed as Log10 FPKM +1) represented by means of a colour scale, in which blue/red
611 represent lowest/highest expression. The evolutionary history of R. *prolixus takeouts* was inferred by using
612 the Maximum Likelihood method in PhyML v3.0. The support values on the bipartitions correspond to SH-

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613 like P values, which were calculated by means of aLRT SH-like test. The LG substitution amino-acid model

614 was used

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Table 1 - Antennal expression (represented as Fragments Per Kilobase Million - FPKM - values) of neuropeptides and their corresponding receptors with FPKM values higher than 1 in at least two of the analysed conditions. Complete names are detailed in Supplementary Table S1 and S2.

<table>
<tr><th>Neuropeptide</th><th>Larvae</th><th>Female</th><th>Male</th><th>Receptor</th><th>Larvae</th><th>Female</th><th>Male</th></tr>
<tr><td>CCAP</td><td>11.3</td><td>9.8</td><td>4.5</td><td>CCAP r</td><td>12.1</td><td>9.8</td><td>14.1</td></tr>
<tr><td rowspan="2">Dh31</td><td rowspan="2">3.4</td><td rowspan="2">3.3</td><td rowspan="2">4.8</td><td>CT/DH r1</td><td>41.8</td><td>81.1</td><td>116.7</td></tr>
<tr><td>CT/DH r3</td><td>7.5</td><td>79.3</td><td>131.6</td></tr>
<tr><td rowspan="2">Dh44</td><td rowspan="2">7.7</td><td rowspan="2">10.7</td><td rowspan="2">12.4</td><td>CRF/DH r1</td><td>6.5</td><td>6.1</td><td>10.9</td></tr>
<tr><td>CRF/DH r2</td><td>4.1</td><td>14.8</td><td>29.2</td></tr>
<tr><td>GPA2</td><td>10.5</td><td>14</td><td>17.5</td><td rowspan="2">GPA2/GPB5 r</td><td rowspan="2">87.9</td><td rowspan="2">54.1</td><td rowspan="2">32.5</td></tr>
<tr><td>GPB5</td><td>1.37</td><td>1.06</td><td>1.02</td></tr>
<tr><td rowspan="2">LK</td><td rowspan="2">8.7</td><td rowspan="2">7.2</td><td rowspan="2">6</td><td>Kinin r 1</td><td>1.3</td><td>1.5</td><td>2.2</td></tr>
<tr><td>Kinin r 2</td><td>0.7</td><td>7.4</td><td>14.1</td></tr>
<tr><td>ITP</td><td>7.5</td><td>20.1</td><td>12</td><td>ITP r</td><td>11.3</td><td>5.2</td><td>5.9</td></tr>
<tr><td>LNPF</td><td>2.5</td><td>5.1</td><td>2.6</td><td>LNPF r1</td><td>13.5</td><td>31.8</td><td>39.8</td></tr>
<tr><td>Ntl</td><td>2.4</td><td>2.3</td><td>2.1</td><td>Ntl r</td><td>1.7</td><td>2.9</td><td>5.4</td></tr>
<tr><td>NP</td><td>5.1</td><td>0.7</td><td>11.5</td><td>NP r</td><td>6.2</td><td>1.6</td><td>8.2</td></tr>
<tr><td>NPLP1</td><td>7.7</td><td>7.1</td><td>7.9</td><td>NPLP r</td><td>1.3</td><td>0.7</td><td>1.3</td></tr>
<tr><td>PDF</td><td>9.6</td><td>5.8</td><td>8.1</td><td>PDF r</td><td>1.2</td><td>1.7</td><td>6.1</td></tr>
<tr><td>RYa</td><td>3.9</td><td>8.9</td><td>8</td><td>RYa r</td><td>3.25</td><td>3.2</td><td>3.87</td></tr>
<tr><td>sNPF</td><td>0.2</td><td>2.5</td><td>1.9</td><td>sNPF r</td><td>5.3</td><td>1.6</td><td>1.5</td></tr>
<tr><td rowspan="2">TK</td><td rowspan="2">3.8</td><td rowspan="2">8.1</td><td rowspan="2">4.8</td><td>TK 86C-like r</td><td>2.1</td><td>7.2</td><td>5.8</td></tr>
<tr><td>TK 99D-like r</td><td>0.2</td><td>1.3</td><td>1.4</td></tr>
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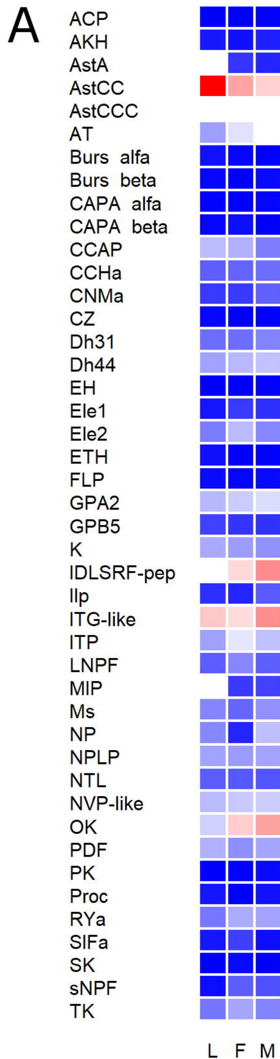
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AKH
AstA
AstCC
AstCCC
AT
Burs alfa
Burs beta
CAPA alfa
CAPA beta
CCAP
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CNMa
CZ
Dh31
Dh44
EH
Ele1
Ele2
ETH
FLP
GPA2
GPB5
K
IDLSRF-pep
Ilp
ITG-like
ITP
LNPF
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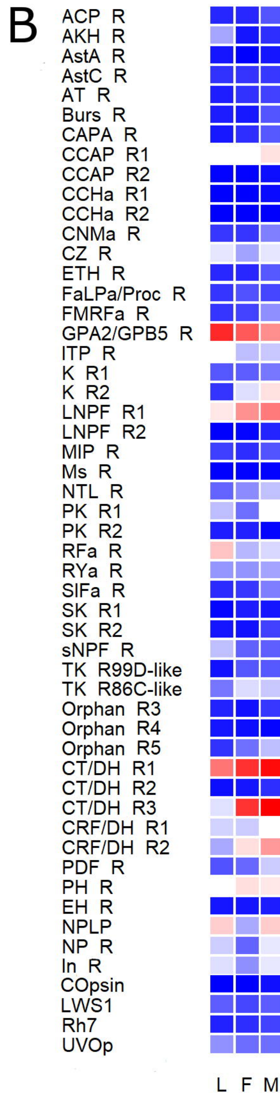
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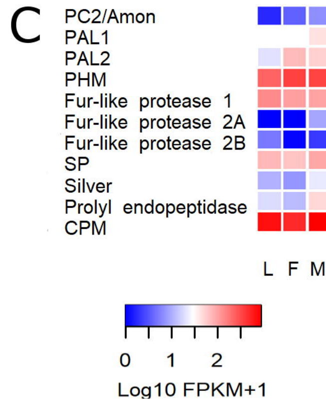
AKH R
AstA R
AstC R
AT R
Burs R
CAPA R
CCAP R1
CCAP R2
CCHa R1
CCHa R2
CNMa R
CZ R
ETH R
FaLPa/Proc R
FMRFa R
GPA2/GPB5 R
ITP R
K R1
K R2
LNPF R1
LNPF R2
MIP R
Ms R
NTL R
PK R1
PK R2
RFa R
RYa R
SIFa R
SK R1
SK R2
sNPF R
TK R99D-like
TK R86C-like
Orphan R3
Orphan R4
Orphan R5
CT/DH R1
CT/DH R2
CT/DH R3
CRF/DH R1
CRF/DH R2
PDF R
PH R
EH R
NPLP
NP R
In R
COpsin
LWS1
Rh7
UVOp
Family A
Family B
Tyrosin Kinase
Guanylyl Cyclase
Opsins
L F M

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PC2/Amon
PAL1
PAL2
PHM
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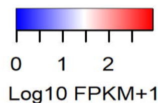
Fur-like protease 2B
SP
Silver
Prolyl endopeptidase
CPM
L F M
0 1 2
Log10 FPKM+1

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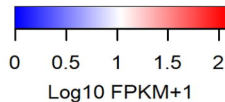
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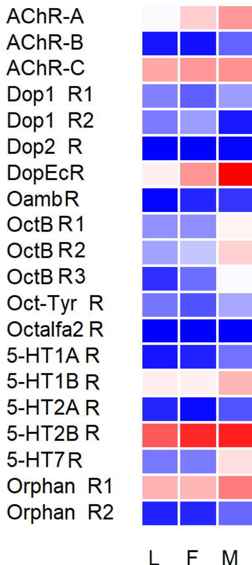
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AChR-A
AChR-B
AChR-C
Dop1 R1
Dop1 R2
Dop2 R
DopEcR
OambR
OctB R1
OctB R2
OctB R3
Oct-Tyr R
Octalfa2 R
5-HT1A R
5-HT1B R
5-HT2A R
5-HT2B R
5-HT7R
Orphan R1
Orphan R2
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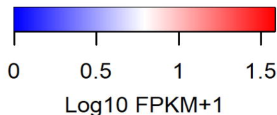
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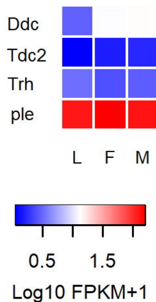
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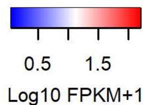
0.5
1.5
Log10 FPKM+1

Dissatisfaction
EcR
Ec induced protein 75B
Ec induced protein 78C
ErR
Ftz TF1
Hepatocyte NF4
HR-like38
HR-like38bis
HR-like39
HR-like4
HR-like46
HR-like46 bis
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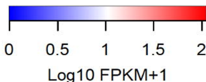
PNR-like
Seven up
Tailless
Ultraspiracle

L F M



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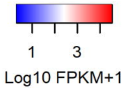

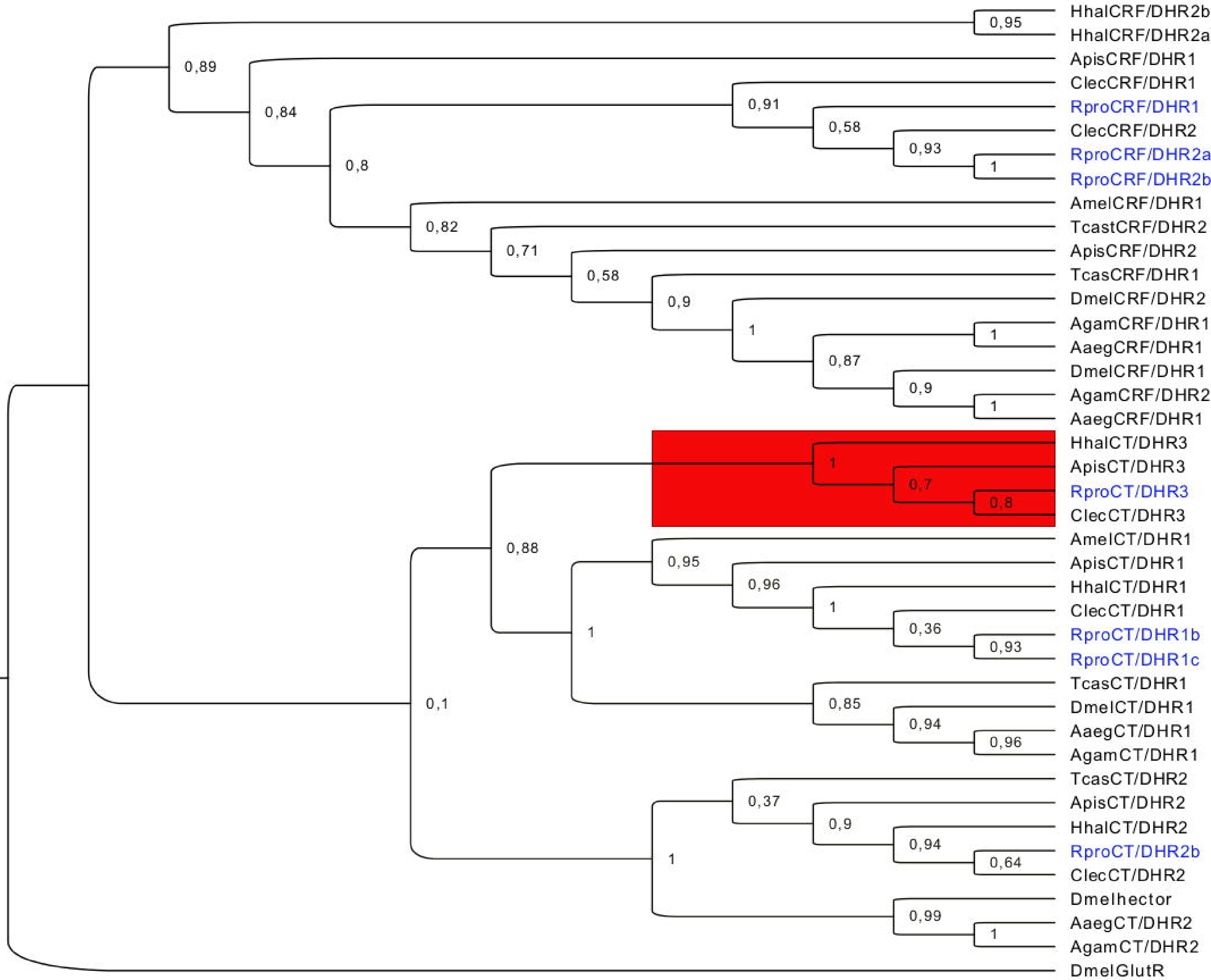

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TcasCRF/DHR1
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ApisCT/DHR3
RproCT/DHR3
ClecCT/DHR3
AmelCT/DHR1
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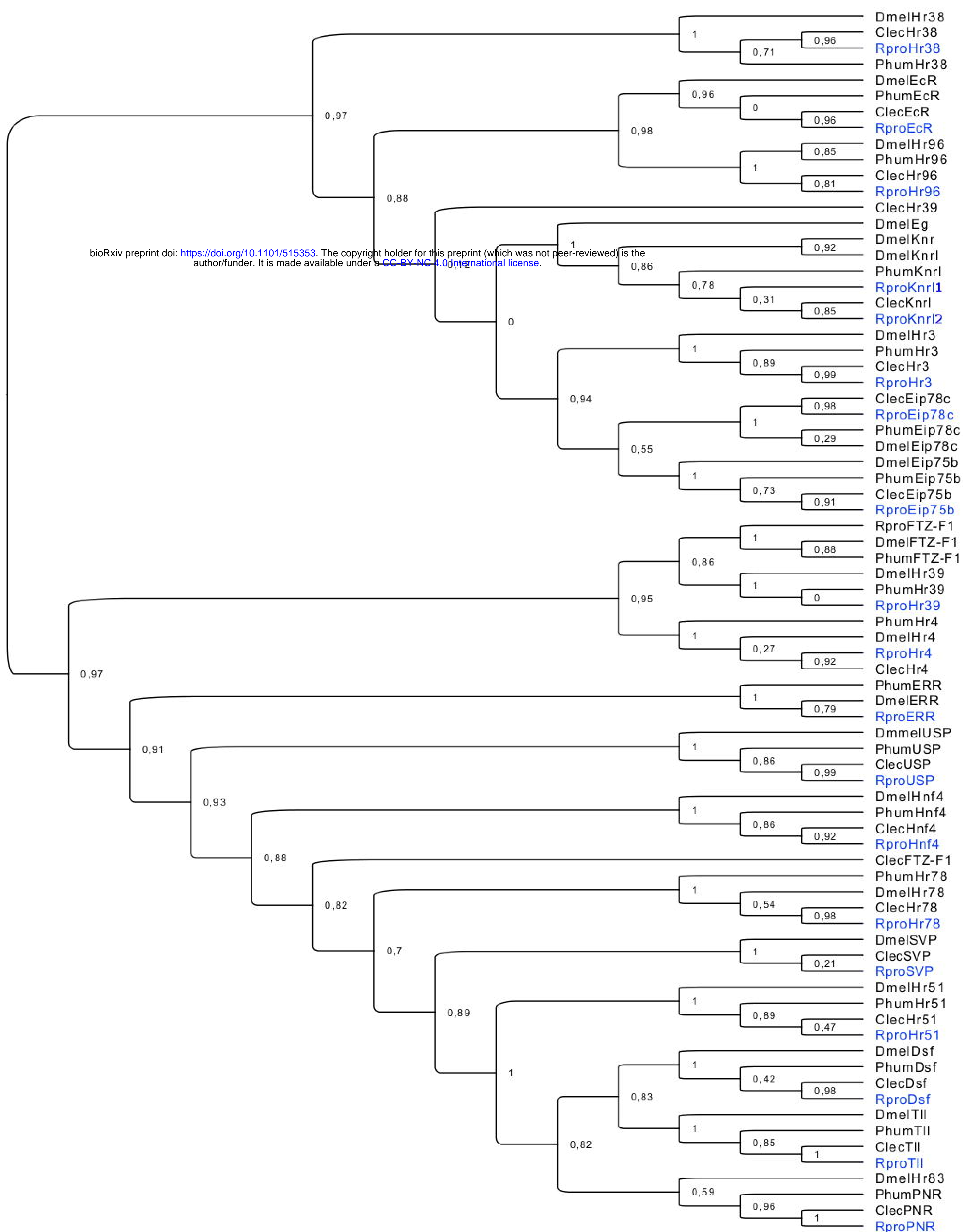

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RproTO2     ----------MW-----TAALVLL--SASAFCQAAKLPKNWI-VCKKSAPDAGDCWKRAM  42
RproTO3     ----------MFKFIV-YLIYLLV--LISDMAQPGKLPKTWK-TCTKGSPEMNECLKGAI  46
RproTO4     -----------MHVL--CMLLMGW--AFIFHVQSAKLPKTWK-TCKKDDPRMNDCLKIAI  44
RproTO5     ---------MMFK-----QLFYIC--AIYGVVESAKLPKTWT-ACKSNDPKKEECLKGAI  43
RproTO6     ------------MTRG-YLLTIFF--CLVGLSVAAKMPSKWK-TCKRSDKNINECLKKAV  44
RproTO7     ----------MY-ILS-SITFGCV--CLYLVSAATVLPESWK-TCKKSDKKLNECLKSSI  45
RproTO8     ---------MIF-----LMILFGL--THCVFGGGKEVPPGVV-LCSRKHPKINDCVRNAI  43
RproTO9     ---------MAR-----QLLTLIA--SVVYILSTPVQGGQPE-LCSLSAKNLPQCLITAI  43
RproTO10    ------MESK-VLFLVLFIGVVCW---TAPARSMNKLPVFLK-SCK-LDKNFDNCMMKNG  48
RproTO11    ------MYCTLVFLL-----------SLVGLSLAVKLPAYVK-TCSRNDPKLSECALRHG  42
RproTO12    -MKTVSLNCMLRV-SLLL--------TALPFTSQLKLPSYIK-TCRQNDPKLNECVVKNG  49
RproTO13    MVQPRSMTAAQIS-MALL--------TIVALSAALKLPSYII-PCKKDDPKLNECAVRHG  50
RproTO14    ------MLCSPLAFSAVVLMFVCSANPAKKDPPVYQLPSYIKRACSRNDPNINKCVVEVG  54
RproTO15    ----------MFLLAVLCTWMVVAPEILAKDLPVYPLPPYVKRACARNDPNLNKCVVEVG  50
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RproTO1     TFAARDLK-DGNANLGILPLDPLRIDQLVIDQ--GQGP-VSVKMVFSNFSISGHRNVEVL  98
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RproTO3     EEAIHELA-DGNPSLGVLPMDPFHFDTITIDQ--GHGP-VSIKLDYTDLDLTGIGDLIIK 102
RproTO4     EEAVHDLV-GGNPSLGVFPLDPMHFDTVSIDQ--GHGP-VSIKLDFKHLEIIGVKDLKIT 100
RproTO5     QHAIRDLSNGGKASLGVLPMDPLHFDMITVDQ--GDGP-VAIKLEFFNLDLIGLKTINVN 100
RproTO6     EEAVKTLK-SGNPSLGVIPLDPLHFNELNIGQ--GSGP-VSINLNFKNMDIHGISTAKVK 100
RproTO7     QTVVRELK-TGNSKFGLPPTEPLLIEEVVLHQ--GNGQAVGLDLTFRKLKMYGLSRVVVD 102
RproTO8     QETMPKFI-SGIKSLDIPSLDPFHVDNLIIDSKRDDGSPVSIDLSWHNVNIKGIKSAKIT 102
RproTO9     QNVIPILV-KGIPRYGVYPMDPMHIDTLDLSN--SPGKTLNVKHKFTNVDLQGLSSAVIR 100
RproTO10    NLAIPTLA-KGDAKWKIPVLDPLKVPSVSISES-SAKS-IALNITLNDLEIYGLKESKLV 105
RproTO11    REMIPKII-PGDPSIRLPRLEPLLLERVEIHPSGNGGS-INMRLVCYKCQVAGLSRASLN 100
RproTO12    RLAIPKFI-NGDTKYRVPRLDPLDINELKVHQG--SRQ-LGLTMSLRDCKVTGLKHAQFI 105
RproTO13    QLAIPKFI-NGDPKYRAPRLDPLDITELRVNQG--TRQ-IGLRMILKNVKIYGLKNTVFT 106
RproTO14    GPAIKTVA-KGDPKYRIPQLDPLHIKELRVQQG--TKQ-VGLELICSDCLMWGLQNTVFK 110
RproTO15    SVALKTVI-KGDPKYRVPVLNPMVIEELIVKQG--TKQ-VGLTLVCKDCKLWGLENTKIV 106
                       *           :*: .  : :           : :     .  : *      .

DmelTO      KVKGFGRDLTAKHEVKIVTKTFSLVGPYNIQGKVLILPISGTGQSNMTMVNVRAIVSFSG 158
RproTO1     SVKNDWKDVYLKAIV---PK-MTLRGKYKMDGKVLTLPIRGEGNCSLDAEDFTSSLHLVL 154
RproTO2     NVKNDWKVVTFNAAN---PR-VTLVSKYVMDGKVLTLPIKGDGPCRIDIDNFKSNFTIRF 154
RproTO3     SVKTDWKEMHFDAEI---PTKVVLDGKYKIDGKVLVLPINGEGHCRIEFTKFKSFAQLKL 159
RproTO4     NLKTDWKEMHVDFIV---PA-VIAVGTYNVTGQVLILPIQGNGFCNLTFTNFSGSGQLKF 156
RproTO5     SVKNDWKSMVVDLIV---PK-LTLRGQYKVNGKVLVLPIKGDGDCKLEFTNYKVIGNLKI 156
RproTO6     RFRADWNNYYLEAEA-TLNVPLVLLGDYTVKGQVLVLPIVGNGKCNLTFDNFVAKLTAKG 159
RproTO7     KVSARYDKDQLSADF-HIDGDFRIESDYTAKGRVLVLPINGAGKNVLKFDNLKGKLDMKF 161
RproTO8     SAKADWDNNMVSFEA-ALTEPVDITGNYNIDGIILILPIKGTGTFDLKLEGFRAHIKVHG 161
RproTO9     HVRLNPKTVEIDVNA-ILSKPVVLTGNYVSQGKILTLPITGRGGGKFNITLINMRAVLKMRG 159
RproTO10    ASRFDVNRKHVVWKI-AVPR-LTLLSKYKVAGRFLVLPITGSGPATVMLESPMLTYKFDY 163
RproTO11    DIKLDLNKKHIDIRL-SIPR-LMVTGKYDVSGKVLVFPITGKGISNITLTDLDVNAGLDW 158
RproTO12    AARTDLKRRHIEWDF-YHPF-ITIAGKYEMSGQVLVLPIRGRGTANITLTNMKTMFKFDF 163
RproTO13    HARTGLRDKHIEWDF-KIPK-IEIISDYEVNGQVLILPITGKGKANVTLTKLDITYKYDW 164
RproTO14    SADVNWEDRKCRWEF-TLDK-MKVTGKYNVTGQVLLLPIVGSGDALINLENLKFSYLYDW 168
RproTO15    KADMNFNTNHHKMDF-TLSK-MRVVGKYNVSGQILLLPISGAGDAEFKFENLKFSIIYDT 164
                                   .    . *     *  .* :** * *      .

DmelTO      KPLVK-NGETYLDVTDLKI-TMKPESSHYHFSNLFNGDKALGDNMNVFLNENSEAIYKET 216
RproTO1     RNNTK-NGKHYFAVEKFDVLKLDAEKGHVHFDNLFNGDKSLGDAMNRFLNANWREILTEI 213
RproTO2     KKISR-GGKEYLEVTKFQL-QFTASNAKLQFDNLFGGNKALGNTMNKFLNENWEEIVNEL 212
RproTO3     KEIEK-GSKHFYEITNFEF-DFDADGVHIQFDNLFNGDKALGDNMNVFLNENWKEILQEL 217
RproTO4     KEIQK-NDKKYYQISHFDF-IFDAEKMDILLENLFNGDKALGDNMNVFLNQNWPEILKEL 214
RproTO5     KEVKK-GDKKHFEVVQFQI-KPTQEKVFIQFDNLFNGDKALGDNMNRFLNENSQEILQEL 214
RproTO6     HEITK-GKEKYMEIDKFTF-DLETSKLRVFLGNLFNGDKALGNNMNVFLNENWQEILKEL 217
RproTO7     NKIKK-GADTYYNVNKCDI-MLDTSRLHLDFKRSSSVNEGLGQNLNTVLNENWKEILTDL 219
RproTO8     KEEMR-DGDKYMMVDRLAF-TFDIDHMEVHYYNLFNGDPVLGESMNSFLNDNWRDIIAEM 219
RproTO9     HQTTK-NGKVHVMMDSVKF-PFKIDKMELLFENLLRGNRLLSDTLNSVLNENWESVLEDM 217
RproTO10    KLVKR-NNEDYLQVTKSDL-KHTTTRLRINFENLFNGDKALGASTNKLINENWEEFNQQL 221
RproTO11    KLIKR-KRHEYSQFIRHKV-TFTASGLKINLSNLFNGDKLLSDNMNMILNANWREVLQDL 216
RproTO12    DLVKKEDGEEYMKVTKTDI-DTDIGNAIFRFNNLFNGDRLLGESMNRFLNENWKEVVKEL 222
RproTO13    ELIKK-NGKEYMNFTSSEL-LFENGRTFFDLKNLFNGDEFLGNNMNRFLNENWREVTKEL 222
RproTO14    TYQKKNNGYTYVILGNSSF-PFEVGHMSIKLENLFNGDPLLGGNMNRFLNEHWQDIMKDL 227
RproTO15    AYEKKSNGRTYLKVVNGSF-PMDAGNLVIRLDNLFNGDKLLGGNMNRFLNENWKEILKDV 223
              :      .   .     .                  .     :  *.    * .:* .    .  :

DmelTO      AKAIDRSFGKLYLGVVKGVFSKLPYAKFFADES   249
RproTO1     TPALSKSFGLAYMRISNRILSKVPGDELFLS*-   244
RproTO2     SPALAQAFGVAMKAVSNKILTQIPFEEINL*--   242
RproTO3     KPAISGAFGAAFKEVGNRVFGKIPIQLISPS--   248
RproTO4     RPAVSKAFSSAFKEVGNRVFSKVPLELISPP*-   245
RproTO5     GPAISRAFGTAFKTISNRIFSKVPSNEINL---   244
RproTO6     KPAISKAFGEAFRSIGNSVFSRIPLNQIAPK*-   248
RproTO7     KPAISKAFGAAFKDLANRVFSKVPLDKVMPA--   250
RproTO8     TPSVEASFSKYFEQVARKVFDHIPIDKIALP--   250
RproTO9     KPSFEEAIGSAFKEFANRIFNRIPQNYKCK---   247
RproTO10    APSVVQSIGAILTQVLSNIVKTVPYENIFTK*-   252
RproTO11    KPSISDTVGQIIRITLNQIFDIIPYSQFFPDS-   248
RproTO12    GAPVVDSISQVFEIILSRITELVPYHLVYTPV*   254
RproTO13    GPAVGEAFSNVFRLLLTRIAAQVPYNDIYLQE*   254
RproTO14    GPAFSRSLAELTTGILTNMARVVPFDIMFPDT-   259
RproTO15    QPALSESLSELSERILNNISALIPMDILFPKK-   255
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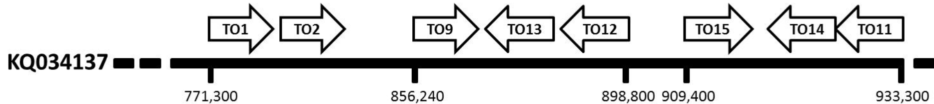
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