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***

4   **Jose Manuel Latorre-Estivalis[1,2][§]; Marcos Sterkel[2]; Sheila Ons[2]; and Marcelo Gustavo Lorenzo[1]**

5   (1) Vector Behaviour and Pathogen Interaction Group, Instituto René Rachou - FIOCRUZ, Belo Horizonte,

6   Minas Gerais, Brazil. Current address: Laboratorio de Neurobiología de Insectos - Centro Regional de

7   Estudios Genómicos – CREG, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina.

8   (2) Laboratorio de Neurobiología de Insectos - Centro Regional de Estudios Genómicos – CREG, Universidad

9   Nacional de La Plata, La Plata, Buenos Aires, Argentina.

10   [§]Corresponding author Jose Manuel Latorre Estivalis: josemlatorre@conicet.gov.ar

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

1. INTRODUCTION

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

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

2. MATERIAL AND METHODS

2.1 Transcriptomic data analysis

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

2.2 *Manual gene curation*

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

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

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

6

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

7

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).

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

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).

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;

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

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 antenna 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

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.

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.

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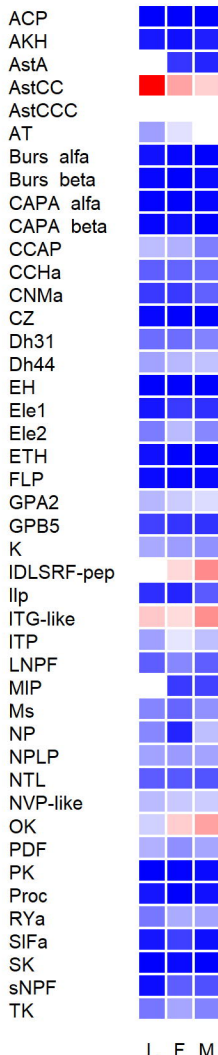
613   like P values, which were calculated by means of aLRT SH-like test. The LG substitution amino-acid model

614   was used

615   **Table 1 - Antennal expression (represented as Fragments Per Kilobase Million - FPKM - values) of**

616   **neuropeptides and their corresponding receptors with FPKM values higher than 1 in at least two of the**

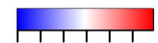617   **analysed conditions.** Complete names are detailed in Supplementary Table S1 and S2.

Neuropeptide	Larvae	Female	Male	Receptor	Larvae	Female	Male
CCAP	11.3	9.8	4.5	CCAP r	12.1	9.8	14.1
Dh31	3.4	3.3	4.8	CT/DH r1	41.8	81.1	116.7
				CT/DH r3	7.5	79.3	131.6
Dh44	7.7	10.7	12.4	CRF/DH r1	6.5	6.1	10.9
				CRF/DH r2	4.1	14.8	29.2
GPA2	10.5	14	17.5	GPA2/GPB5 r	87.9	54.1	32.5
GPB5	1.37	1.06	1.02				
LK	8.7	7.2	6	Kinin r 1	1.3	1.5	2.2
				Kinin r 2	0.7	7.4	14.1
ITP	7.5	20.1	12	ITP r	11.3	5.2	5.9
LNPF	2.5	5.1	2.6	LNPF r1	13.5	31.8	39.8
Ntl	2.4	2.3	2.1	Ntl r	1.7	2.9	5.4
NP	5.1	0.7	11.5	NP r	6.2	1.6	8.2
NPLP1	7.7	7.1	7.9	NPLP r	1.3	0.7	1.3
PDF	9.6	5.8	8.1	PDF r	1.2	1.7	6.1
RYa	3.9	8.9	8	RYa r	3.25	3.2	3.87
sNPF	0.2	2.5	1.9	sNPF r	5.3	1.6	1.5
TK	3.8	8.1	4.8	TK 86C-like r	2.1	7.2	5.8
				TK 99D-like r	0.2	1.3	1.4

618

22

A

ACP
AKH
AstA
AstCC
AstCCC
AT
Burs alfa
Burs beta
CAPA alfa
CAPA beta
CCAP
CCHa
CNMa
CZ
Dh31
Dh44
EH
Ele1
Ele2
ETH
FLP
GPA2
GPB5
K
IDLSRF-pep
Ilp
ITG-like
ITP
LNPF
MIP
Ms
NP
NPLP
NTL
NVP-like
OK
PDF
PK
Proc
RYa
SIFa
SK
sNPF
TK

L F M

Log10 FPKM+1

B

ACP R
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

Family A

CT/DH R1
CT/DH R2
CT/DH R3
CRF/DH R1
CRF/DH R2
PDF R
PH R
EH R

Family B

NPLP
NP R
In R

Tyrosin Kinase
Guanylyl Cyclase
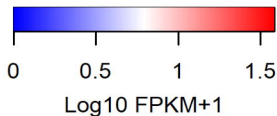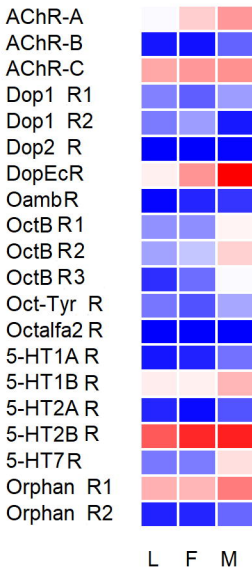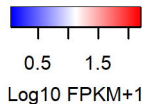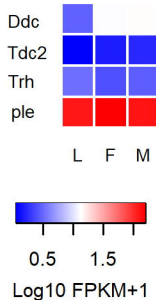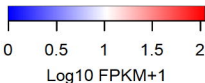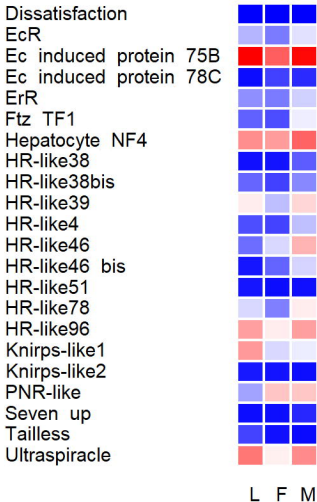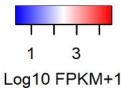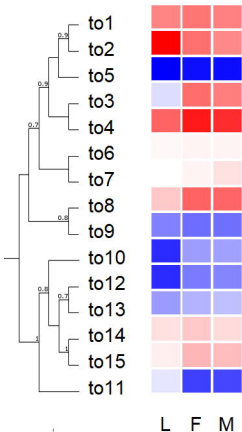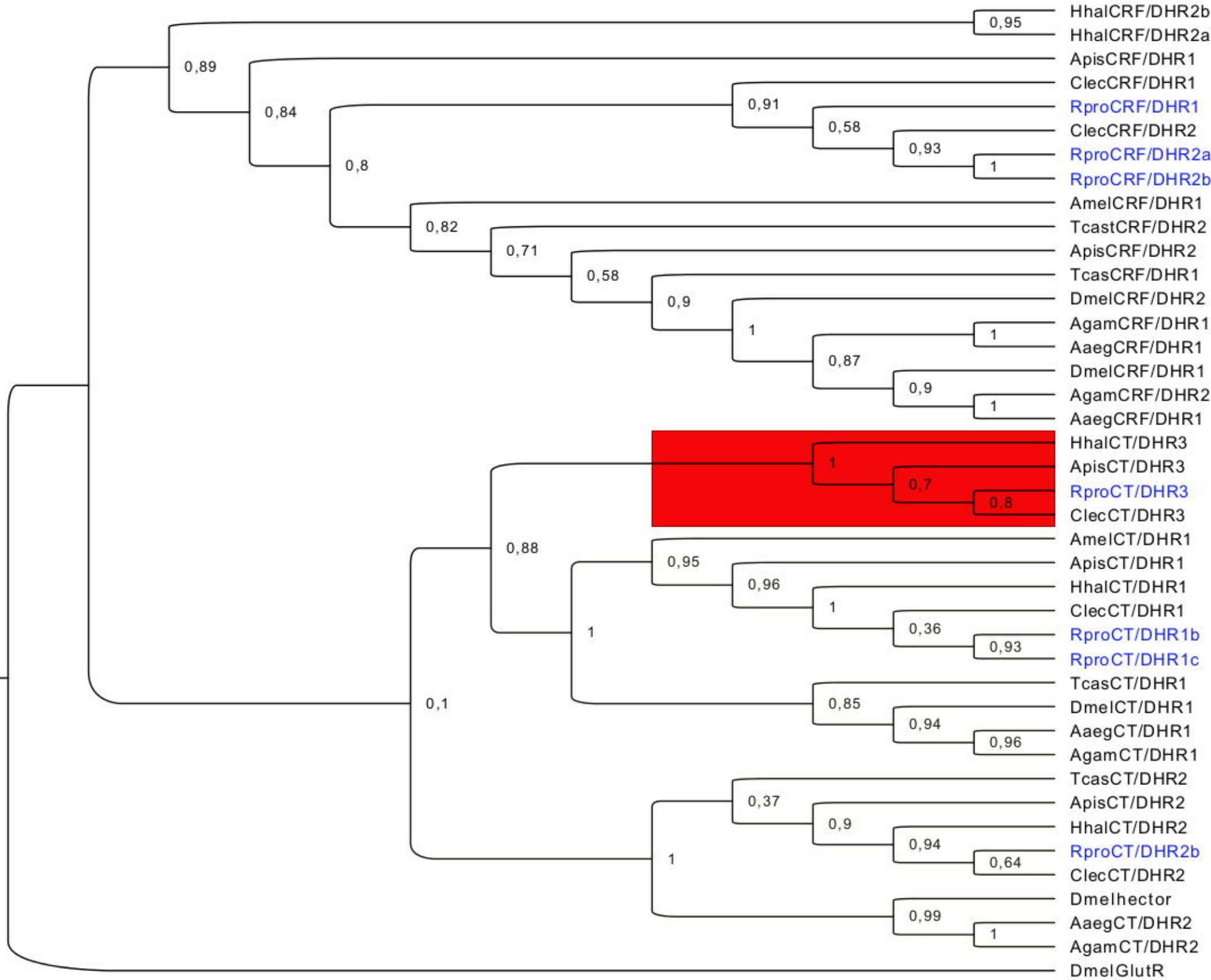
COpsin
LWS1
Rh7
UVOp

Opsins

L F M

Log10 FPKM+1

C

PC2/Amon
PAL1
PAL2
PHM
Fur-like protease 1
Fur-like protease 2A
Fur-like protease 2B
SP
Silver
Prolyl endopeptidase
CPM

L F M

Log10 FPKM+1

A

	L	F	M
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			

Log10 FPKM+1

B

	L	F	M
Ddc			
Tdc2			
Trh			
ple			

Log10 FPKM+1

	L	F	M
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			
HR-like51			
HR-like78			
HR-like96			
Knirps-like1			
Knirps-like2			
PNR-like			
Seven up			
Tailless			
Ultraspiracle			

0 0.5 1 1.5 2

Log10 FPKM+1

Log10 FPKM+1
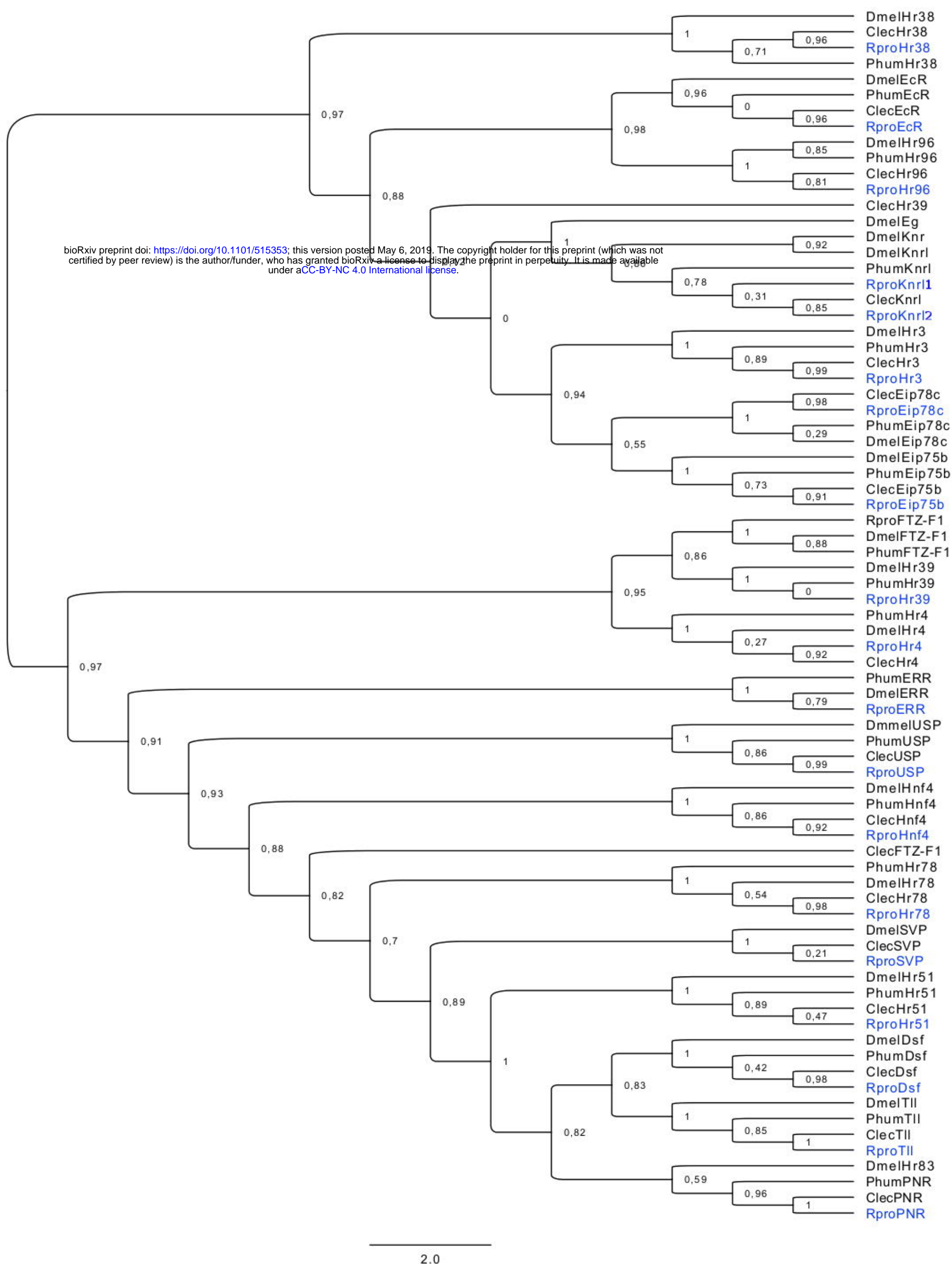
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RproTO2    ----------MW-----TAALVLL--SASAFCQAAKLPKNWI-VCKKSAPDAGDCWKRAM  42
RproTO3    -----MFKFIV-YLIYLLV--LISDMAQPGKLPKTWK-TCTKGSPEMNECLKGAI  46
RproTO4    ------MHVL--CMLLMGW--AFIFHVQSAKLPKTWK-TCKKDDPRMNDCLKIAI  44
RproTO5    --------MMFK-----QLFYIC--AIYGVVESAKLPKTWT-ACKSNDPKKEECLKGAI  43
RproTO6    --------MTRG-YLLTIFF--CLVGLSVAAKMPSKWK-TCKRSDKNINECLKKAV  44
RproTO7    ----------MY--ILS-SITFGCV--CLYLVSAATVLPESWK-ICKKSDKKLNECLKSSI  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
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RproTO3    EEAIHELA-DGNPSLGVLPMDPFHFDTITIDQ--GHGP-VSIKLDYTDLDLTGIGDLLIK  102
RproTO4    EEAVHDLV-GGNPSLGVFPLDPMHFDTVSIDQ--GHGP-VSIKLDFKHLEIIGVKDLKIT  100
RproTO5    QHAIRDLSNGGKASLGVLPMDPLHFDMITVDQ--GDGP-VAIKLEFFNLDLIGLKTINVN  100
RproTO6    EEAVKTLK-SGNPSLGVIPLDPLHFNELNIGQ--GSGP-VSINLNFKNMDIHGISTAKVK  100
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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
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RproTO1    SVRNDWKDVYLKAIV---PK-MTLRGKYKMDGKVLTLPIRGEGNCSLDAEDFTSSLHLVL  154
RproTO2    NVKNDWKVVTFNAAN---PR-VTLVSKYVNDGKVLTLPIRGDGPCRIDIDNFKSNFTIRF  154
RproTO3    SVKTDWKEMHFDAEI---PTKVVLDGKYKIDGKVLVLPINGEGHCRIEFTKFKSFAQLKL  159
RproTO4    NLKTDWKEMHVDFIV---PA-VIAVGTYNVTGQVLLLPIQGNGFCNLTFTNFSGSGQLKF  156
RproTO5    SVRNDWKSMVVDLIV---PK-LTLRGQYKVNGKVLVLPIGKGDGCKLEFTNYKVIGNLKI  156
RproTO6    RFRADWNNYYLEAEA-TLNVPLVLLGDYTVKGQVLVLPIVGNAGCNLTFDNFVAKLTAKG  159
RproTO7    KVSARYDKDQLSADF-HIDGDFRIESDYTAKGRVLVLPINGAGKNVLKFDNLKGKLDMKF  161
RproTO8    SAKADWDNNMVSFEA-ALTEPVDITGNYNIDGIILILPIKGTGTFDLKLEGFRAHIKVHG  161
RproTO9    HVRLNPKTVEIDVNA-ILSKPVVTLDGKYVSQGKVLVLPIGGGKFNITLINMRAVLKMRG  159
RproTO10   ASRFDVNRKHVVWKI-AVPR-LTLLSKYKVAGRFLVLPITGSGPATVMLESPMLTYKFDY  163
RproTO11   DIKLDLNKKHIDIRL-SIPR-LMVTGKYVSGKVLVFPITGKGISNITLTDLDVNAGLDW  158
RproTO12   AARTDLKRRHIEWDF-YHPF-ITIAGKYEMSGQVLVLPIRGRGTANITLTNMKTMFKFDF  163
RproTO13   HARTGLRDKHIEWDF-KIPK-IEIISDYEVNGQVLLLPIGKGKANVTLTKLDITYKYDW  164
RproTO14   SADVNWEDRKCRWEF-TLDK-MKVTGKYNVTGQVLLLPIVGSGDALINLENLKFSYLYDW  168
RproTO15   KADMNFNTNHHKMDF-TLSK-MRVVGKYNVSGQILLLPISGAGDAEFKFENLKFSIIYDT  164
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RproTO1    RNNTK-NGKHYFAVEKFDVLKLDAEKGHVHFDNLFNGDKSLGDAMNRFLNENSREILTEI  213
RproTO2    KKISR-GGKEYLEVTKFQL-QFTASNAKLQFDNLFGGNKALGNTMNKFLNENNEEIVNEL  212
RproTO3    KEIEK-GSKHFYEITNFEF-DFDADGVHIQFDNLFNGDKALGDNMNVFLNENKEILQEL  217
RproTO4    KEIQK-NDKKYYQISHFDF-IFDAEKMDILENLFNGDKALGDNMNVFLNQNWPEILKEL  214
RproTO5    KEVKK-GDKKHFEVVQFQI-KPTQEKVFIQFDNLFNGDKALGDNMNRFLNENSQEILQEL  214
RproTO6    HEITK-GKEKYMEIDKFTF-DLETSKLRVFLGNLFNGDKALGNNMNVFLNEMQEILKEL  217
RproTO7    NKIKK-GADTYYNVNKCDI-MLDTSRLHLDFKRSSSVNEGLGQNLNTVLNEWKEILTDL  219
RproTO8    KEEMR-DGDKYMMVDRLAF-TFDIDHMEVHYYNLFNGDPVLGESMNSFLNDNWRDIIAEM  219
RproTO9    HQTTK-NGKVHVMMDSVKF-PFKIDKMELLFENLLRGNRLLSDTLNSVLNEWESVLEDM  217
RproTO10   KLVKR-NNEDYLQVTKSDL-KHTTTRLRINFENLFNGDKALGASTNRFLKLINENWEEFNQQL  221
RproTO11   KLIKR-KRHEYSQFIRHKV-TFTASGLKINLSNLFNGDKLLSDNMNMILNANWREVLQDL  216
RproTO12   DLVKKEDGEEYMKVTKTDI-DTDIGNAIFRFNNLFNGDRLLGESMNRFLNENWKEVVKEL  222
RproTO13   ELIKK-NGKEYMNFTSSEL-LFENGRTFFDLKNLFNGDEFLGNNMNRFLNENWREVTKEL  222
RproTO14   TYQKKNNGYTYVILGNSSF-PFEVGHMSIKLENLFNGDPLLGGNMNRFLNEHWQDIMKDL  227
RproTO15   AYEKKSNGRTYLKVVNGSF-PMDAGNLVIRLDNLFNGDKLLGGNMNRFLNENWKEILKDV  223
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RproTO1    TPALSKSFGLAYMRISNRILSKVPGDELFLS*-  244
RproTO2    SPALAQAFGVAMKAVSNKILTQIPFEEINL*--  242
RproTO3    KPAISGAFGAAFKEVGNRVFGKIPIQLISPS--  248
RproTO4    RPAVSKAFSSAFKEVGNRVFSKVPLELISPP*-  245
RproTO5    GPAISRAFGTAFKTISNRIFSKVPSNEINL---  244
RproTO6    KPAISKAFGEAFRSIGNSVFSRIPLNQIAPK*-  248
RproTO7    KPAISKAFGAAFKDLANRVFSKVPLDKVMPA--  250
RproTO8    TPSVEASFSKYFEQVARKVFDHIPIDKIALP--  250
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RproTO10   APSVVQSIGAILTQVLSNIVKTVPYENIFTK*-  252
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RproTO14   GPAFSRSLAELTTGILTNMARVVPFDIMFPDT-  259
RproTO15   QPALSESLSELSERILNNISALIPMDILFPKK-  255
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