Supporting Information

Mirabeau and Joly 10.1073/pnas.1219956110

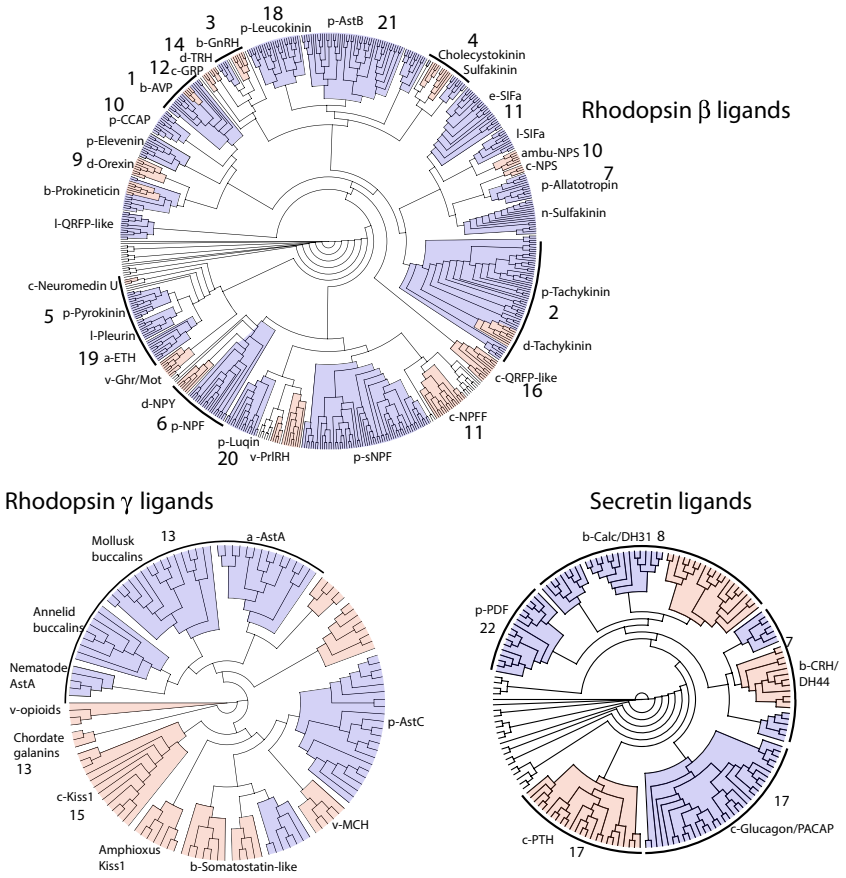

Fig. S1. Dendrogram of predicted bilaterian peptides. Neighbor-joining trees of neuropeptide ligands of rhodopsin β and γ and secretin receptors. The distance used is LAKdist (*Methods*). The color (blue for protostomes and pink for deuterostomes) and prefix code for species is the same as in Fig. 2; in addition, v- stands for vertebrates and n- for nematodes. Solid arcs indicate deuterostome (d-) and protostome (p-) peptide families, including d-gonadotropin-releasing hormone (GnRH) and p-adipokinetic hormone (AKH), d-cholecystokinin (CCK) and p-sulfakinin (SK), and bilaterian (b-) peptide families, such as tachykinin-like peptides and vasopressin-like peptides.

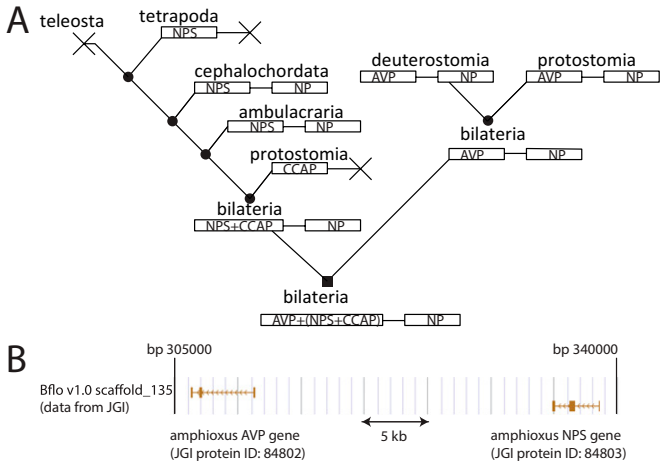

Fig. S2. Evolutionary scenario for NPS/CCAP neuropeptide precursors. (*A*) In an ancestor of extant bilaterians, a duplication of an AVP + NPS/CCAP–NP gene must have occurred, giving rise to an AVP–NP and NPS/CCAP–NP gene. The neurophysin domain was then lost independently in protostomes and tetrapods. This scenario is supported by both our receptor analysis, which shows a close association between bilaterian AVPR and NPSR, and (*B*) the neighboring tandem position of AVP and NPS peptide genes in the amphioxus genome, indicating that they are the product of an ancient duplication. AVP, arginine vasopressin; Bflo, *Branchiostoma floridae*; CCAP, crustacean cardioactive peptide; NPS, neuropeptide S; v1.0, version 1.0 of the Joint Genome Institute genome assembly.

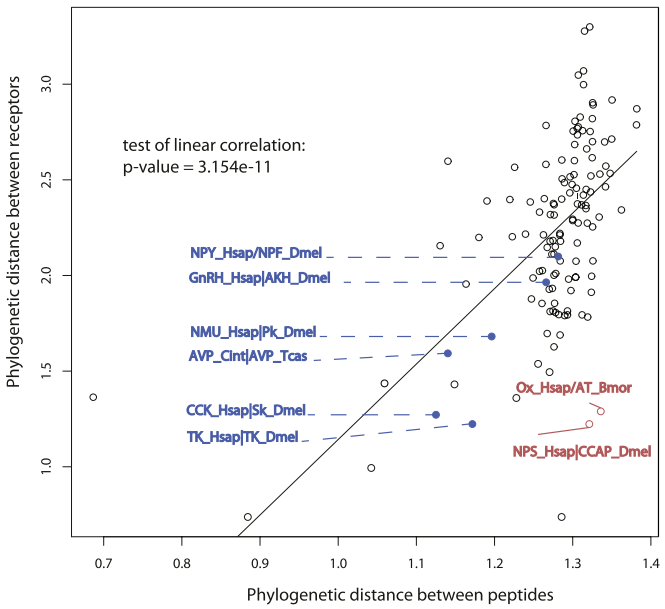

Fig. S3. Coevolution between peptides and receptors. A clear correlation between peptide distances and receptor distances across bilaterian peptidergic systems (PSs) ($P = 3.1e\text{-}11$) suggests that a majority of receptors from the rhodopsin β family have coevolved with their cognate ligands since the emergence of bilaterians. The distance between any two peptides or receptors is plotted on the *x*- and *y*-axes, respectively. This distance corresponds to the distance between leaves induced by the phylogenetic trees. Only known pairs of peptide-receptors according to the literature (supporting information is given at http://neuroevo.org) were considered. Blue points correspond to PSs that have already been hypothesized to be homologous, including mammalian CCK and ecdysozoan SK, or mammalian GnRH and ecdysozoan AKH (1). In red are shown the relationships between two systems [human orexin (Ox) versus silkmoth allatotropin (AT) and human NPS vs. drosophila CCAP] for which receptors are clearly related, but for which no homology was detected between peptides with our procedure (*Methods*).

1. Janssen T, Lindemans M, Meelkop E, Temmerman L, Schoofs L (2010) Coevolution of neuropeptidergic signaling systems: From worm to man. *Ann N Y Acad Sci* 1200:1–14.

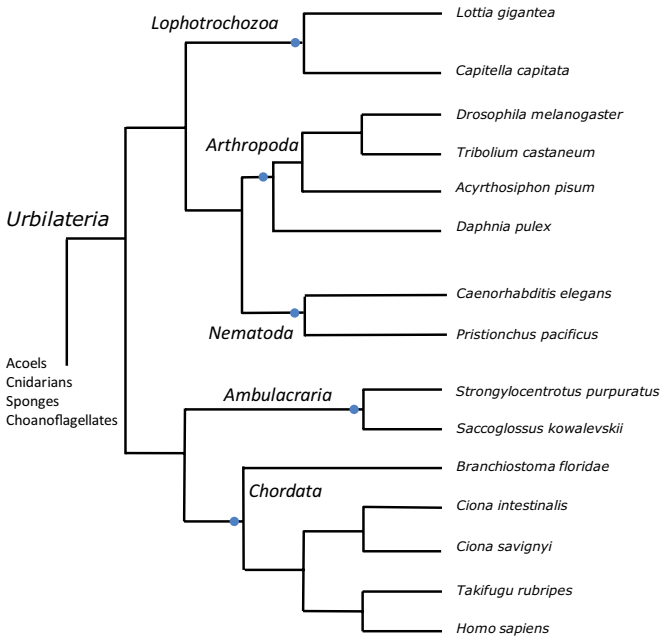

Fig. S4. Bilaterian genomes used for systematic receptor and peptide search. We mined the genomes of species belonging to five of the following major bilaterian phylogenetic groups: Lophotrochozoa, Arthropoda, Nematoda, Ambulacraria and Chordata.

Dataset S1. Annotated peptide and precursor alignments of the different bilaterian families

Dataset S1