Characterization of a GnRH-type signalling system in an echinoderm

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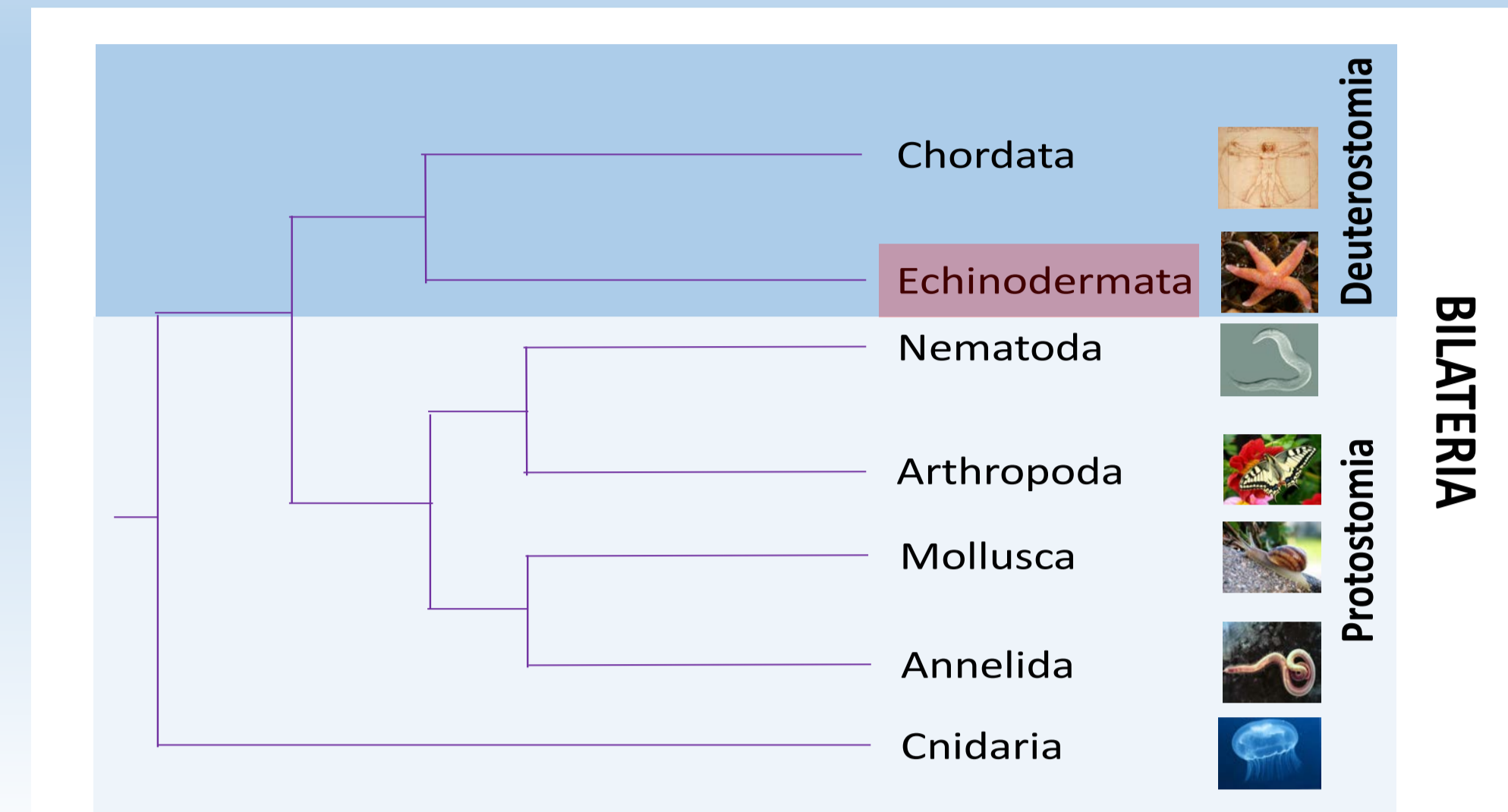
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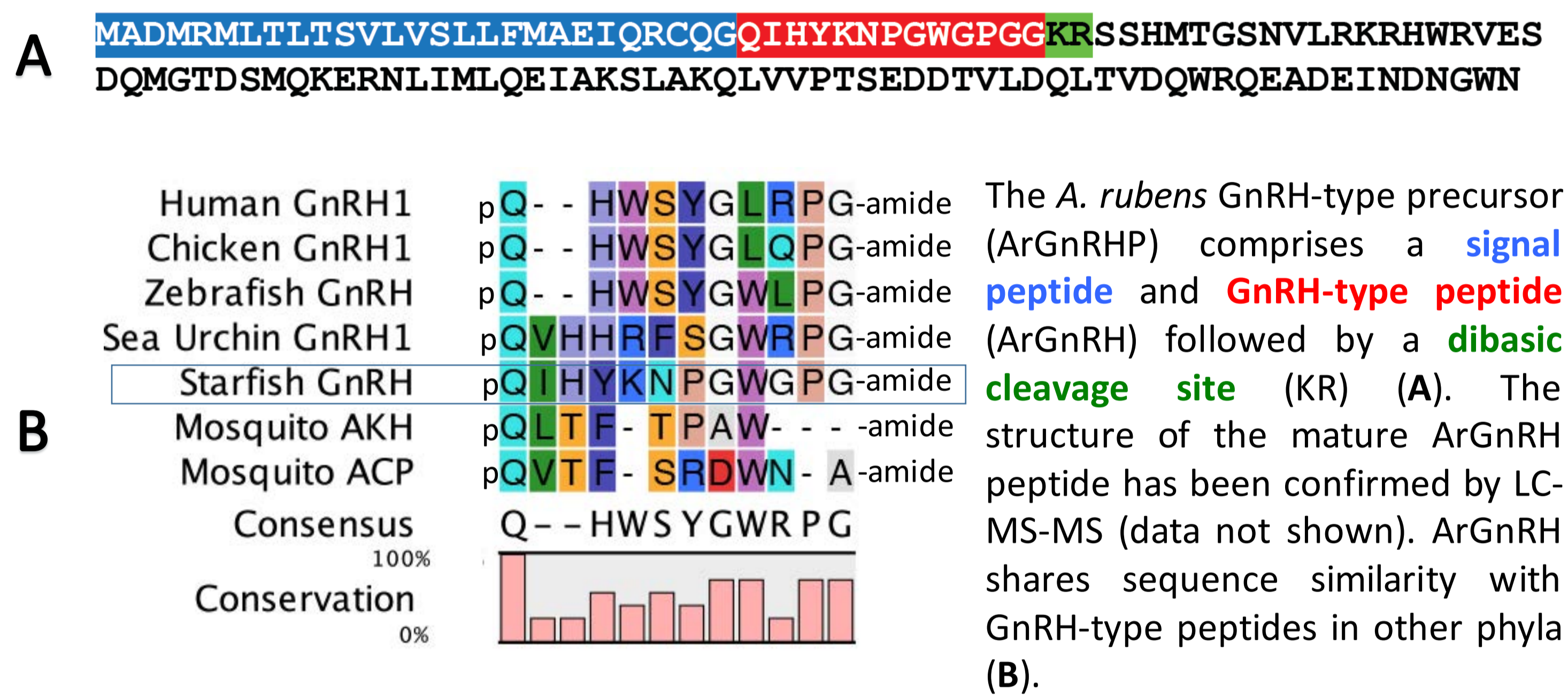
Background

❖ Neuropeptides are well-characterised signalling molecules in vertebrates and in some invertebrates (e.g. arthropods).

❖ Deuterostomian invertebrates (e.g. echinoderms) are "evolutionary intermediates" of vertebrates and arthropods and therefore can provide insights on the evolution of neuropeptide signalling.

❖ Using the starfish *Asterias rubens* as a model system we have identified forty neuropeptide precursors, including homologs of many mammalian hormones (Semmens et al., submitted).

❖ Here we have characterised the **gonadotropin-releasing hormone** (GnRH) signalling system in *A. rubens*.
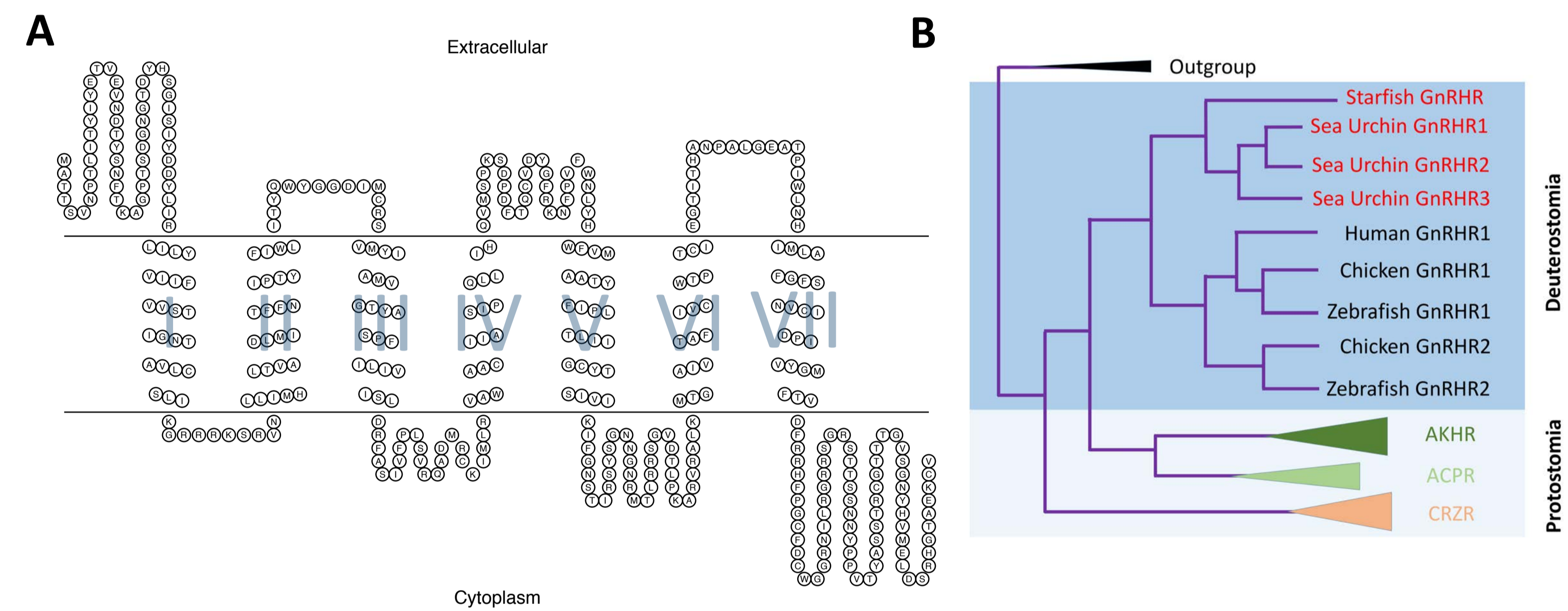
Animal Phylogeny

Results

Asterias rubens GnRH-type neuropeptide precursor

The *A. rubens* GnRH-type precursor (ArGnRHP) comprises a **signal peptide** and **GnRH-type peptide** (ArGnRH) followed by a **dibasic cleavage site** (KR) (**A**). The structure of the mature ArGnRH peptide has been confirmed by LC-MS-MS (data not shown). ArGnRH shares sequence similarity with GnRH-type peptides in other phyla (**B**).

Expression pattern of GnRH-type neuropeptide precursor in starfish *A. rubens*

ArGnRHP transcripts were only detected in the **radial nerve cords** and **circumoral nerve ring** of the starfish nervous system (**B,C,E**). Expression was localized in cells (▲) in both the **ectoneural (e)** and **hyponeural (h)** parts of the nerve cords and nerve ring. The anatomy of the starfish *A.rubens* is shown in **A** and **D**. **D** shows a longitudinal section of the starfish which is cut though the dashed line in **A**. The white point in **A** indicates the postion of the Madreporite.
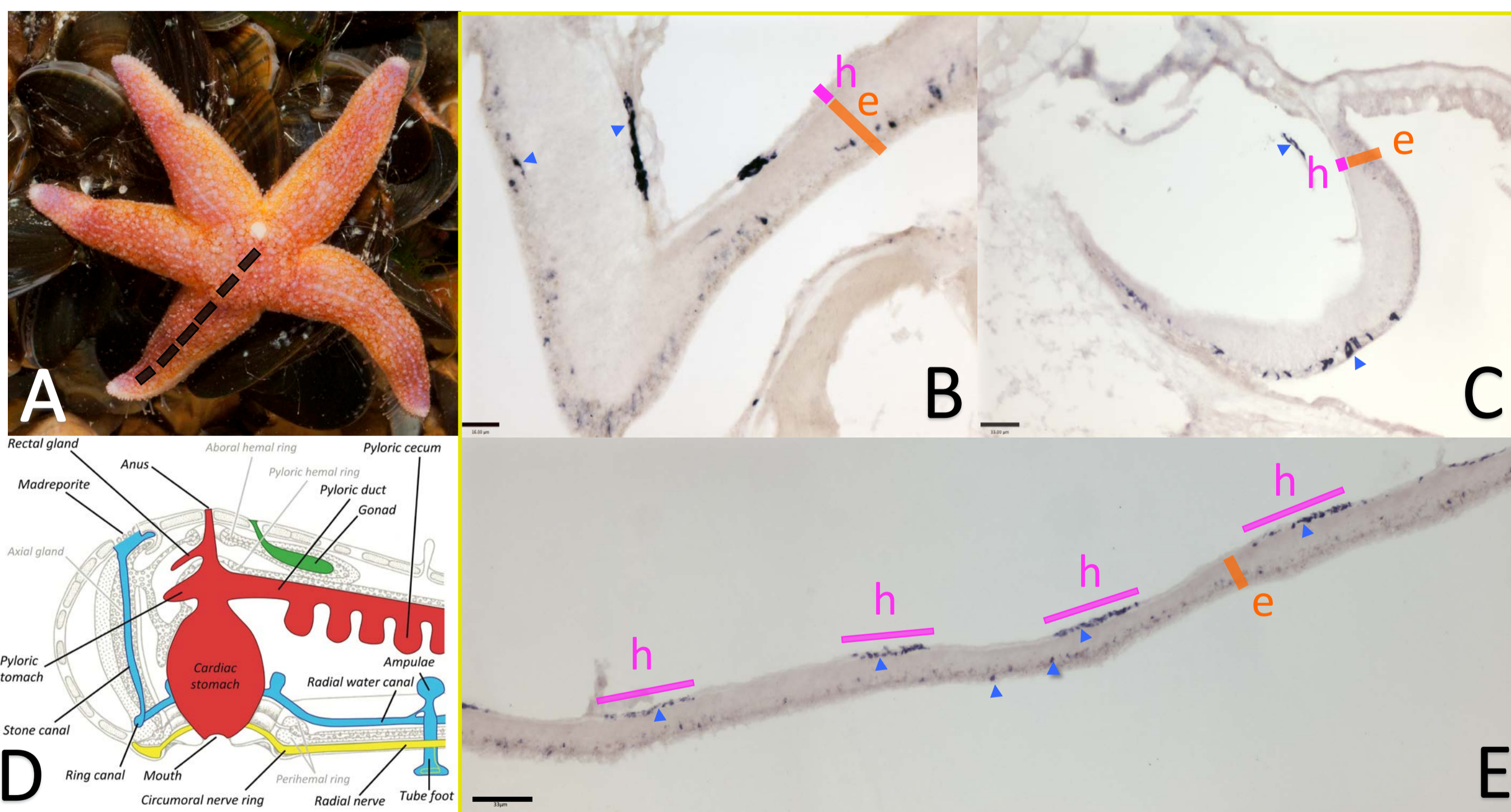
Asterias rubens GnRH-type neuropeptide receptor

A. The *A. rubens* GnRH-type receptor (ArGnRHR) is a **G-protein coupled type receptor** with seven putative transmembrane domains.
B. Phylogenetic analysis (maximum likelihood) reveals that **ArGnRHR is an ortholog of vertebrate GnRH-type receptors**. Protostomian GnRH-type receptors that are activated by **Adipokinetic hormone** (AKH), **AKH/Corazonin-related peptides** (ACP), and **Corazonin** (CRZ) are also shown.

Pharmacological characterization of *Asterias rubens* GnRH-type receptor

$EC_{50} = 6.03 \times 10^{-10}$ M
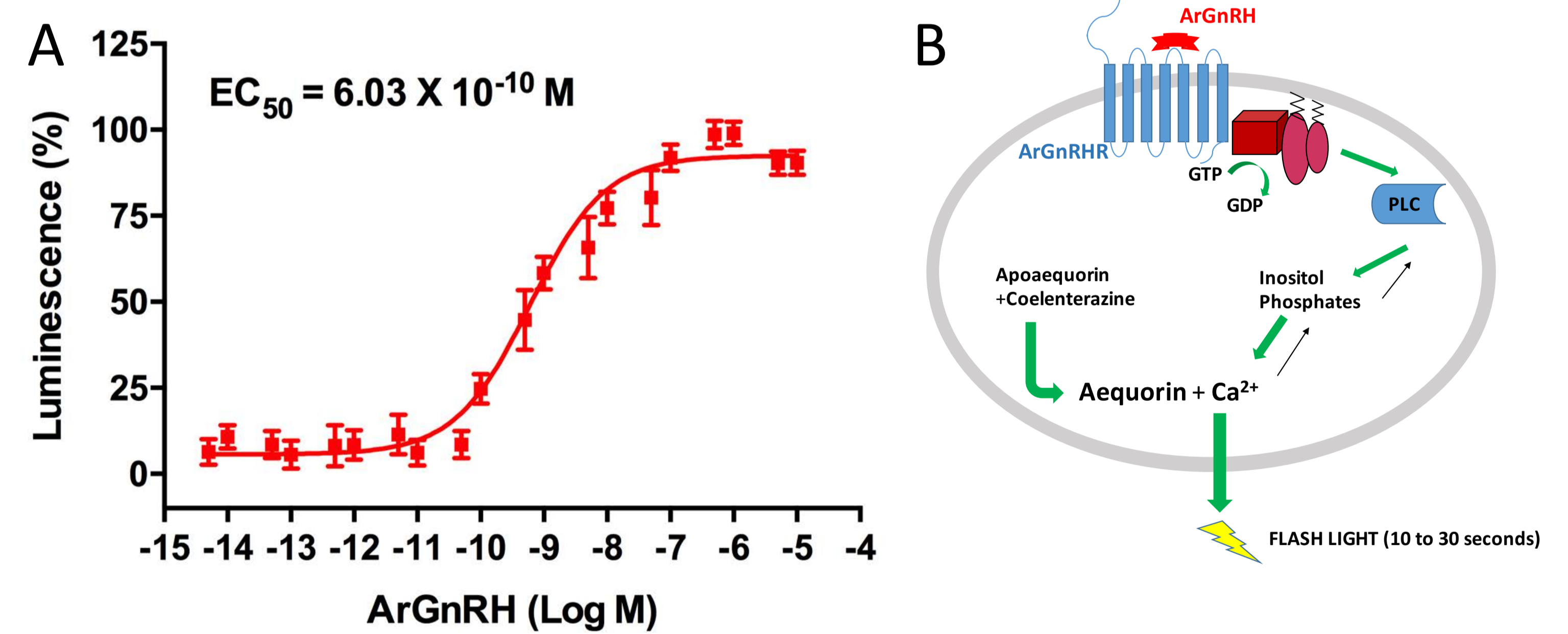
ArGnRH is a ligand for ArGnRHR expressed in CHO cells overexpressing the mitochondrial targeted apo-aequorin(A), using a method illustrated in (B).

Methods

1. ArGnRHP and ArGnRHR cDNAs were **cloned** from *A. rubens* radial nerve cDNA and then **sequenced**.
2. The structure of ArGnRH was determined using **LC-MS-MS**.
3. Mapping of the expression of ArGnRHP in *A. rubens* was accomplished using **mRNA *in situ* hybridization** methods.
4. **Heterologous calcium mobilization assay** was used to characterise ArGnRHR.

Summary

✓ This is the **first characterization of a GnRH signaling system in an echinoderm**.

✓ Expression of ArGnRH is restricted to the nerve cords and nerve ring in starfish.

✓ The **physiological roles** of ArGnRH in starfish are **not yet known**.

✓ On-going studies are addressing this issue using immunocytochemical and pharmacological techniques.