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The decapod red pigment-concentrating hormone (Panbo-RPCH) is the first identified neuropeptide of the order Plecoptera and is interpreted as homoplastic character state

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ABSTRACT

This paper presents the first neuropeptide structure, identified by mass spectrometry, in two species of Plectoptera (stoneflies) and in one species of the coleopteran family Lycidae. In all three species, the octapeptide Panbo-RPCH (first identified in *Pandalus borealis* as a red pigment-concentrating hormone: pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp amide) is present. A review of the literature available on invertebrate neuropeptides that are identified or predicted from expressed sequence tags, transcriptome shotgun assemblies, and from fully sequenced genomes, show that Panbo-RPCH is found in Malacostraca (Crustacea) and certain hemipteran Heteroptera (Insecta). To date, Panbo-RPCH has not been shown present in non-Malacostracan crustaceans, nor in basal taxa of the Insecta (Archaeognatha, Zygentoma, Ephemeroptera, Odonata). The present data adds to knowledge on the distribution of Panbo-RPCH, and when taking into account the most accepted, current phylogenetics of the Crustacea–Hexapoda relationship, this distribution of Panbo-RPCH in Malacostraca, Plecoptera, some hemipteran Heteroptera and in Coleoptera (Lycidae) can best be explained by homoplasy, implying parallel evolution.

1. Introduction

About 90 years ago it was already demonstrated that extracts from certain glands of crustaceans and insects cause “blanching” in shrimps, i.e. a change in general integumental appearance from a dark to a light colour (reviewed in Rao, 2001; Gäde and Marco, 2006). In 1972 the complete structure of the active principle for this blanching effect was elucidated as the first invertebrate neuropeptide, and was named the red pigment-concentrating hormone (RPCH); it was isolated from the eyestalks of the prawn *Pandalus borealis* (Fernlund and Josefsson, 1972). This octapeptide, now denoted Panbo-RPCH, affects primarily the concentration of the red pigment granules in epithelial chromatophores in various crustacean species but is also known for a number of other functions (Rao, 2001; Gäde and Marco, 2006; Christie et al., 2010a,b; Sathapondecha et al., 2014).

In 2003 it was demonstrated for the first time that Panbo-RPCH was synthesized and stored in the retrocerebral glands, the corpora cardiaca (CC), of an insect as well, viz. in the green stinkbug *Nezara viridula* (Hemiptera: Heteroptera: Pentatomidae) (Gäde et al., 2003). Here its function was shown to increase the concentration of lipids in the haemolymph, thus a true adipokinetic effect, whereas it failed to cause an increase in the concentration of carbohydrates in the stinkbug (Gäde et al., 2003). The presence of Panbo-RPCH in Pentatomidae was confirmed in three (Predel et al., 2008) and five more species (Kodrik et al., 2010), while in the edible inflated stinkbug *Encosternum delegorguei* (Hemiptera: Heteroptera: Tessaratomidae), Panbo-RPCH is produced together with another octapeptide of the adipokinetic hormone (AKH)/RPCH family, viz. Schgr-AKH-II (see Table 1 for structure; Gäde and Marco, 2009).

The question arises as to whether this distribution of Panbo-RPCH in crustaceans and insects is wholly unexpected. Morphological evidence (for example: Zrzavy and Stys, 1997) and molecular analyses (for example: Friedrich and Tautz, 1995; Giribet et al., 2001; Regier and Shultz, 2001; Richter, 2002) are in favour of a monophyletic Crustacea–Hexapoda clade, termed Pancrustacea or Tetraconata. It is unclear, however, which crustacean taxon represents the sister group to the Hexapoda. Two crustacean taxa have been favoured in this regard, Branchiopoda and Remipedia: (i) molecular support for the Branchiopoda as sister group of insects is presented by the work of Glenner et al. (2006) and is corroborated by phylogenetic work based on morphological data (Schram and Koenemann, 2004). (ii) Support for Remipedia and Hexapoda as sister taxa comes also from

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Table 1
Peptide similarity with reference to Panbo-RPCH.

Name	Primary structure
Crustacean similarities	
Panbo-RPCH	pGlu – Leu – Asn – Phe – Ser – Pro – Gly – Trp amide
Anaim-AKH	pGlu – **Val** – Asn – Phe – Ser – Pro – **Ser** – Trp amide
Dappu-RPCH	pGlu – **Val** – Asn – Phe – Ser – **Thr** – **Ser** – Trp amide
Argsi-RPCH	pGlu – **Val** – Asn – Phe – Ser – **Lys** – **Ser** – Trp amide
Plecoptera/Neoptera similarities	
Panbo-RPCH	pGlu – Leu – Asn – Phe – Ser – Pro – Gly – Trp amide
Manto-CC	pGlu – **Val** – Asn – Phe – Ser – Pro – Gly – Trp amide
Locmi-AKH-II	pGlu – Leu – Asn – Phe – Ser – **Ala** – Gly – Trp amide
Schgr-AKH-II	pGlu – Leu – Asn – Phe – Ser – **Thr** – Gly – Trp amide

Residues in bold differ from those in Panbo-RPCH.

morphological (Fahnenbruck et al., 2004; Harzsch, 2006; Koenemann et al., 2009) and molecular work (Regier et al., 2008, 2010; von Reumont et al., 2012). In a recent review article by Jenner (2010), however, a detailed critique is supplied that each proposed Crustacea-Hexapoda sister taxon relationship is still very speculative. Nonetheless, it is not too surprising that Panbo-RPCH does occur in the sister group of the Crustacea, the insects.

A second question then arises as to whether this currently known presence of Panbo-RPCH in certain hemipteran families is – in light of phylogeny – only a direct symplesiomorphic retention by a quite advanced group of paraneopteran insects, or not? To answer this question we must know whether Panbo-RPCH is present in (a) the more basal crustacean taxa, and (b) in the more basal insect taxa. In the current study, we present experimental data on the presence of Panbo-RPCH in two new taxa: a number of species from the order Plecoptera (stoneflies) and a species of net-winged beetles (Coleoptera: Lycidae). Additionally, we screened the existing literature on Panbo-RPCH and report here on evidence of the expressed peptide or of genomic information on Panbo-RPCH in Crustacea and insects.

With respect to neuropeptides in Plecoptera, we are aware of only two reports: one based on immunocytochemical findings with antisera raised against pigment-dispersing hormone (PDH), prothoracicotropic hormone (PTTH) and eclosion hormone (EH), and another study dealing with heterologous *in vivo* bioassays. PDH-positive neurons were, thus, detected in the optic lobe of the stonefly *Perla burmeisteriana* (Plecoptera: Perlidae) and two clusters of PTTH-positive cells were observed in the brain; no immunoreactivity to EH could be shown in the cephalic nervous system of this stonefly species (Zavodska et al., 2003). In the other study, albino locusts were injected with a brain extract from *Oyamia gibba* (Plecoptera: Perlidae) and consequently developed a dark colour, suggesting that this stonefly species produces a form of corazonin (Tanaka, 2006). In both cases the neuropeptides involved were not further investigated, thus, no neuropeptide has been structurally analysed in any species of the Plecoptera. The same is true for the coleopteran family Lycidae. The current report is, thus, the first one dealing with the full structural characterisation of a neuropeptide in these taxa.

2. Materials and methods

2.1. Insects

2.1.1. Plecoptera

All specimens were adult insects of unknown age and gender. *Perla marginata* (Perlidae: Perlinae; 11 specimens) and *Isoperla* sp. (identification is not absolutely clear: it may be *Isoperla grammatica* or *Isoperla oxylepis*) (Perlodidae: Isoperlinae; 5 specimens) were collected in June 2011 in vegetation at the edge of the Wutach river close to the confluence with the Gauchach near Ewattingen (Baden Württemberg Province, Germany).

2.1.2. Coleoptera: Lycidae

Adult specimens (10 individuals) of the net-winged beetle *Lycus* sp. were collected in the austral spring (September) 2009 and 2010 on private land of the farm Traveller's Rest, near Clanwilliam in South Africa.

All insects were kept alive in small containers on ice until the evening when CC material was dissected into 80% methanol.

2.2. Biological assays

A well-known and established heterologous bioassay was performed to assess the hypertrehalosaemic potency of a CC extract from Plecoptera. Adult male American cockroaches, *Periplaneta americana*, of unspecified age purchased from a local dealer in Pretoria, RSA, were used in the bioassay. Cockroaches were maintained in captivity, and the amount of total carbohydrates (measured as anthrone-positive material) was determined, as described previously (Gäde, 1980).

2.3. Dissection of corpora cardiaca, peptide extraction, mass spectrometry and sequence analysis

The CC of the two plecopteran species and of the net-winged beetle were dissected and prepared as outlined previously (Gäde et al., 1984). The vacuum-centrifuged dried extracts were either dissolved in distilled water for heterologous bioassays (see Section 2.2.), or in 15% acetonitrile plus 0.1% trifluoroacetic acid (TFA) for reversed-phase high performance liquid chromatography (RP-HPLC) according to previously published methods (Gäde, 1985), or in 50 μl of aqueous 0.1% formic acid for liquid-chromatography tandem positive ion electrospray mass spectrometry (LC–MS^n) on an LTQ XL linear ion trap instrument (Thermo Fisher Scientific, San Jose, California, USA), as described in detail elsewhere (Kodrik et al., 2010).

2.4. Synthetic peptides

The synthetic cardioacceleratory (= hypertrehalosaemic) hormone I of the American cockroach *P. americana* (denoted Peram-CAH-I) and the crustacean red pigment-concentrating hormone (denoted Panbo-RPCH) were purchased previously from Peninsula Laboratories (now Bachem Americas Inc., California, USA). The Ile^2-Panbo-RPCH analogue was custom-synthesized previously by PolyPeptide Laboratories s.r.o. (Praha, Czech Republic).

3. Results

3.1. Screening of existing literature for presence of Panbo-RPCH

3.1.1. Crustacea

We find no information on Panbo-RPCH as expressed peptide or as predicted peptide from expressed sequence tags (ESTs) or transcriptome shotgun assembly (TSA) sources in the crustacean classes Remipedia (Christie, 2014a), Branchiopoda (see below), Maxillopoda (for example, Christie, 2014c for Copepoda; Yan et al., 2012 for Cirripedia) or in Malacostraca other than in the orders Decapoda (see, for example, Gäde, 2009), Isopoda (Zrala et al., 2010) and Amphipoda (Christie, 2014d). In the Branchiopoda, order Cladocera, family Daphniidae and in the maxillopodan subclass Branchiura, family Argulidae, however, modified RPCHs have been identified. Three species of the genus *Daphnia*, i.e. *Daphnia pulex*, *Daphnia magna*, *Daphnia* (=*Ctenodaphnia*) *carinata* do express or genomically encode for a peptide, code-named Dappu-RPCH (Christie et al., 2008, 2010a,b; Marco and Gäde, 2010; Christie et al., 2011; Dircksen et al., 2011), which differs at three out of eight