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Identification and characterization of androgenic gland specific insulin-like peptide-encoding transcripts in two spiny lobster species: *Sagmariasus verreauxi* and *Jasus edwardsii*

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ABSTRACT

In this study we describe, for the first time in spiny lobsters, the androgenic gland and its putative hormone. The androgenic gland in crustaceans is the key regulator of crustacean masculinity. The transcript encoding the insulin-like androgenic gland specific factor has recently been identified and characterized in a number of decapod crustacean species including commercially important crabs, crayfish, prawns and shrimps. This insulin-like factor has proven to be the androgenic gland masculinizing hormone, and is absent in females. While the androgenic gland and its putative hormone have been identified in all other commercially valuable groups, none had been identified in lobsters. We identified and characterized the androgenic glands of two spiny lobster species (*Sagmariasus verreauxi* and *Jasus edwardsii*) and conducted a transcriptomic analysis of the *S. verreauxi* androgenic gland. Bioinformatics analysis led to the discovery and characterization of the insulin-like androgenic gland specific factors in both species studied. Changes in androgenic gland cell size and quantity between sub-adult and sexually mature males were evident. The transcriptomic database established for the *S. verreauxi* androgenic gland might enable to elucidate the mechanisms through which the insulin-like factor is secreted, transported to the target cells and how it triggers the physiological effects of sexual differentiation towards maleness and maintenance of the male gonad.

1. Introduction

The androgenic gland (AG) was first identified as a male reproductive accessory gland in a decapod crustacean species by Cronin in (1947). Its key role in crustacean masculinity was then established in many crustacean species from the class Malacostraca, initially by a ground-breaking series of studies by Charniaux-Cotton (reviewed in Charniaux-Cotton (1962)). By removing the gland from males and grafting into females in several species from different orders, opposite gender sexual characteristics transpired, suggesting that the AG regulates masculinity in all malacostracan crustacean species. After a long period where the nature of the compound/s produced and secreted by the AG was debated (e.g. Berreur-Bonnenfant et al., 1973; King, 1964), the first AG active protein extract was purified from a terrestrial isopod species by Hasegawa et al. in (1987). In 1999, this compound was sequenced and determined to be an N-glycosylated, insulin-like peptide (Martin et al., 1999; Okuno et al., 1999).

Malacostraca is the largest and most diverse class of crustaceans. Besides amphipods where the AG function was first established (Charniaux-Cotton, 1954) and isopods where the AG hormone (AGH) was first identified (Martin et al., 1999; Okuno et al., 1999), it also includes the commercially important decapods which include crabs (infraorder Brachyura), crayfish (infraorder Astacidea), clawed and spiny lobsters (infraorders Astacidea and Achelata, respectively), prawns and shrimps (infraorder Dendrobranchiata). In the years following the discovery of the insulin-like AGH attempts to identify homologous sequences from other species by means of degenerate primers polymerase chain reaction (PCR) were only successful in a few other isopod species (Ohira et al., 2003), but not in the commercially important decapods. The first decapod AG-specific insulin-like encoding sequence was retrieved through a suppressive subtractive hybridization cDNA library of the AG in the red claw crayfish *Cherax quadricarinatus* (Manor et al., 2007), followed by a similar library in the giant

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freshwater prawn *Macrobrachium rosenbergii*, where the function of the insulin-like AG factor (IAG) was established for the first time in decapods (Ventura et al., 2009). The transcript encoding *M. rosenbergii* IAG (*Mr-IAG*) was silenced via double stranded RNA (dsRNA) in males where both spermatogenesis and male secondary sexual characteristics were completely arrested throughout the silencing period. *Cq-IAG* silencing in *C. quadricarinatus* intersex individuals (with an arrested ovary and a female gonopore in one side and an active testis, a male gonopore and an AG in the other side) showed a shift toward feminine characteristics. The shift included testicular degeneration and ovarian activation (Rosen et al., 2010). IAGs have now been identified in as many as ten other decapod species based on homology, including two crab species (Chung et al., 2011; Sroyraya et al., 2010), another crayfish species (GenBank accession number ACD91988), four other prawn species (Banzai et al., 2012; Ma et al., 2013) and three shrimp species (Banzai et al., 2011; Li et al., 2012; Mareddy et al., 2011) (reviewed by Ventura et al. (2011)), but none in lobsters.

The commercial merit of manipulating the AG in decapod crustacean aquaculture has long been discussed with the aim of producing monosex populations, a desired commodity for many farmers. This could be achieved through intervention with the AG to manipulate phenotypic sex, regardless of the genetic background (Ventura and Sagi, 2012). A complete sex change of males into functional females using AG removal in decapods was reported by Sagi et al. (1986). This was later established as a biotechnological solution for all-male giant prawn production (Aflalo et al., 2006). Hindered by low success rates and lengthy and laborious quality control of the process, this technology was recently improved using genetic sex markers identified in prawns (Ventura et al., 2011a) and the ability to change genetic males into fully functional phenotypic females using silencing of Mr-IAG prior to the development of external sexual characteristics (Ventura et al., 2012). Attempts to translate this method for other decapod crustacean species have not yet been reported.

The spiny lobster (family Palinuridae) aquaculture industry is primarily reliant on wild catch or harvesting of juveniles and farming them to product size (Jeffs, 2010). Wild spiny lobster fisheries have been declining world-wide due to overfishing, habitat destruction, disease and climate change (Fitzgibbon, 2013; Jeffs, 2010). The ongoing endeavor to establish a commercially sustainable closed life cycle aquaculture for spiny lobsters has progressed significantly in recent years (Fitzgibbon and Battaglene, 2012; Cox et al., 2011), with two candidate species: the Eastern (*Sagmariasus verreauxi*) and the Southern (*Jasus edwardsii*) rock lobsters. *S. verreauxi* is the largest spiny lobster species in the world reaching sexual maturity at greater than 150–170 mm carapace length (~1.4–2.0 kg) whilst *J. edwardsii* is sexually mature at 60–120 mm carapace length (approximately 0.1–0.9 kg) (Booth, 2006). The aquaculture industry would benefit from novel biotechnologies that maximize production, including the possibility of generating monosex populations. In this study we report for the first time the identification of the AG in spiny lobsters, a transcriptome of the AG in *S. verreauxi* and characterization of AG-specific insulin-like encoding transcripts in *S. verreauxi* and *J. edwardsii*.

2. Materials and methods

2.1. Animals

Juvenile (3–5 g, with no gonad evident) and sub-adult (1.16–1.36 kg, with premature gonads) *S. verreauxi* individuals were cultured from egg and adults (3.5 kg) cultured from wild-caught puerulus at Institute for Marine and Antarctic Studies (IMAS), Hobart, Tasmania, Australia under previously described

Table 1
Primers used in this study.

Primer name	Primer sequence (5′–3′):
*Sv-IAG*_3′-RACE_F	CATGAGGACGGTGAAAGTCGATTGTG
*Sv-IAG*_5′-RACE_R	AGCGGAGGAGTCAAAGAGGAGGGTTT
*Je-IAG*_deg_F	GAYTTYGAYTGYGGNSAYYT
*Je-IAG*_5′-RACE_R	TTATCCTTCCATCAGGTAGCAG
*Sv-IAG*_ORF_F	ATGTTGGCGCCAATCCTGCTCA
*Sv-IAG*_ORF_R	TCCCTCCGTCAGAAAACAGTAATG
Sv16S_F	AAGGGGGTACTAGAGATTGTAAGATT
Sv16S_R	TTTGTTTTTCGTTCACCCATT

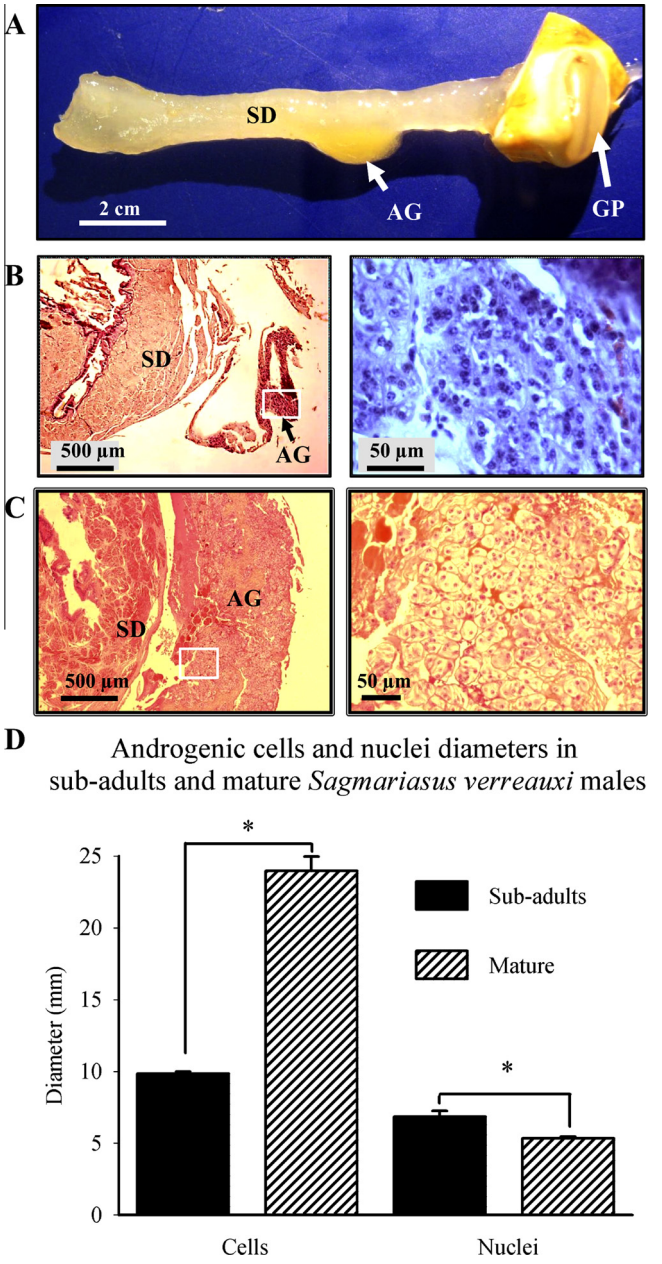

Fig. 1. Identification of the androgenic gland in a mature *S. verreauxi* male. (A) The distal part of *S. verreauxi* mature male reproductive tract (sperm duct – SD) with the androgenic gland (AG) firmly attached approximately 2 cm from the gonopore (GP). (B) Cross section of the SD with a small cluster of AG cells loosely attached in a sub-adult male stained with H&E. Right photo is magnification of the box in the left photo. (C) Cross section of the SD with AG firmly attached in a mature male stained with H&E. Right photo is magnification of the box in the left photo. (D) Cells and nuclei average diameters differ between sub-adults and mature *S. verreauxi* males (asterisk represent significance of $P < 0.05$ using one-way ANOVA).