

The decapod researcher's guide to the galaxy of sex determination

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Abstract Sex determination systems in *Animalia* encompass a diverse array of genes, functioning in complex regulatory networks. This diversity is even pronounced within taxonomic ranks and the crustacean Order *Decapoda* is no exception. The commercial importance of the decapods and the ambition to develop their potential in aquaculture has resulted in the necessity to better understand the processes of sexual development. However, due to a lagging understanding of the regulation of sex determination, systems characterised in other model species often serve as the basis for these investigations. This work presents a collated summary of the current information of sex determination in *Decapoda*, including all determined chromosomal mechanisms and identified “sex-regulator” homologues, often focussing on genes characterised in the model arthropod *Drosophila melanogaster* (namely *Sxl*, *Tra*, *Tra-2*, *Fru* and *Dsx*), the nematode *Caenorhabditis elegans* (*Fem-1* and *Mab-3*) and *Mammalia* (*Sry*, *Sox9*, *Foxl2* and

Dmrt1). Although homologue analyses such as these offer a good method to guide investigations in non-model species, the low conservation and variability of sex determination systems cautions against the assumption of conserved functionality. Thus, we propose a better suited approach to guide studies into sex determination in *Decapoda*, primarily relating to the functionally conserved sex regulators, the *Dmrts*.

Keywords Sex determination · Sex chromosome · *Malacostraca* · *Decapoda* · *Isopoda* · Doublesex and mab-3-related transcription factor (*Dmrt*)

Sexual differentiation in *Malacostraca*

The integrated signalling cascades of sexual development make it difficult to clearly distinguish the processes of sex determination from sexual differentiation (Matson & Zarkower, 2012). However, in the decapods (*Crustacea*, *Malacostraca*), as members of the *Malacostraca*, the onset of male sexual differentiation is more clearly defined, due to the unique involvement of a male-specific endocrine gland known as the androgenic gland (AG). First characterised in the sister Order, *Amphipoda* (*Crustacea*, *Malacostraca*), the AG was determined to be the sole regulator of male primary and secondary sexual differentiation (Charniaux-Cotton, 1954). It was then

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in a second sister Order, *Isopoda*, that the specific regulatory hormone was first isolated, defined as an insulin-like peptide and so termed the insulin-like androgenic gland hormone (AGH) (Hasegawa et al., 1987; Martin et al., 1999; Okuno et al., 1999; Suzuki, 1999).

As a sex-differentiating factor shared by all malacostracans, an understanding of the regulation of male sexual differentiation in *Decapoda* soon followed, now known to be regulated through a unique developmental axis known as the X-organ–sinus-gland neuroendocrine complex (XO–SG)—AG—Testis axis. In brief, the XO–SG (located in the eyestalk) secretes an array of neuropeptides including the gonad-inhibiting hormone (GIH) (also known as vitellogenesis-inhibiting hormone in females) and moult-inhibiting hormone (MIH) (Rodríguez et al., 2007; Nagaraju, 2011), both belonging to the crustacean hyperglycaemic hormone (CHH) family. In males, these neurohormones do not act directly on the gonad but instead modulate the proliferation of the AG; in the absence of their inhibitory signal, the AG develops bilaterally on each of the posterior sections of the sperm ducts (Charniaux-Cotton, 1954; Charniaux-Cotton, 1958; Charniaux-Cotton et al., 1966; Sagi et al., 1997). Once established, the male-specific AG is solely responsible for the synthesis and secretion of the insulin-like hormone, named IAG in the decapods, which in turn stimulates both testicular differentiation (Rodríguez et al. 2007) and the broad tissue effects of male sexual dimorphism through to full reproductive capacity (Manor et al., 2007; Martin et al., 1999; Okuno et al., 1999; Rosen et al., 2010; Ventura et al., 2009; reviewed in Ventura et al. (2011a)).

The resulting sexual systems

Although the male sex-differentiating influence of IAG is a unifying feature of malacostracans, the hormone's functional interaction with the broader networks of sexual development is not so conserved. While most decapods are gonochoristic, meaning that they develop into one distinct sex, multiple sexual systems have been described. First, there is a prevalence of complex gonochoristic species, where, although sex is genetically determined by a distinct genotype, simultaneous intersex (both male and female) phenotypes occur. This is particularly

pronounced in certain Infraorders, such as the *Astacidea* (Grilo and Rosa, 2017); an example being the red-claw crayfish (Sagi et al., 1996), which can present as one of the seven distinct intersex phenotypes, all of which develop from a ZW (female) genotype (Parnes et al., 2003).

There are also examples of protandrous hermaphroditic species, where individuals are born as males and later develop into females. This appears to be common in the *Caridea*, such as the Peppermint shrimp (*Lysmata wurdemanni*) (Bortolini & Bauer, 2016; Zhang et al., 2017) and is a process regulated through the sex-differentiating effects of IAG (Zhang et al. 2017). Such hermaphroditism has also been documented in the *Thalassinidae* and *Anomura* (Subramoniam, 2017). To complicate things further, the shrimp species, *L. wurdemanni*, can also exist as a simultaneous hermaphrodite, where an individual can function as a male and a female at any one time (a protandrous simultaneous hermaphrodite) (Bauer & Holt, 1998).

More staggeringly, the Order also contains one documented parthenogenic species. The marbled crayfish (*Procambarus fallax* forma *virginalis*), only exists as female, of which all offspring is genetically identical to the mother and therefore, female (Scholtz et al., 2003; Vogt et al., 2015). Finally, to truly demonstrate the plasticity of the IAG-mediated system, one should consider the sister Order, *Isopoda*, where the influence of the AG is superseded by the feminisation effects of the bacterial endosymbiont, *Wolbachia*. This parasitism is thought to prevent the initial differentiation of the male-specific AG (Rigaud et al., 1997; Bouchon et al., 2008; Cordaux et al., 2011), allowing for female development. So dramatic are these AG-disruptive effects, not only do they result in total feminisation of ZZ males but ultimately, the loss and re-emergence of the W chromosome (Leclercq et al., 2016; Cordaux & Gilbert 2017).

How is this diversity in sexual systems achieved?

Based on our understanding of the conserved mode of sexual differentiation amongst these species, as well as the resulting diversity of sexual systems, the regulatory axis of the malacostracan AG, although ubiquitous, is certainly labile. This suggests that the preceding regulation of sex determination, which

serves as the foundation of sexual development within each species, may be responsible for the diversity of sexual systems observed. Sex determination mechanisms are known to be highly divergent across *Animalia*, even across closely related species, particularly amongst those genes acting at the very top of the genetic cascades, the master sex determinants (Bachtrog et al., 2014). This variability is owing to the relaxed evolutionary restraint on sex determination genes (Meiklejohn et al., 2003; Parsch & Ellegren 2013), readily apparent in the increased rates of several fundamental evolutionary phenomena: (1) Gene duplication and neofunctionalization: examples are found in the master sex determinants of medaka (*Oryzias latipes*) (Matsuda et al., 2002; Nanda et al., 2002) and the African clawed frog (*Xenopus laevis*) (Yoshimoto et al., 2008), both of which have evolved through the sex-specific gene duplication of autosomal *Dmrt1* genes. Neofunctionalization can result in the recruitment of entirely unrelated genes, such as the immune-related gene duplicate *SdY*, acting as the master sex determinant in the rainbow trout (Yano et al., 2012) and more staggeringly still, the *Wolbachia* bacterial element gained through horizontal gene transfer to become the master female sex determinant in the common pill bug (*Armadillidium vulgare*) (Leclercq et al., 2016). (2) Hierarchical rearrangement: resulting in the loss of function between seemingly well conserved genes across species, such as the master sex determinant *Sxl* in *Drosophila melanogaster*, a gene which has been expelled from the master regulatory role in the fly species *Musca domestica* and *Ceratitis capitata* (Meise et al., 1998), where it actually lacks any role in sex determination. (3) The increased occurrence of functional mutations: like the single nucleotide mutation in the male pufferfish (*Takifugu rubripes*) anti-Müllerian hormone receptor (*Amhr2*), a polymorphism which now acts as the master male sex-determining signal (Kamiya et al., 2012). (4) Altered translational and temporal gene expression patterns (Bachtrog et al., 2014; Beukeboom & Perrin 2014) and (5) epigenetic effects (Piferrer 2013). These factors culminate to make the characterisation of each sex determination system highly cryptic.

Thus, considering the diversity of sexual systems amongst *Decapoda* and the apparent plasticity of IAG endocrinology, it is apparent that to fully understand the regulation of sexual development, we must

integrate the genetic networks that serve as the basis of each system. Furthermore (knowing the evolutionary characteristics and diversification of sex determination genes) to gain an accurate understanding of this highly complex regulatory process, this characterisation must be tackled on a species-by-species basis. In response, this review has collated the current research findings regarding the molecular basis of sex determination in *Decapoda*, in an attempt to serve both as a reference for preliminary investigations and to provide a critical evaluation of the current data. To provide evolutionary context, we have included the sister Order *Isopoda* (*Crustacea*, *Malacostraca*), which shares the commonality of the AG and the model crustacean species *Daphnia pulex* and *D. magna* (*Crustacea*, *Branchiopoda*) from the sister Class *Branchiopoda*. It is thought that *Insecta* evolved from the freshwater branchiopod crustaceans around 410 MYA (Glennier et al., 2006). Hence the *Daphnia* sp. are included as a phylogenetic link between the decapods and the insects (*Hexapoda*, *Insecta*), from which a significant proportion of the model sex determination genes have been characterised.

The genetic (chromosomal) modes of sex determination characterised to date are presented, followed by a list of candidate genes implicated in sex determination in *Decapoda*. These genes were primarily identified through transcriptomic homologue screening and therefore the list is heavily biased by systems characterised in the model species *D. melanogaster*, *Caenorhabditis elegans* and *Mammalia*. Given the high evolutionary rates of sex determination systems, this review highlights the significance of functional genomics to ensure that genes are assigned with appropriate functions. To reiterate such, we have included a brief functional description of the genes most commonly targeted for homologue analyses. It is of note that there is only a single gene family common to all three model systems: namely *Drosophila-Dsx*, *C. elegans Mab-3* and *Mammalia-Dmrt1* (denoted with an * in Table 2). These genes are from the DM domain transcription factor family, collectively known as the *doublesex and male abnormal-3-related transcription factors (Dmrts)*.

Functional definitions of sex-regulator genes in model species

The fruit fly, *Drosophila melanogaster*
(Arthropoda, Insecta)

Sex lethal: pre-mRNA splicing protein

Sex lethal (Sxl) is a splicing factor, defined by an RNA-binding domain. This gene is responsive to the higher X: Autosome chromosome ratio in females. The X chromosome gene products (e.g. *Runt*, *Sisterless-A* and *Sisterless-B*) bind to the *Sxl* promoter and induce its activation. Thus, Sxl is the master sex determinant for female development. Its primary target is *Tra* mRNA (Hashiyama et al., 2011; Beukeboom & Perrin, 2014).

Transformer: pre-mRNA splicing protein

Transformer (Tra) is an mRNA splicing factor, defined by an RNA-recognition motif and an arginine/serine-rich domain followed by a proline-rich region. The action of Sxl results in the active splice variant of *Tra* in females. Tra then acts as a downstream splicing factor, regulating female-specific splicing of target RNAs (Kulathinal et al., 2003).

Transformer-2: pre-mRNA splicing protein

Transformer-2 (Tra-2) is an mRNA splicing factor, defined by an RNA-recognition motif and arginine/serine-rich domain. Tra-2 is also a downstream splicing factor, which is constitutively produced (in both males and females) but only in females can it act in concert with Tra to regulate female-specific splicing of target RNAs. A primary target of Tra and Tra-2 is *Dsx* mRNA (Amrein et al., 1990).

*Doublesex: DM domain, zinc finger protein (transcription factor)**

Doublesex (Dsx) is a transcription factor defined by a zinc finger domain, termed the DM domain. In the presence of the Tra–Tra-2 complex, the female splice variant of *Dsx* is produced, generating the Dsx^F protein. Dsx^F works in concert with an array of regulatory genes as the major effector of sexual

development (Hoshijima et al., 1991; Beukeboom & Perrin 2014).

Fruitless: zinc finger protein (transcription factor)

Fruitless (Fru) is a transcription factor defined by its zinc fingers. *Fru* is a male-promoting gene, regulating development of the male central nervous system and male sexual behaviour. *Fru* is spliced in the absence of the female Tra–Tra-2 complex, carried out by non-sex-specific splicing machinery (Billeter et al., 2006).

The nematode, *Caenorhabditis elegans*
(Nematoda, Chromadorea)

Feminization of XX and XO animals-1: Ankyrin repeat-containing protein

Feminization of XX and XO animals-1 (Fem-1) is a second messenger protein, defined by ankyrin repeats. It acts in male sex determination, as a signal-transducing regulator between the membrane receptor Tra-2 (not to be confused with the mRNA splicing factor, Tra-2 in *Drosophila*) and the transcription factor Tra-1. It works in concert with Fem-2 and Fem-3 (Yi et al., 2000; Haag 2005).

*Male Abnormal-3: DM domain, zinc finger protein (transcription factor)**

Male Abnormal-3 (Mab-3) is a transcription factor defined by a novel zinc finger domain, termed the DM domain. In males, Her-1 binds the Tra-2 receptor, releasing Fem-1,2,3 which then sequester Tra-1. This in turn activates the major effector *Mab-3*. *Mab-3* is vital for the male sexual differentiation of the peripheral nervous system and the intestine, causing the repression of vitellogenesis (Yi et al., 2000; Haag 2005).

Mammals (Chordata, Mammalia)

Sex-determining region Y: HMG-box DNA-binding protein (transcription factor)

Sex-determining region Y (Sry) is the master sex determinant in males. Sry, along with SF-1, binds and activates *Sox9*. Its function is transient, after its activation of *Sox9*, other major effector genes are

responsible for the continuity of its signal (Sinclair et al., 1990; Matson et al., 2011).

Sex-determining region Y-box 9: HMG-box DNA-binding protein (transcription factor)

Sex-determining region Y-box 9 (Sox9) is a transcription factor defined by its Sry-related HMG-box. Once activated by Sry it is responsible for upregulating the expression of the male-promoting *Dmrt1* via Fgf9. These genes then function in an auto-regulating loop, maintaining their own expression, stimulating (and maintaining) the male-specific programme of development (Jakob & Lovell-Badge 2011; Matson et al., 2011).

*Doublesex and male abnormal-3-related transcription factor 1: DM domain, zinc finger protein (transcription factor)**

Doublesex and male abnormal-3-related transcription factor 1 (Dmrt1) is a transcription factor defined by a novel zinc finger domain, termed the DM domain. It is upregulated in response to Sox9 via Fgf9. Dmrt1 works in concert with an array of regulatory genes as the major effector of male sexual differentiation, specifically testicular development and maintenance. It also acts to inhibit the female developmental pathway (e.g. by suppressing *Foxl2*) (Matson et al., 2011).

Forkhead box L2: Forkhead box DNA-binding protein (transcription factor)

Forkhead box L2 (Foxl2) is a transcription factor that is defined by a unique DNA-binding domain. In the absence of the Sry-driven expression of *Sox9*, *Foxl2* expression ensues and acts to inhibit the male pathway, whilst promoting the female pathway through the action of *Rspo1*, *Wnt4* and *B-catenin* (Matson et al., 2011).

Acting at the top of the cascade: the sex chromosomes















The summary presented in Table 1 provides clear demonstration of the diversity of sex determination modes present in *Crustacea*. Both *D. pulex* (Crease

et al., 1989; Chen et al., 2014) and *D. magna* (Kleiven et al., 1992; Kato et al., 2011) are subject to environmental sex determination, through cyclic parthenogenesis. This contrasts to the decapods where, thus far, genetic sex determination is common across the Order, although both XY/XX (heterogametic male) and ZW/ZZ (homogametic male) mechanisms exist sporadically across families (Chandler et al., 2016b). In *Isopoda*, male homogamety (ZZ) seems to be more common than male heterogamety (XY) (Becking et al., 2017). There is also strong evidence for frequent sex chromosome turnover, with an estimated three to thirteen heterogametic transitions occurring, accounting for the dramatic XY and ZW diversity observed within *Genera* (Becking et al., 2017).

This suggests that the disruptive effects of *Wolbachia* have had significant consequence on the evolution of sex determination mechanisms in isopods. Furthermore, the dramatic influence of *Wolbachia* has given rise to a third, distinct mode of sex determination: cytoplasmic. Genetic and environmental (including social) modes of sex determination are both extensively characterised across species (Beukeboom & Perrin 2014), but cytoplasmic sex determination appears to be specific to the *Crustacea*, resulting from the feminising effect of parasites (Rigaud et al., 1997). Hence, this mode of inheritance described in the isopods, accounts for a third mode of sex determination in the already diverse regulation of sexual development in *Crustacea*.

One unifying factor shared by the malacostracans, is the potential for sex to be manipulated, whether by parasitic infection, acting to prevent the differentiation of the AG in males (*Isopoda*) (Rigaud et al., 1997; Bouchon et al., 2008; Cordaux et al., 2011), or the RNAi-induced suppression of IAG expression (*Decapoda*) (Ventura et al., 2012); both causing fully functional feminisation. Taken together, this suggests that both sexes, irrelevant of their genetic background, have the genetic potential to develop as male or female, suggesting that the sex chromosomes must be predominantly homologous. This is further supported by the viability of WW females and YY males observed in both isopods (Rigaud et al., 1997; Becking et al., 2017) and decapods (Shpak et al., 2016), therefore suggesting that the sex chromosomes of these Orders are in the primary stages of differentiation (Charlesworth 1991; Rigaud et al., 1997),

Table 1 Modes of sex determination in *Branchiopoda*, *Isopoda* and *Decapoda*. Unless otherwise indicated, modes are genetic

Mechanism	Taxonomy	Species	Reference
Environmental: cyclic parthenogenesis	<i>Branchiopoda</i>	 <i>Daphnia pulex</i>	Crease et al. (1989); Chen et al. (2014)
Environmental: cyclic parthenogenesis	<i>Branchiopoda</i>	 <i>Daphnia magna</i>	Kleiven et al. (1992); Kato et al. (2008)
Genetic: ZZ/ZW homogametic ♂ ZZ ♂ + f = ZZ ♀ Cytoplasmic: ZZ ♂ + Wolbachia = ♀ viable WW ♀	<i>Malacostraca</i> <i>Isopoda</i> Oniscidea	 <i>Armadillidium vulgare</i>	Rigaud et al. (1997); Cordaux et al. (2011); Leclercq et al. (2016); Becking et al. (2017); Cordaux and Gilbert (2017)
Genetic: XY/XX heterogametic ♂ (diversity achieved through heterogametic transitions) viable YY ♂ [§]	<i>Malacostraca</i> <i>Isopoda</i> Oniscidea	 <i>Armadillidium nasatum</i> <i>Armadillo officinalis</i> <i>Asellus aquaticus</i> <i>Helleria brevicornis</i> [§] <i>Porcellio dilatatus dilatatus</i> [§]	Juchault and Legrand (1964); Rocchi et al. (1984); Becking et al. (2017)
Genetic: ZZ/ZW homogametic ♂ (diversity achieved through heterogametic transitions) viable WW ♀	<i>Malacostraca</i> <i>Isopoda</i> Oniscidea	 <i>Armadillidium depressum</i> <i>A. granulatum</i> <i>A. maculatum</i> <i>A. nasatum</i> <i>Eluma purpurascens</i> <i>Oniscus asellus</i> <i>Porcellio dilatatus petiti</i> <i>P. laevis</i> <i>P. scaber</i> <i>Trachelipus rathkei</i>	Juchault and Legrand (1972); Legrand et al. (1974); Juchault and Legrand (1979); Mittal and Pahwa (1980); Mittal and Pahwa (1981); Juchault and Rigaud (1995); Becking et al. (2017)
XY/XX heterogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Achelata	 Spiny lobster <i>Sagmariasus verreauxi</i> (Eastern) <i>Panulirus marginatus</i> (Hawaiian) (?)	Chandler et al. (2017) Shaklee (1983)
ZZ/ZW homogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Caridea	 Freshwater prawn <i>Macrobrachium rosenbergii</i>	Sagi and Cohen (1990); Malecha et al. (1992); Ventura et al. (2011b); Jiang and Qiu (2013)
X ₁ X ₁ X ₂ X ₂ X ₃ X ₃ X ₁ X ₂ Y heterogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Caridea	 Rockpool prawn <i>Palaemon elegans</i>	Torrecilla et al., (2017)
Absence of heteromorphic sex chromosomes	<i>Malacostraca</i> <i>Decapoda</i> Caridea	 Common prawn <i>P. serratus</i>	Torrecilla et al., (2017)
ZZ/ZW homogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Astacidea	 Red-claw crayfish <i>Cherax quadricarinatus</i>	Parnes et al. (2003)
XY/XX heterogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Astacidea	 European freshwater crayfish <i>Austropotamobius pallipes</i> <i>A. torrentium</i>	Mlinarec et al. (2016)
ZZ/ZW homogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Dendrobranchiata	 Penaeid shrimp <i>Fenneropenaeus chinensis</i> <i>Litopenaeus vannamei</i> <i>Marsupenaeus japonicus</i> <i>Penaeus monodon</i>	Li et al. (2003) Zhang et al. (2007); Gopal et al. (2010) Li et al. (2003); Preston et al. (2004); Coman et al. (2008) Benzie et al. (2001); Preechaphol et al. (2007)
ZZ/ZW homogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Brachyura	 Chinese mitten crab <i>Eriocheir sinensis</i>	Cui et al. (2015)
XY/XX heterogametic ♂	<i>Malacostraca</i> <i>Decapoda</i> Brachyura	 Crucifix crab <i>Charybdis feriatius</i>	Trino et al. (1999)

perhaps only differing in the one master sex-determining gene responsible for flipping the male/female switch.

The mechanism: evaluating the role of sex-regulator genes

The data presented in Table 2 clearly illustrate how transcriptomics (and genomics) have greatly advanced the identification of new gene families in the decapods. From an evolutionary perspective, this has enhanced our understanding of the divergence of these genes both within the Order and across *Animalia*. However, with regards to the functional context of sexual development, these data must be interpreted with a critical understanding of the evolutionary rates that define sex-determining genes.

The *Tra* homologues provide an apt example: the *Tra* genes are known to be highly divergent in sequence even amongst *Drosophila* sp. (Kulathinal et al., 2003); in other non-*Drosophilid* fly species such as the housefly (*M. domestica*) (Hediger et al., 2010) and medfly (*C. capitata*) (Pane et al., 2002) the *Tra* orthologues show functional divergence, having evolved up the hierarchy (expelling *Sxl*) to adopt the master sex-determining role; and in the distantly related decapods, thus far *Tra* homologues appear to be lacking.

There is some level of conservation within *Insecta*, as a functional *Tra* orthologue (termed *Feminiser*, *Fem*) also acts as the female master sex determinant in the honey bee (*Apis mellifera*) (Hasselmann et al., 2008). *Fem* lacks complete conservation of the sequence motifs described in *Drosophila* but does contain the same arginine/serine and proline-rich domain organisation and one conserved sequence motif, also described in *C. capitata* (Pane et al., 2002). Although diverged in both sequence and function across these three species (telling of significant independent evolution over the ~ 300 million years that separates the Orders), the conserved role of the three orthologues is indicative of a *Tra*-based ancestral pathway of sex determination in *Insecta* (Hasselmann et al., 2008). Furthermore, the discovery of *Fem* suggests that the complementary sex determiner (*csd*) gene may not be the universal master sex determinant across hymenopteran insects (bees, ants and wasps), as

was previously thought (Hasselmann et al., 2008; Heimpel & Boer 2008).

It is interesting to note that the brachiopods *D. pulex* (Chen et al., 2014) and *D. magna* (Kato et al., 2010) do have *Tra* homologues; however, both show even greater divergence in domain organisation from that of *Drosophila* *Tra*. Furthermore, it is only in *D. magna* that a function in sexual development has been suggested, not in the female sex determination pathway (as with the insect sp.), but in the maintenance of male phenotype (Chen et al., 2014). This evolutionary emergence and divergence of *Tra* is in support of the proposed decapod—branchiopod—hexapod trajectory of the *Pancrustacea* (Glenner et al., 2006). A similar story is true of the highly conserved *Sxl* splice variants, which although seemingly well conserved in sequence throughout *Insecta* and *Decapoda* (Table 2), have not retained conserved functionality. These evolutionary patterns exemplify why the functional conservation of the genes summarised in this work need to be appropriately evaluated; a conserved function to that described in each model species should not be assumed a priori.

Dmrts: the functionally conserved sex regulators

There is however, a gene family that does display the functional conservation that appears to be somewhat assumed in other cases: the *Dmrts*. Indeed, the *Dmrts* are the only gene family with a conserved function in sex determination across *Animalia* (Kopp 2012; Beukeboom & Perrin 2014), being identified in all investigated species to date, with the only exception being the sponge *Amphimedon queenslandica* (Wexler et al., 2014) (which may well reflect its lack of tissue differentiation). There has been good progress in the identification of *Dmrts* in the decapods, with homologues identified in seven species comprising the *Penaeidae* (prawns), *Palinuridae* (lobsters), *Palaeomonidae* (shrimp) and the *Portunidae* (crab). Although from our data collation, they appear yet to be identified in the isopods. Indeed, this work may suggest that the characterisation of the *Dmrts* in *Isopoda* is a promising avenue of further study regarding the mechanism by which the *f* element disrupts the native pathway of sexual development.

The functional conservation of the model genes characterised in *D. melanogaster* (*Dsx*), *C. elegans*

Table 2 Sex-regulator homologues identified in *Decapoda*, based on model systems characterised in *Drosophila melanogaster*, *Caenorhabditis elegans* and *Mammalia*; (p) denotes partial sequences and (?) indicates lack of conclusive data

Gene	Species	Expression pattern / function	Reference/GenBank accession number
DM domain (<i>Dmrt</i>)*: characterised across <i>Animalia</i>, model species include <i>D. melanogaster Doublesex (Dsx)</i>, <i>C. elegans Male abnormal 3 (Mab-3)</i> and Mammalian <i>Doublesex and mab-3-related transcription factor 1 (Dmrt1)</i> A transcription factor defined by novel zinc finger domain, termed the DM domain: hold a conserved function in sex determination and differentiation, often adopting a downstream major effector role.			
<i>Dmrt3</i> (<i>Dmrt93B</i> -like)	<i>Daphnia pulex</i>	No data (identified in sequenced genome)	Colbourne et al. (2011) <i>Dmrt3</i> : EFX89054.1
<i>DapmaDmrt11E</i> <i>DapmaDmrt99B</i> <i>DapmaDmrt99B (DM-less)</i> <i>DapmaDmrt93B</i>	<i>Daphnia magna</i>	<i>Dmrt11E</i> and <i>Dmrt99B</i> ovary biased <i>Dmrt93B</i> testis specific Not involved in SD	Kato et al. (2008) <i>DapmaDmrt11E</i> : BAG12871.1 <i>DapmaDmrt99B</i> : BAG12873.1 <i>DapmaDmrt93B</i> : BAG12872.1
<i>DapmaDsx1αβ</i> <i>DapmaDsx2</i>	<i>Daphnia magna</i>	<i>Dsx1</i> and <i>Dsx2</i> male-biased <i>Dsx1</i> responsible for male SD	Kato et al. (2011) <i>DapmaDsx1α</i> : BAJ78307.1 <i>DapmaDsx1β</i> : BAJ78308.1 <i>DapmaDsx2</i> : BAJ78309.1
	<i>Isopoda</i>	<i>None identified</i>	
<i>Sv-iDmrt1</i> <i>Sv-Dmrt11E</i> <i>Sv-Dsx</i> <i>Sv-DMA</i> (p)	Eastern spiny lobster <i>Sagmariasus verreauxi</i>	<i>Sv-iDmrt1</i> : gonad and antennal gland- biased <i>Sv-Dmrt11E</i> : AG-biased <i>Sv-Dsx</i> : gonad, antennal gland and AG <i>Sv-DMA</i> : gonad specific	Chandler et al. (2016a) <i>Sv-iDmrt1</i> : KY427006 <i>Sv-Dmrt11E</i> : KY427008 <i>Sv-Dsx</i> : KY427007!
<i>Sv-iDMY</i>	Eastern spiny lobster <i>Sagmariasus verreauxi</i>	<i>Sv-iDMY</i> : male genome specific paralogue of <i>iDmrt1</i> <i>iDMY</i> > <i>iDmrt1</i> during embryogenesis and acts as dominant negative suppressor to transcriptionally active <i>iDmrt1</i> Authors suggest <i>Sv-iDMY</i> to be master sex determinant	Chandler et al. (2017) <i>Sv-iDMY</i> : KY427009!
<i>Dmrt1</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	No data (identified in pooled tissue library)	Ma et al. (2012)
<i>MroDmrt11E</i> <i>MroDmrt99B</i>	Freshwater prawn <i>Macrobrachium rosenbergii</i>	Increasing in developmental stages Testis biased in adults <i>MroDmrt11E</i> stimulates expression of <i>MroLAG</i>	Yu et al. (2014) <i>MroDmrt11E</i> : AH147024.1 <i>MroDmrt99B</i> : AH147025.1
<i>Dsx</i>	Whiteleg shrimp <i>Litopenaeus vannamei</i>	Expression in testis > ovary	Peng et al. (2015) TSA: GDUV00000000 Unigene35364
<i>Dsx</i>	Chinese white shrimp <i>Fenneropenaeus chinensis</i>	No data (direct NCBI submission)	Li et al. (2012) <i>Dsx</i> : AFU60552.1
<i>Dmrt1</i> (p) (<i>Dmrt11E</i> -like)	Banana shrimp <i>Fenneropenaeus merguensis</i>	Specific to hepatopancreas	Powell et al. (2015)
<i>Dmrt5/A2</i> (p) <i>Dmrt2a</i> (p) (<i>Dmrt11E</i> -like) <i>Dmrt2b</i> <i>Dmrt2c</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	No data (direct NCBI submission) All sequences are partial	Wang et al. (2007) <i>Dmrt5</i> (p): ABP04228.1 <i>Dmrt2a</i> (p): ABP04225.1 <i>Dmrt2b</i> (p): ABP04226.1 <i>Dmrt2c</i> (p): ABP04227.1
<i>EsDmrt-like</i> (<i>Dmrt99B</i> -like)	Chinese mitten crab <i>Eriocheir sinensis</i>	Testis specific, greater in immature testis	Zhang and Qiu (2010) <i>EsDmrt-like</i> : ADH15934.1
<i>Dmrt1</i> <i>Dsx</i> <i>Dmrt2</i> (<i>Dmrt11E</i> -like) <i>Dmrt5/A2</i> <i>Dmrt11E</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	<i>Dmrt1</i> specific to first juvenile instar (post sex-determination), located on putative autosome Not involved on SD <i>Dsx</i> specific to megalopa and first juvenile instar	Cui et al. (2015)
<i>EsDmrt2</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	Low comparable expression in males and females (RPKM < 3) Authors describe a >1.5x FC in males (whole body male and female transcriptomes)	Liu et al. (2015)
<i>Dmrt1</i> (p) (<i>Dmrt99B</i> -like)	Mud crab <i>Scylla paramamosain</i>	Testis specific (only ovary and testis tissue analysed) Authors note conserved testis specificity with <i>E. sinensis</i>	Gao et al. (2014)

Table 2 continued

Sxl: characterised in <i>D. melanogaster</i> Splicing factor, defined by RNA binding domain: acts as master sex determinant for female sex determination.			
<i>Sxl1</i> <i>Sxl2</i>	<i>Daphnia pulex</i>	No data (genome)	Colbourne et al. (2011) <i>Sxl1</i> : EFX75394.1 <i>Sxl2</i> : EFX75395.1
	<i>Isopoda</i>	None identified	
<i>Sv-Sxl1</i> <i>Sv-Sxl2</i>	Eastern spiny lobster <i>Sagmariasus verreauxi</i>	<i>Sxl1</i> and <i>Sxl2</i> show broad male and female expression	Chandler et al. (2016a)
<i>MnSxl1</i> <i>MnSxl2</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	<i>Sxl1</i> and <i>Sxl2</i> show broad male, female and developmental expression Authors suggest a function in development	Zhang et al. (2013b) <i>MnSxl1</i> : AGI44577.1 <i>MnSxl2</i> : AGI44578.1
<i>MrSxl1</i> <i>MrSxl2</i> <i>MrSxl3</i>	Freshwater prawn <i>Macrobrachium rosenbergii</i>	No data (direct NCBI submission)	McMillan et al. (2015) <i>Sxl1</i> : APO14321.1 <i>Sxl2</i> : APO14322.1 <i>Sxl3</i> : APO14323.1
<i>Sxl1</i>	Banana shrimp <i>Fenneropenaeus merguensis</i>	Broad male and female expression	Powell et al. (2015)
<i>EsSxl1</i> <i>EsSxl2</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	<i>Sxl1</i> and <i>Sxl2</i> show broad male and female expression Not a female sex-determinant	Shen et al. (2014) <i>Sxl1</i> : AHA33390.1 <i>Sxl2</i> : AHA33391.1 Cui et al. (2015)
Tra: characterised in <i>D. melanogaster</i> A specialised downstream mRNA splicing factor, defined by an RNA recognition motif and an arginine/serine rich domain followed by a proline rich region: acts as a female-specific splicing factor in female sex determination (functions in complex with Tra-2).			
<i>DptrA</i>	<i>Daphnia pulex</i>	Different domain organisation from insect Tra. Higher expression in sexually reproductive males, with correlative spatial expression patterns in male-specific appendages DptrA may influence change in reproductive state and maintain male-phenotype	Chen et al. (2014) <i>DptrA</i> : KC685625.1
<i>DmagTra</i>	<i>Daphnia magna</i>	Different domain organisation from insect Tra. No sexual-dimorphic expression or splicing No function in sex-determination	Kato et al. (2010) <i>DmagTra</i> : BAI66432.1
	<i>Isopoda</i>	None identified	
	<i>Decapoda</i>	None identified	
Those genes identified in <i>D. pulex</i> and <i>D. magna</i> are the only examples in <i>Crustacea</i> (Shen et al., 2014). Even within <i>Insecta</i> , <i>Tra</i> is a rapidly evolving element of sex determination and is one of the most divergent sex-regulatory proteins (Kulathinal et al., 2003). For example, in the housefly (<i>Musca domestica</i>) (Hediger et al., 2010) and medfly (<i>Ceratitis capitata</i>) (Pane et al., 2002) where <i>Sxl</i> is non-functional (Meisse 1998), <i>Tra</i> has adopted the master female sex-determining role.			
Tra-2: characterised in <i>D. melanogaster</i> A downstream mRNA splicing factor, defined by RNA recognition motif and an Arginine/Serine rich domain: acts as a splicing factor for female sex-determination in concert with Tra			
<i>Tra-2</i>	<i>Daphnia pulex</i>	No data (genome)	Colbourne et al. (2011) <i>Tra-2</i> : EFX90042.1
<i>Tra-2</i> (17 isoforms)	<i>Daphnia magna</i>	No data (direct submission)	Gilbert et al. (2015) Bioproject: IPRJNA284518
	<i>Isopoda</i>	None identified	
<i>Sv-TRA-2</i> 9 protein isoforms	Eastern spiny lobster <i>Sagmariasus verreauxi</i>	Basal level or testis-biased expression	Chandler et al. (2016a)
<i>Mntra-2</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	Broad male, female and developmental expression	Zhang et al. (2013a) <i>Mntra-2</i> : AGI50962.1
<i>FcTra-2a</i> <i>FcTra-2b</i> <i>FcTra-2c</i> (alternatively spliced)	Chinese white shrimp <i>Fenneropenaeus chinensis</i>	<i>FcTra-2c</i> shows biased expression in post-larval developmental stages, female juveniles and ovary in adult Authors suggest a function in female sex-determination	Li et al. (2012) <i>FcTra-2a</i> : AFU60540.1 <i>FcTra-2b</i> : AFU60544.1 <i>FcTra-2c</i> : AFU60542.1
<i>Tra-2b</i> <i>Tra-2c</i>	Banana shrimp <i>Fenneropenaeus merguensis</i>	Broad expression in males and females	Powell et al. (2015)
<i>PMTRA-2</i> <i>PMTRA-2v</i> (variant)	Giant tiger shrimp <i>Penaeus monodon</i>	Increasing with development and gonad biased in adults	Leelatanawit et al. (2009) <i>PMTRA-2</i> : ACD13597.1 <i>PMTRA-2v</i> : ACD13598.1

Table 2 continued

<i>TRA-2</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	Broad male and female expression	Cui et al. (2015)
<i>EsTRA-2</i> <i>EsTRA-2v</i> (variant)	Chinese mitten crab <i>Eriocheir sinensis</i>	Similarly expressed in males and females (whole body male and female transcriptomes) Authors highlight that all <i>Drosophila Sxl-Tra-2-Dsx</i> elements have been identified in <i>E. sinensis</i> suggesting a similar sex determination pathway may exist.	Liu et al. (2015)
		These conclusions must be interpreted with caution, considering the lack of TRA and female sex-specificity SXL.	
It must be remembered that in <i>D. melanogaster</i> Tra and Tra-2 work in concert to regulate the female specific splicing of <i>Dsx</i> . Considering that no Tra homologues have been identified in decapods, it is highly unlikely that Tra-2 will hold a conserved function to that described in <i>Drosophila</i> Sp.			
Fem-1: characterised in <i>C. elegans</i>			
A second messenger protein, defined by ankyrin-repeats: acts in male sex-determination as signal-transducing regulator between the membrane protein Tra-2 and the transcription factor Tra-1, works in concert with Fem-2 and Fem-3.			
	<i>Daphnia pulex</i>	None identified	
<i>Fem-1 A</i> <i>Fem-1 B</i> <i>Fem-1 C</i> (multiple isoforms)	<i>Daphnia magna</i>	No data (direct submission)	Gilbert et al. (2015) Bioproject: PRJNA284518
	<i>Isopoda</i>	None identified	
<i>Fem-1</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	No data (identified in pooled tissue library)	Ma et al. (2012); Jin et al. (2013); Qiao et al. (2015)
<i>MnFem-1</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	<i>MnFem-1</i> specific to ovary (immature and mature) <i>MnFem-1</i> RNA in follicular cells and <i>MnFem-1</i> protein in vitellogenic oocyte Authors suggest a role in oogenesis/vitellogenesis <i>MnFem-1</i> expression in unfertilised egg to cleavage which precedes <i>IAG</i> expression in blastula Authors suggest a role in female sex determination/sexual differentiation; also suggest maternal inheritance (also suggested in <i>E. sinensis</i>).	Ma et al. (2016a)
<i>MnFem1b</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	<i>MnFem1b</i> expressed in all tissues and male biased in all, most significantly in gonads (testis) <i>MnFem1b</i> expressed through development, peaking after metamorphosis Authors note conservation of testis-bias of <i>Fem1b</i> with <i>E. sinensis</i> Authors suggest a role in metamorphosis and external sexual differentiation	Rahman et al. (2016) <i>MnFem1b</i> : KT258023
<i>Fem-1</i>	Whiteleg shrimp <i>Litopenaeus vannamei</i>	No data (direct NCBI submission)	Galindo-Torres et al. (2013) <i>Fem-1</i> : AHA90856.1
<i>Fem-1-like</i> 7 isoforms	Whiteleg shrimp <i>Litopenaeus vannamei</i>	Expression testis > ovary	Peng et al. (2015) TSA Database: GDUV00000000
			Unigene24042, Unigene9434, Unigene9463, Unigene24047, Unigene11407, Unigene2036, Unigene15025
<i>Fem-1</i>	Banana shrimp <i>Fenneropenaeus merguensis</i>	Broad expression in males and females	Powell et al. (2015)
<i>Fem-1c</i>	Giant tiger shrimp <i>Peneaus monodon</i>	No data (genomic study) Pattern of SNP segregation suggests gene is within sex determining locus region on chromosome	Robinson et al. (2014)
<i>EsFem-1a</i> <i>EsFem-1b</i> <i>EsFem-1c</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	Highly expressed in early embryonic development. In adults expression is sexually-dimorphic <i>EsFem-1a</i> was highest in hepatopancreas <i>EsFem-1b</i> in testis <i>EsFem-1c</i> in muscle <i>EsFem-1</i> suggested to be maternally inherited, providing protection to germ line through cell differentiation	Cui et al. (2015) Song et al. (2015) <i>EsFem-1a</i> : AKS25864.1 <i>EsFem-1b</i> : AKS25865.1 <i>EsFem-1c</i> : AKS25866.1

Table 2 continued

<i>EsFem-1</i> <i>EsFem-1b</i> <i>EsFem-1c</i> <i>EsFem-1hc</i> (homolog <i>c-like</i>)	Chinese mitten crab <i>Eriocheir sinensis</i>	Similarly expressed in males and females (whole body male and female transcriptomes) Authors state function of Fem in SD remains elusive	Liu et al. (2015)
<i>FEM-1</i>	Mud crab <i>Scylla paramamosain</i>	Equally expressed in testis and ovary (NB: only ovary and testis tissue analysed)	Gao et al. (2014)
Additional genes identified with putative function in sex determination in Decapoda (and Isopoda)			
Mostly from genes characterised in <i>Mammalia</i>			
<i>Vrille</i> (<i>Vri</i>), a bZIP transcription factor	<i>Daphnia magna</i>	This bZIP transcription factor, <i>Vrille</i> (<i>Vri</i>) is responsible for the male-specific up-regulation of <i>Dsx1</i> (which is the master sex determinant in males). <i>Vrille</i> is a homologue of <i>Drosophila</i> 's circadian clock gene, <i>Vri</i> . Hence this is an example of how an apparently functionally unrelated homologue has been co-opted to act in sex determination in <i>D. magna</i> (exemplifying the plasticity of sex determination systems).	Mohamad Ishak et al. (2017)
<i>f element</i> (~3 Mb gained through horizontal gene transfer from <i>Wolbachia</i>)	<i>Armadillidium vulgare</i>	This genetic element is a section of the <i>Wolbachia</i> genome, that has been horizontally transferred into the <i>A. vulgare</i> genome, to act as a feminising element, even in the absence of <i>Wolbachia</i> infection. Thus, this inserted region ultimately constitutes the new W (female sex-determining) chromosome. The specific causative gene is yet to be elucidated.	Leclercq et al. (2016)
<i>SRY</i> (HMG-box) <i>HMG-box a</i> <i>HMG-box b</i> <i>SOX2</i> <i>SOX14</i> <i>SOX19</i> <i>ZFY1</i> + more	Oriental river prawn <i>Macrobrachium nipponense</i>	No data (identified in pooled tissue library) Authors suggest lack of a single sex-determinant	Ma et al. (2012)
<i>Fru</i>	Oriental river prawn <i>Macrobrachium nipponense</i>	No data (identified in testis cDNA library)	Qiao et al. (2012)
<i>SOX14</i> + more	Oriental river prawn <i>Macrobrachium nipponense</i>	Some differential express data between testis and ovary	Qiao et al. (2015)
<i>Mnms13</i> (male-specific lethal 3)	Oriental river prawn <i>Macrobrachium nipponense</i>	<i>Mnms13</i> expressed in all tissues, general male-biased expression, highest in testis Authors suggest a role similar to that characterised in <i>Drosophila</i>, involved in dosage compensation, upregulating X chromosome in males, thus is involved in mediating sexual differentiation (NB: chromosomal mechanism is currently unknown in this species)	Zhang et al. (2015)
<i>SRY</i> transcription factor binding site <i>SOX5</i> transcription factor binding site	Oriental river prawn <i>Macrobrachium nipponense</i>	Transcription factor binding sites found in the 5' flanking region of <i>Mn-IAG</i> , indicative of a regulatory role upstream of IAG. Authors note a conservation in these sites with other species (eg. mud crab, <i>S. paramamosain</i>)	Ma et al. (2016b)
<i>SRY</i> <i>SOX9</i> <i>Foxl2</i>	Whiteleg shrimp <i>Litopenaeus vannamei</i>	Expression testis > ovary Even the female sex determination-related <i>Foxl2</i> Authors highlight that the function of these genes in sex determination remain to be examined.	Peng et al. (2015) TSA Database: GDUV00000000 Unigene7231; CL86.Contig, Unigene2458; Unigene24037
<i>Fru</i> <i>SOX5</i> + more	Banana shrimp <i>Fenneropenaeus merguensis</i>	<i>FcFru</i> expressed in gonads, never chord and hepatopancreas <i>SOX5</i> in AG, eyestalk, testis and hepatopancreas	Powell et al. (2015)
<i>Ankyrin-2</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	Biased in early developmental stages. Located on putative sex-chromosome Authors suggest <i>Ankyrin-2</i> as a putative sex-determinant	Cui et al. (2015)

Table 2 continued

<i>EsFru</i> <i>EsFru-like</i> <i>EsSRY</i> <i>EsSRY8</i> <i>EsSOX2</i> <i>EsSOX5</i> <i>EsSOX8</i> <i>EsSOX14</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	<i>Es-SRY</i> shows low comparable expression in males and females (RPKM < 3) but authors describe a >1.5x FC in males All other transcripts are similarly expressed in males and females (whole body male and female transcriptomes) Identification of many SD elements suggests complex sex determination pathway	Liu et al. (2015)
<i>EsSOXB1</i> + more			
<i>Esfru1</i> <i>Esfru2</i>	Chinese mitten crab <i>Eriocheir sinensis</i>	<i>Esfru1</i> and 2 are encoded by the same genomic locus and generated by alternative splicing of pre-mRNA. Authors suggest Esfru1 to be involved in female-specific neuronal development. And Esfru2 (male biased expression) to participate in male sexual development.	Li et al. (2017) <i>Esfru1</i> : KY464984 <i>Esfru2</i> : KY464985
<i>SRY</i> transcription factor binding site	Mud crab <i>Scylla paramamosain</i>	Transcription factor binding sites found in the 5' flanking region of <i>Sp-1AG</i> , indicative of a regulatory role upstream of <i>1AG</i> .	Zhang et al. (2014)

In cases where the authors have arbitrarily named *Dmrt* genes but we have shown they fall into a given clade, the clade name is given after in brackets. Authors' conclusions are highlighted in bold

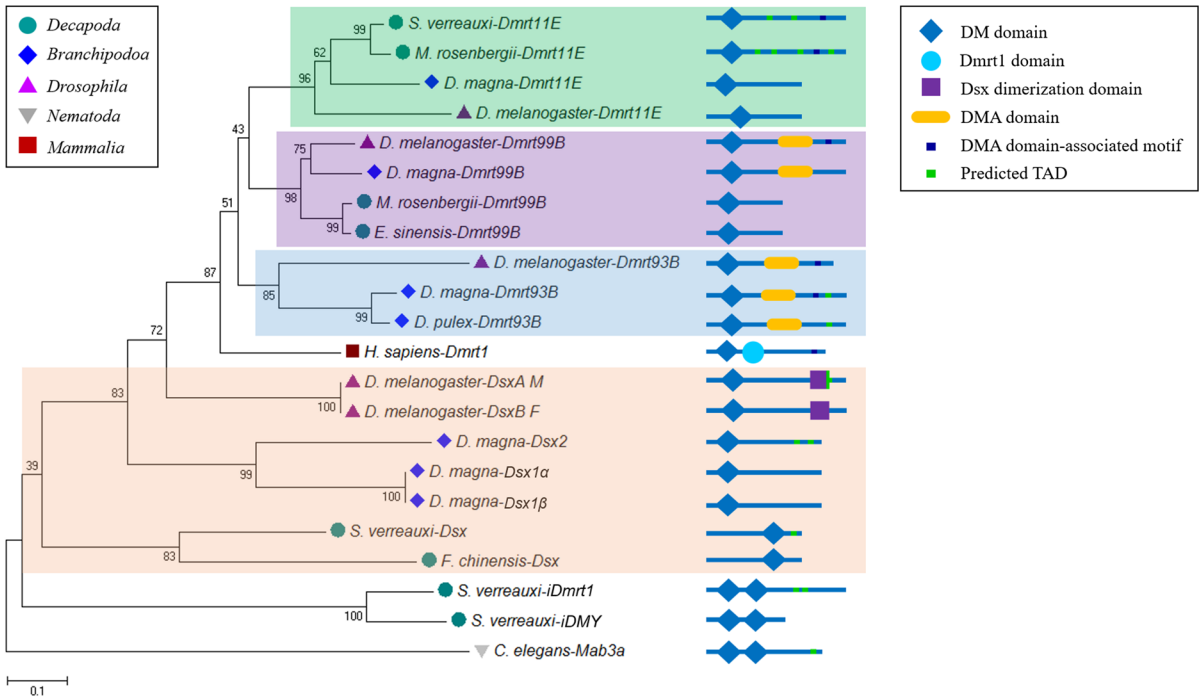
(*Mab-3*) and *Mammalia* (*Dmrt1*) is readily illustrated in *D. magna*. In this branchiopod, a *Dmrt* homologue (*Dsx1*) was determined to be the master male sex determinant (Kato et al., 2011). It is of note however, that four additional *Dmrts* were also identified, *Dmrt11E*, *Dmrt93B*, *Dmrt99B* (Kato et al., 2008) and *Dsx2* (Kato et al., 2011) and determined to have no clear function in sexual development. This highlights the critical importance of the functional analyses to reliably determine the explicit regulatory role of *Dapma-Dsx1*. The functional conservation of the *Dmrts* continues in the decapods, as recently a Y-chromosome-linked *Dmrt* was identified in the Eastern spiny lobster (*S. verreauxi*) (Chandler et al., 2017). This gene, termed as *Sv-iDMY*, offers the third example of a sex-linked *Dmrt* across *Animalia* (joining medaka *DMY* and frog *DM-W*) and is the first example in an invertebrate. As is the case in both medaka and frog, our functional analyses suggest that *Sv-iDMY* has evolved as the master sex determinant in this decapod species (Chandler et al., 2017).

To better illustrate the evolutionary and mechanistic features of the *Dmrt* family, Fig. 1 presents the phylogeny of all the available decapod *Dmrt* sequences, as well as those from *D. pulex* and *D. magna* and the model *Dsx*, *Mab-3* and *Dmrt1*. Figure 1A shows the full protein sequence,

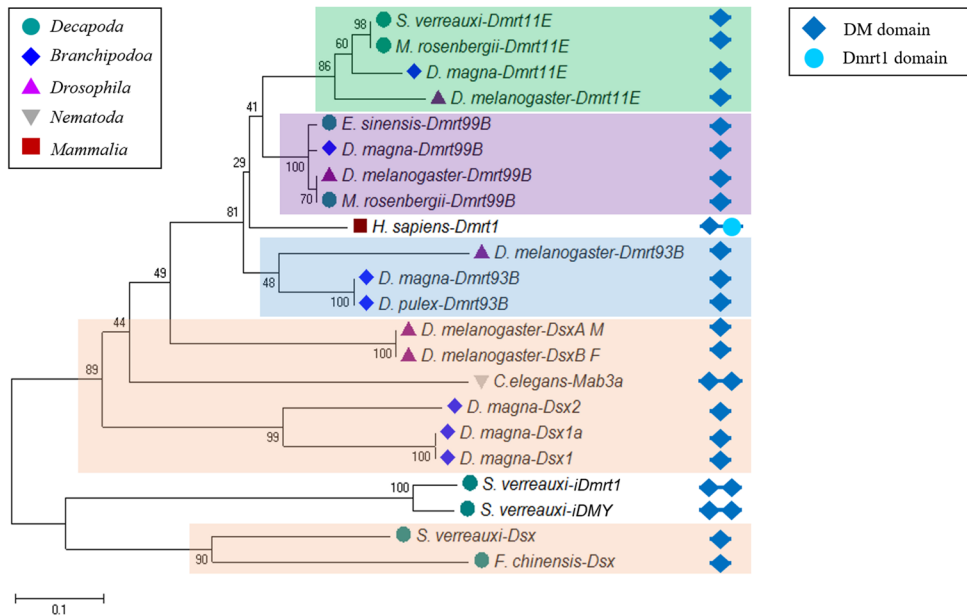
Fig. 1 Neighbour-joining phylogram and domain illustration of *Dmrts* in *Decapoda*, alongside model comparative species. **A** Displays phylogeny of full length *Dmrt* peptides, TAD predictions were conducted using the Nine Amino Acids Transactivation Domain (9aaTAD) Prediction Tool (Piskacek et al., 2007) as described in (Chandler et al., 2017). **B** Displays phylogeny of isolated DM domains. Throughout, the *Dmrt11E* cluster is highlighted in green, 99B cluster in purple, 93B cluster in blue and *Dsx* cluster in orange. Domain illustration and taxonomy keys are provided in figure. Bootstrap values are shown at each node and were performed with 1000 replicates to ensure reliability; scale bar indicates number of amino acid substitutions per site. All decapod GenBank Accession Numbers are given in Table 2, with the addition of *H. sapiens* *Dmrt1* (Q9Y5R6.2), *D. melanogaster* *Dmrt11E* (AAF48261.2), *Dmrt99B* (AAF56919.1), *Dmrt93B* (AAF55843.1), *Dsx*^M (AAF54169.1), *Dsx*^F (AAN13385.1) and *C. elegans* *Mab-3* (O18214.1)

highlighting the clear clustering of *Dmrt* subclasses, but more significantly, the pronounced variation in domain organisation outside of the DM domain itself. In contrast, Fig. 1B shows the phylogeny of the DM domains only, emphasising the extreme conservation of the DNA-binding domain that defines the family. Taken together, Fig. 1 demonstrates how the mechanistic diversity of this family is achieved: routed in the sequence and domain variation outside of the DM domain itself. The only exception being the

A



B



specialised repeat DM domain (defined as the Dmrt1 domain) of *H. sapiens* Dmrt1, which defines all

vertebrate Dmrt1 homologues and is not present in any invertebrates thus far (Wexler et al., 2014).

The mechanistic diversity of the Dmrts

A pronounced example of the domain variation illustrated in Fig. 1A is the DMA domain. This domain has been identified in multiple animal taxa, including the decapods, cnidarians and the ctenophores and tends to be associated with the Dmrt93B and 99B clades, lacking from Dsx homologues (Wexler et al., 2014). However, a function for this domain is yet to be determined. The transactivation domain (TAD) however, has an explicit functional significance, responsible for the transcriptional activation of the bound Dmrt-DNA complex (Mapp & Ansari 2007; Piskacek et al., 2007; Beukeboom & Perrin 2014). Hence its occurrence has dramatic potential to shape the mechanistic action of each Dmrt. In cases where the TAD is absent, such as *Drosophila* Dsx^F (Fig. 1A), additional coactivators (hermaphrodite (HER) and intersex (IX)) are required to achieve transcriptional activity (Pultz & Baker 1995; Garrett-Engele et al., 2002). Or indeed, in the absence of such coactivators, there is potential for the Dmrt to exert suppressive mechanisms, such as the antagonistic mechanisms described for DM-W (Yoshimoto et al., 2006; Yoshimoto et al., 2008) and iDMY (Chandler et al., 2017). When one considers the functional significance of each additional domain, it becomes apparent how the seemingly well conserved Dmrts adopt the diversity of mechanisms observed across species. Moreover, this domain variation points towards the varied necessity to recruit additional genes to support functionality (explaining the diversity of described sex determination networks), from which the integration of co-regulatory genes can be better elucidated.

The DM domain that defines the Dmrts

In contrast, that depicted in Fig. 1B, reiterates the dramatic sequence conservation of the DNA-binding domain that defines the Dmrt family; the DM domain is that responsible for binding the target DNA. This commonality across species therefore suggests that DNA-binding motifs, which constitute the response elements targeted by each Dmrt, must also be relatively well conserved. Indeed, such conservation of the Dmrt-binding motif has already been demonstrated amongst *Drosophila* sp. (Luo et al., 2011). This

conservation therefore offers a perfect method by which to bridge the gap, identifying the regulatory genes that act downstream of these key node regulators, through the identification of the conserved DM domain-binding motif. This has already been done in *Mammalia* (Murphy et al., 2010), *X. laevis* (Herpin et al., 2010) and *Drosophila* (Luo et al., 2011), informing of the auto and cross-regulatory effects of the Dmrt genes (Murphy et al., 2010), the mechanisms by which they coordinate their own expression (Herpin et al., 2010), as well as the extent of divergence of the DNA-binding motif itself (Luo et al., 2011). Similar promoter analysis in the decapods would facilitate the identification of genomic-binding motifs, from which candidate response genes can be elucidated and fully investigated through the more telling expression patterns gained through transcriptomics. Similar analyses have been conducted on a gene-by-gene basis, such as that conducted for the genomic regions of IAG in *S. paramamosain* (Zhang et al., 2014) and *M. nipponense* (Ma et al., 2016b), identifying a range of transcription factor binding sites in the 5' promoter region (Table 2). Although informative, when one considers the interconnected nature of genetic networks, a genome-wide analysis would prove a far more powerful tool to accurately assemble the regulatory map of sex determination.

Future directions

In summary, this review serves as a detailed reference for those interested in sex determination in *Decapoda*, offering a list of putative candidates that (in most cases) can act to guide further functional investigations. However, we also intend for the critical assessment presented throughout this summary, to highlight the risks associated with the arbitrary identification of target genes without an appropriate consideration of functional conservation. Thus, we urge, that when evaluating each homologous candidate, one considers the divergence that defines the rapidly evolving genes of sex determination.

The spatial expression analyses that constitute the majority of the molecular studies presented, are a sound starting point for functional investigations; they are however indicative and not conclusive. This, considered with the fact that *Decapoda* is a non-model

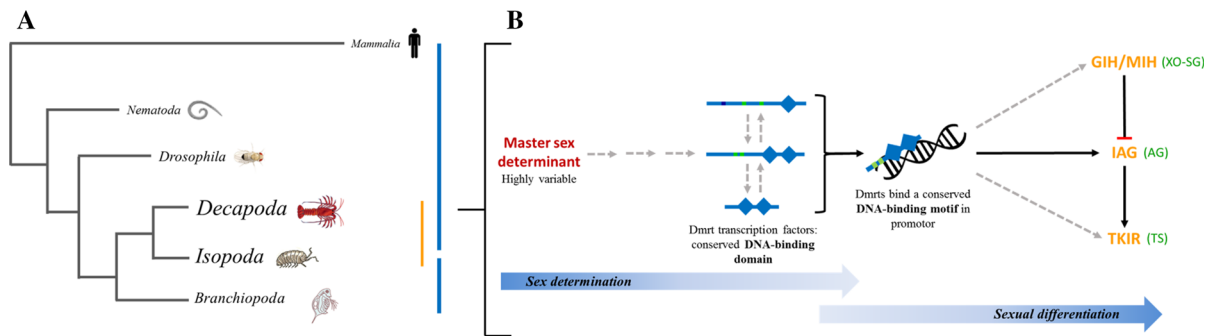


Fig. 2 **A** Illustration of the evolutionary relationships of the focal taxa discussed in this work, emphasising the extreme evolutionary distance over which the *Dmrts* are conserved, highlighted by the blue bar (note that the *Dmrts* remain yet to be identified in the isopods). The shared role of *IAG* in the malacostracan Orders is highlighted in orange. **B** Revised

Order, advises that the best approach to advance current understanding should build on what is known in *these* species, rather than relying on that characterised in others. As described above, the well characterised (and conserved) function of *IAG* in sexual differentiation, offers an ideal functional basis with which to integrate putative candidates.

Following this, we advocate for a greater emphasis to be placed on the *Dmrt* genes, which of all the candidate homologues, are known to have the most significant functional conservation. Work should aim to thoroughly investigate the spatial and temporal expression of the *Dmrts* in *Decapoda*. The use of genome-guided promoter analyses would inform of *Dmrt* interconnectivity, as well as facilitate the identification of, as yet, unknown response genes. In conjunction with (or, as is often the case, in the absence of) genomic resources, the use of *RNAi* knockdown is a well-suited tool to begin to understand the transcriptional integration of each *Dmrt* with each other, with *IAG* and with the regulatory elements of *IAG*, such as the CHH hormones (e.g. *GIH*) and the *TKIR* receptor (Aizen et al., 2016; Sharabi et al., 2016). Yu et al., (2014), present a sound example of such, using *RNAi* approaches to determine that of two *Dmrts* (both of which showed notable testicular-biased expression), *Dmrt11E* but not *Dmrt99B*, was functionally involved in the regulation of *IAG* expression. We therefore conclude with Fig. 2, presenting our revised suggestion of the regulatory axis of sexual development in *Decapoda*, emphasising the network-like qualities of the system, rather than the linear ones

depiction of the XO–SG–AG–TS axis of the *Malacostraca* (*Decapoda*), with inclusion of the *Dmrts*. Proven interactions are displayed in black line and those that hold a promising avenue for future work are shown in grey. The red-ended line indicates the inhibitory signal of *GIH/MIH*; all endocrine factors are shown in orange and tissues in green

described at present. In following these suggested avenues of future research, we can begin to identify and integrate associative genes, assembling a functionally valid understanding of sexual development in the decapods.

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